World Health Organization (WHO) Nipah Research & Development (R&D) Roadmap

Taskforce Meeting

Summary Report

July 31 & August 1, 2023
Wellcome Trust, London, UK

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Background and Meeting Objectives

The Nipah Research and Development (R&D) Roadmap provides a 6-year framework beginning in 2024 for identifying the vision, underpinning strategic goals, and prioritizing areas and activities for accelerating the collaborative development of medical countermeasures (MCMs) – diagnostics, therapeutics, and vaccines – against Nipah virus infection. The roadmap is a key component of the World Health Organization's (WHO's) R&D Blueprint Initiative, and was first drafted in 2017/2018 with input from subject matter experts, including a core group of taskforce members. WHO now aims to finalize and formally launch the Nipah R&D Roadmap, and the draft has been updated to incorporate recent scientific advances and research. The roadmap will be delivered to WHO in late 2023 for publication on the WHO website.

The meeting objectives include the following:

1. Briefly recap each of the four topic areas of the Nipah R&D Roadmap (cross-cutting, diagnostics, therapeutics, and vaccines).
2. Review the goals and milestones in each section and develop consensus on the wording and timelines for each milestone, as time allows.
3. Identify the milestones or issues that are highest priority.
4. Discuss challenges and opportunities for implementing the roadmap goals and milestones.

Meeting Participants and Observers

Meeting participants and observers joined both in-person and virtually. Those who participated virtually are noted with an asterisk (*). Sushmita Barmen was also present in-person as the meeting organizer for Wellcome.

Meeting Participants		
Christopher Broder	Tabitha Kazaglis*	Michael Osterholm
Emmie de Wit	Eve Lackritz	Julie Ostrowsky
Petra Fay	Stephen Luby	Shahana Parveen
Alex Freiberg	Alison Mack	Marie-Pierre Preziosi
Pierre Formenty*	Anje Mehr*	Mahmudur Rahman
Josie Golding	Joel Montgomery	Christina Spiropoulou
Emily Gurley	Kristine Moore	Anaelia Siya Temu*
Kim Halpin	Nicolina Moua*	Angela Ulrich

Guest Observers		
Zakiul Hassan	Cathy Roth	Laura Mazzola

Cross-Cutting Issues in the Nipah R&D Roadmap

Emily Gurley served as the facilitator for discussion of cross-cutting issues in the draft roadmap.

Emily began the session with a review of important advances, barriers, challenges, and key needs for Nipah (NiV) vaccines. Key points from that discussion:

- Various entities (e.g., the National Institute of Allergy and Infectious Diseases [NIAID]) have been providing financial support for this work (Spiropoulou, Gurley).
- Researchers and developers have made significant contributions to NiV vaccines in the last 5 years; that's been the main R&D push, but we're now coming to a bit of a roadblock (Spiropoulou).
- The discovery of other henipaviruses, such as Langya, raises the importance of NiV and henipaviruses in general (Montgomery).
- Surveillance is lacking (Montgomery); the more we look for henipaviruses, the more we'll find and they will continue to be a global problem (Broder).
- The Centers for Disease Control and Prevention (CDC) supports NiV activities, but not to the same extent as NIAID; for example, surveillance isn't adequately supported in Bangladesh, India, or Southeast Asia (Montgomery). CDC's investment in Bangladesh has been crucial, but more effort is needed (Luby).
- More surveillance is needed in Bangladesh and India as well as other countries (e.g., Philippines, Malaysia). Undiagnosed NiV infections likely are occurring and we need to better understand the burden of disease and transmission; we are potentially missing a lot of cases (Montgomery, Luby).
- We also need better surveillance of bats to improve our ecological understanding, including in SE Asia, where there are likely more strains of NiV circulating in wildlife than we are currently aware of (Montgomery).
- CDC is working on modifying case definitions and respiratory surveillance. We are missing cases because our surveillance is not good enough, and this affects diagnostics and other MCMs. Identifying cases earlier in illness (e.g., respiratory phase) is critical but not yet accomplished (Montgomery).
- Surveillance for Japanese encephalitis (JE) is ongoing in Bangladesh, including the collection and characterization of blood samples. These samples could potentially also be tested for NiV if funding can be made available (Rahman).
- In the past, incident-based surveillance was conducted, but funding has weakened and now only large clusters of infections are identified, missing sporadic cases. The JE surveillance program could be leveraged, and this is being pushed by the WHO South-East Asia Regional Office (SEARO), along with JE vaccination (Rahman).
- Surveillance is the key to making a business case for development of NiV MCMs (Gurley).
- Minimally invasive diagnostic methods are needed that avoid cultural reluctance for postmortem exams (Halpin).
- Bangladesh has made significant advancements over the last 5 years in postmortem exams through minimally invasive sampling. These updates could possibly be added to the epidemiology/surveillance section of the roadmap (Gurley).

- Risk communication is important and potentially a cross-cutting issue; it should be addressed somewhere in the roadmap. The roadmap also should address the need to involve media, policymakers, and the general public in NiV prevention efforts (Rahman).
- Political buy-in is important for surveillance, reporting, and prevention (Gurley).
- We should consider linking NiV to other surveillance efforts, including influenza and postmortem work. If we can create a common platform and case definitions, we can identify cases for further testing (Parveen).
- The Coalition for Epidemic Preparedness Innovations (CEPI) is engaged in discussion with regulatory authorities on advancements in licensure and approval of NiV MCMs (Rahman).
- The long-term goal is to have a rapid, bedside diagnostic test. Antibodies/antigens are available that could be used to develop such a test, but investment is lacking (multiple participants).

Emily then led a discussion of the draft roadmap strategic goals and milestones for cross-cutting issues. Discussion highlights are detailed in the tables below.

Cross-Cutting Issues: Strategic Goal 1 and Aligned Milestones		
Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 1: Identify additional and ongoing sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV MCMs.	• No comments.	• No edits suggested.
Milestone 1: By 2025, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.	• Persuading people that this issue is a reasonable investment is challenging, given few recognized cases (Luby). • It might be worth emphasizing the risks posed by gain-of-function research with NiV, especially given the democratization of synthetic biological tools (Luby). All disease roadmaps should acknowledge this risk (Gurley). • The COVID-19 pandemic showed what can happen and how either humans or nature can make viruses bigger threats. Even without nefarious actors, nature will give us more challenging viruses; roadmaps should acknowledge potential for enhanced transmission (Osterholm). • Three quarters of the world's population is at risk from NiV and henipaviruses, not just SE Asia. Henipaviruses	• The group agreed that the value proposition is still needed (and should remain a milestone). • Add phrasing to the Introduction to acknowledge recent experience with coronaviruses to capture the point about nature and humans both capable of more challenging viruses. • Remove "potential" from point #1 of the milestone; emphasize that this is a global threat that requires

	should be emphasized as the virus being focused on, as it is more inclusive (Montgomery). - While milestones under this Strategic Goal are important and should be addressed soon, no timelines should be earlier than 2024, as the roadmap will not be published until 2024 (Moore).	global engagement (Luby, Gurley). - Modify milestone language to the “global threat of NiV and related henipaviruses” (Moore, Gurley). - Add a comment to the Introduction about the risk to the global population. - Change the timeline from 2025 to 2024.
Milestone 2: By 2026, create a funding plan based on the value proposition for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.	- Since 2017, the funding situation has improved (e.g., CEPI, Gates Foundation). The shortfall of indirect funds is the major current roadblock (Broder). - An expert able to provide economic calculations to inform a clearly-articulated value proposition is important for NiV MCMs (Preziosi). - A value proposition has been developed for Lassa fever therapeutics; the group could consider reaching out to the team that developed that proposition (Hassan). - In addition to a solid economic calculation for NiV MCMs, we need the vision and explanation of importance from NiV experts (Gurley).	- Change the timeline from 2026 to 2025 (Gurley, Moore).
Milestone 3: By 2026, develop a coordinated strategy for promoting and incentivizing greater industry engagement in R&D for NiV MCMs.	- CEPI and pharmaceutical companies in Bangladesh are discussing stockpiling vaccines; they might also be interested in stockpiling monoclonal antibodies (mAbs) (Rahman). - At a CEPI meeting in June 2023, concern was expressed that mAb therapies are not feasible due to expense, even though their cost has been reduced drastically the last 5-6 years. Because NiV and Hendra do not mutate a lot, mAbs could be useful for a longer period of time and they could be combined in cocktails (of >1 antibody) to prevent viral escape (Broder).	- Add mention of indirect costs and shortfalls. - Add in-country industry partners. - Change the timeline from 2026 to 2025 (Gurley, Moore).

	• Indirect costs and shortfalls are lacking but needed for industry partners to produce GMP products that are ready; the money is not currently there (Broder). • Industry interest in NiV MCMs may have changed from what it was previously as a result of the COVID-19 pandemic (Roth). • In addition to industry work on MCMs, there are US federal scientists and industry partners who will produce countermeasures for the public benefit. It is important to promote that the technology for the MCMs is available and that it be given to affected countries so those countries can evaluate and manufacture for their own use (Broder). • Participants raised the importance of engagement of in-country industry partners.	

Cross-Cutting Issues: Strategic Goal 2 and Aligned Milestones		
Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to better define the disease burden, risk factors for infection, and risk of spillover events in affected countries.	• Instead of “affected” countries, use “countries at risk” or “countries with appropriate reservoir hosts” (Gurley, Rahman, Moore).	• Change goal language from “affected” countries to “at-risk” countries and include reservoirs in the revised Strategic Goal (Moore).
Milestone 1: By 2025, develop a plan for enhancing human NiV surveillance in India and Bangladesh outside of existing surveillance areas (i.e., where cases previously have been identified). This should include securing funding, identifying additional surveillance catchment areas, engaging key partners in those areas, generating standardized surveillance protocols, and conducting training for implementation.	• Discussion for milestones 1 and 2 (which currently separate development and initiation of a NiV surveillance plan) had significant overlap, and the group agreed that they should be combined. • Consider adding leveraging of existing surveillance platforms for other disease, such JE (Rahman). • It is also important to collaborate with organizations already in place, e.g., CDC and WHO (Parveen). • Surveillance should not be restricted to human surveillance; instead, this should include all relevant	• Combine milestones 1 and 2; the revised milestone timeline should be 2025 (Gurley, Moore). *[Note: In the edited version, original milestone 1 is now milestone 3.]* • Add a new milestone on human surveillance outside of India and Bangladesh with

Milestone 2: By 2026, initiate enhanced human NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), promote early case detection, and better define the disease burden in India and Bangladesh, particularly outside of areas where cases previously have been identified.	efforts, such as surveillance in domestic animals and wildlife (Roth). • Surveillance must be clearly embedded in comprehensive strategies for prevention and control. We want India and Bangladesh to lead in surveillance and extend their leadership to other countries (Formenty). This highlights the importance of broadening surveillance to include henipaviruses, and the importance of disease prevention (Gurley). • One Health issues are important in relation to surveillance for spillover into domestic animals and livestock (Broder). Such issues are an important part of a value proposition (Gurley). Tests for some diseases are required of certain species for international transportation (Halpin). • Instead of using the word “Nipah” throughout the roadmap, it should say “Nipah and Nipah-like” or “Henipavirus” (Gurley, Broder). • Hendra virus genotype 2 (HeV-g2) taught us that PCR tests will miss related viruses, and therefore we need serology on samples (Broder). • Add a milestone on conducting human surveillance in areas other than India and Bangladesh.	a timeline of 2025. *[Note: In the edited version this new milestone is now milestone 4.]* • Add a milestone on conducting surveillance outside of India and Bangladesh in animals with a timeline of 2025. *[Note: In the edited version this new milestone is now milestone 5.]* • Expand language in the roadmap, as appropriate, from just “Nipah” to include Nipah-like and/or Henipaviruses (Gurley, Broder).
Milestone 3: By 2026, generate and implement standardized protocols for case investigation that are aimed at identifying risk factors for primary NiV infection and at conducting case-contact studies to better understand chains of transmission ([Hedge 2023]()).	• Incorporate One Health for this milestone (Rahman, Gurley). • Bangladesh already has a protocol for this, and the milestone could be moved to 2024 (Rahman). However, it might take time for India to meet this milestone (Luby). • WHO could help by hosting a standardized protocol and, if spillovers happen in other countries, they could go through WHO to avoid inter-country tension (Luby). • WHO has developed several standardized protocols for other diseases, such as Crimean Congo Hemorrhagic Fever (CCHF). Part of that work involves WHO developing a standardized protocol for appropriate and in-depth case	• Change the timeline from 2026 to 2024. • This will now be milestone 1 in the edited version because of the date change. • Add One Health language to the milestone.

	investigation. This is needed well before 2026 (Formenty). • Bangladesh and India have protocols in place; the push is to share, modify, and expand into other countries, and to have funding for that work (Montgomery). • "Standardizing" includes adaptability to individual countries/regions (Gurley, Osterholm).	
Milestone 4: By 2026, develop plans for conducting additional research in India and Bangladesh to identify the potential for and drivers of spillover events, particularly in areas where NiV cases have not yet been identified.	• Additional research should not be restricted to India and Bangladesh; for example, Malaysia has requested work on such protocols (Formenty, Gurley).	• Change the timeline from 2026 to 2025. • Revise milestone so it is not restricted to India and Bangladesh (Formenty, Gurley). Instead, refer to at-risk countries. *[Milestone 6 in the edited version.]*
Additional considerations	• The following comment was made in another section: Country-level capacity will need to be assessed and gaps identified as part of standard clinical protocol development (Parveen). This work could be included in one of the surveillance-related issues (Gurley). • Later in the discussion, participants suggested the following milestone (originally milestone 4 under Strategic Goa 4) be moved to this goal: By 2026, establish capacity at selected surveillance sites in India and Bangladesh to investigate NiV-related deaths by using post-mortem minimally invasive tissue sampling to enhance understanding of NiV disease pathogenesis (Bassat 2021).	• Consider adding the following milestone to this goal: "By 2025, assess country-level capacity in affected countries to implement standardized clinical protocols for case investigation and address key gaps in implementation." *[Milestone 2 in the edited version.]* • Move the following milestone to this goal: "By 2026, establish capacity at selected surveillance sites in India and Bangladesh to investigate NiV-related deaths by using post-mortem minimally invasive tissue sampling to enhance

		understanding of NiV disease pathogenesis ([Bassat 2021](#)).” *[Milestone 7 in the edited version.]*

Cross-Cutting Issues: Strategic Goal 3 and Aligned Milestones

Strategic Goal or Milestone	Discussion highlights	Suggested actions
Strategic Goal 3: Determine the requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure or approval of NiV MCMs by engaging an international regulatory working group representing NRAs in affected areas and other key international stakeholders.	• No comments.	• No edits suggested (although the comment below on changing the wording from an international regulatory group also applies to the wording of the goal).
Milestone 1: By 2024, conduct scenario planning to clarify the regulatory procedures and determine the acceptable pathways for approval and emergency use authorization of NiV MCMs (including vaccines, novel candidate therapeutics, repurposed therapeutics, and diagnostics) in countries at highest risk for NiV outbreaks (i.e., Bangladesh and India) through an international NiV-focused regulatory group.	Discussion and comments for Milestones 1 and 2 are combined in the following bullets: • The group touched on CEPI’s accomplishments specific to vaccines, but noted that this milestone is broader than just vaccines (Gurley, Moore). • In 2018, NIAID and country partners (e.g., Bangladesh) created a protocol for use of mAbs. However, this was not the work of an established international regulatory group (Broder). • Regulatory issues are major barriers to industry engagement and private sector participation. It would be helpful to understand these issues better and have an advocacy strategy to overcome those issues (Luby). • Look to WHO’s work with the African Vaccine Regulatory Forum (AVAREF), which allows for interfacing with regulators. It might be possible to create a subgroup from that group for relevant discussions (Roth). • WHO’s work with African regulators to involve the regulators on reviewing protocols and dossiers – mostly for vaccines – has been very positive, allowing for	• Replace “international NiV-focused regulatory group” with “international forum of regulators” or similar language (Moore, Preziosi). • Marie-Pierre Preziosi will ask a colleague at WHO for input on this milestone wording. • Milestone language should clarify that this is scenario planning to identify gaps (Moore, Gurley). • Remove “countries at highest risk for NiV outbreaks (i.e.,)” and “through an international focused regulatory group.” • No change to timeline.
Milestone 2: By 2025, determine if any key gaps exist for regulatory approval		• Milestone 2 should be reworded to “develop

and emergency use authorization of candidate NiV MCMs in affected countries and develop strategies to address those gaps, with specified timelines for completion.	progress made on regulatory harmonization. Broader efforts are being led now, such as working on mAbs (Preziosi). • The regulatory agencies in India and Pakistan are already involved, and therefore the focus should first be with them, followed by engaging others more broadly. There is a potential for bottlenecks if the work goes too broad at first (Gurley). • What constitutes a regulatory body (Gurley)? Examples provided were FDA, EMA, national regulatory agencies (Moore). • International regulatory groups cannot focus on individual diseases; their reviews are thematic. As such, the milestone wording should be adjusted and also expanded to include fast review of adaptive platform trials (Preziosi). • We need engagement from the countries themselves (Formenty). • An understanding of how this work is coordinated should be linked to this milestone, perhaps through WHO. For example, the WHO portal could be adapted for this purpose (Parveen, Gurley, Rahman, Preziosi).	strategies" and focus on developing strategies to address the gaps identified as part of milestone 1 (Moore, Gurley). • No change to timeline.
Milestone 3: By 2026, ensure that any issues affecting approval and emergency use of candidate MCMs in affected countries have been resolved and that consensus has been achieved on the necessary regulatory steps and procedures.	• The group noted that this milestone may not be realistic. It may be better for focus on a system for monitoring regulatory issues. • Assessing the need/capacity to conduct clinical trials must be included; it does not exist now (Hassan). • Capacity is a "key gap," so consider having a nod to capacity here, as this is a cross-cutting issue (Gurley). A milestone on plans/protocols for emergency use of candidate NiV MCMs is needed.	• Revise this milestone to creating a system for monitoring regulatory issues for licensure and use of candidate NiV MCMs. • Add another milestone on developing plans for emergency use of candidate NiV MCMs. • Add another milestone on assessing capacity to conduct clinical trials and field studies

		of MCMs, particularly during outbreaks.

Cross-Cutting Issues: Strategic Goal 4 and Aligned Milestones

Strategic Goal or Milestone	Discussion highlights	Suggested actions
Strategic Goal 4: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.	• Consider expanding to include non-animal models, such as organoids. Several countries have non-animal models in place, mainly for basic research (Halpin). • Possible edits suggested include adding "non-animal models" to the goal language and editing the language to "in vitro and in vivo models" (Gurley, de Wit). Instead, the group agreed to revise the goal by just shortening the language to remove reference to animal models.	• Delete "in human and animal models" (Gurley, Moore). • Consider adding non-animal models activities (e.g., organoids, using AI technology, etc.) to the additional research priorities.
Milestone 1: By 2025, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.	• CEPI, NIAID, and CDC are already doing this work (multiple participants). • Unclear whether this milestone should be kept or if it should be moved to research priorities (Gurley). • We should not lock into challenge strain, etc.; we need to know more about epidemiology (de Wit). • We need standardized assays, reagents, etc., for clinical trials, but these are continually improved and revised. These resources are needed now; some are available and some are in-progress (multiple participants).	• Change timeline from 2025 to 2024.
Milestone 2: By 2025, conduct additional research to further optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs.	• Consider changing to "standardized" animal models, since they are well-defined already (Freiberg). • Milestone could potentially be combined with an earlier milestone on assay standardization (Freiberg). • Discussion on whether we do or do not know which animal model is the best for MCM development, and whether it is the African green monkey (AGM) or if the AGM is just the best that we have currently (Broder, de Wit). • There is a lack of understanding on pathogenesis in humans (de Wit), but that is data we may never have (Broder).	• Leave the milestone as is for now. • Consider adding a new milestone on better understanding human pathology (Gurley, Moore). Wording related to this could potentially be "pathogenesis investigation protocol" or "standardized human pathology investigation" (Gurley). *[New milestone 4.]*

	- Any pathogenesis investigation has to be linked to surveillance: picking up cases early, developing MCMs to address early disease, understanding disease progression, optimizing animal models, etc. (Broder). - We need to do better clinical investigation to better optimize animal models of early-stage disease/pathophysiology (Montgomery). - The International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) developed clinical characterization protocols for a number of diseases. They are designed to be simple, capture evolution, natural history of disease, and can be made targeted and sophisticated. They were widely used for COVID-19 and other diseases. Such an approach would fit both surveillance and identifying cases where they happen (Roth). - Country-level capacity will need to be assessed and gaps identified as part of standard clinical protocol development (Parveen). This work could be included in one of the surveillance-related issues (Gurley).	
Milestone 3: By 2026, conduct sequencing of NiV strains from existing clinical samples obtained from past NiV cases to assess variability of NiV strains in India and Bangladesh (CEPI 2023a).	- This is an ongoing activity (Gurley).	- Delete this milestone (Gurley) and instead add to additional research priorities.
Milestone 4: By 2026, establish capacity at selected surveillance sites in India and Bangladesh to investigate NiV-related deaths by using post-mortem minimally invasive tissue sampling to enhance understanding of NiV disease pathogenesis (Bassat 2021).	- Participants suggested that this milestone be moved to Strategic Goal 2.	- Move this milestone to the surveillance-focused Strategic Goal 2 (Moore).
Milestone 5: By 2028, conduct research in animal models to determine if strain variability impacts efficacy of promising	- The timeline for this milestone seems far off (Gurley). - Much of this may not require animal models (de Wit).	- Change milestone language to de-emphasize animal models, i.e., “conduct

NiV MCMs or the accuracy of diagnostic tests.		research to determine if strain variability…" (Moore). • Move up the timeline by one year.

Diagnostics in the Nipah R&D Roadmap

Joel Montgomery served as facilitator for discussion on diagnostics in the draft roadmap.

Joel led the group in a review of important advances, barriers, challenges, and key needs for NiV diagnostics. Key points from that discussion:

- At the previous meeting of this group, two diagnostic platforms were discussed: a gene expert platform developed in India, possibly being moved to Bangladesh, and Linfa Wang's lateral flow platform (Gurley). Meeting participants were not aware of those diagnostic platforms progressing further (i.e., no one was aware of additional announcements or updates).
- Challenges for NiV diagnostics include lack of RDTs (e.g., lateral flow tests) or bedside diagnostics. Of note, bedside diagnostics would require changes to infection control practices.
- The CDC serology assay is low-cost and could be helpful in ecological studies, but supporting research has not yet been published; also, this assay can't differentiate IgM from IgG antibodies. CDC is also working with FIND to evaluate rapid diagnostic tests (RDTs); progress with RDTs will be driven by the perceived need for these tests in the US or Europe (Montgomery).
- Even severe cases of NiV are often not diagnosed; regular use of an RDT for severely ill patients could be justified (Gurley).
- Improved diagnostics are needed for the following reasons: (1) improve early detection when therapeutic intervention is viable; (2) ensure adequate infection prevention and control (IPC) practices; and (3) enhance surveillance to better understand the burden of disease. Point-of-care (POC) diagnostics are particularly useful for outbreaks and IPC implementation. RDTs can be used in rural settings that lack adequate surveillance or specimen transportation. Diagnostics can also be useful in postmortem surveillance (Broder).
- RDTs, despite limitations, are useful in remote locations, such as where NiV outbreaks have occurred. Tests that have longer shelf lives and resist heat and humidity (e.g., patch technologies) are being pursued. However, there is no market for rapid POC tests that would save frontline healthcare workers (HCWs) during an outbreak (Roth, Broder).
- POC tests for henipaviruses (i.e., broader than just tests for NiV) would increase the geographic area where the tests could be used (Gurley).
- Serially testing of contacts during an outbreak could be used to gather data for developing tests for early diagnosis (Montgomery, Broder).
- Patients generally do not present to the hospital before day 4 of illness, at which point they are severely ill. We want tests to work early, but we have no way to evaluate those tests. One approach would be to retest (2 days after initial testing) suspected cases that initially test negative if they are still symptomatic (Gurley).

Joel then led a discussion of the draft roadmap strategic goals and milestones for NiV diagnostics. Discussion highlights are detailed in the tables below.

Diagnostics: Strategic Goal 1 and Aligned Milestones		
Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.	• Some participants were unclear on what was meant by a virtual repository.	• Delete the words "virtual reference" in front of repository.
Milestone 1: By 2026, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).	Discussion of milestones 1 and 2 was as follows: • Discussion on what would be needed with a "virtual reference repository" highlighted the following: centralized registry for samples located in/owned by multiple countries; requests can be made but not necessarily granted; location is known and documented; control over samples is maintained in-country; bilateral agreements between individual researchers (Montgomery, Broder, Rahman, Moore, Osterholm). • The repository of samples "to be collected in future outbreaks" in milestone 2 is centralized within countries, whereas in milestone 1 the samples remain in labs and their locations are known through an online registry/catalogue. For the repository in milestone 1, a governance structure dictates rules for gaining access to samples (Broder, Mazzola, Osterholm). • The goal is to have samples accessible for diagnostic assay evaluation; a repository of well-characterized samples (Montgomery). • A governance structure is critical for several reasons, including that sample sharing must conform to the Nagoya Protocol and a prioritization of sharing requests for limited samples. This will not just involve India and Bangladesh, but also CDC (Rahman, Mazzola, Montgomery).	• Remove the words "virtual reference" and change to "repositories" (Moore, Rahman, Gurley). • The milestone should note that the samples are "well-documented" (i.e., full clinical history of patient), as well as "well-characterized" (multiple participants). • Potential milestone language to address the previous bullet: "well-characterized with a recommended set of metadata (i.e., age, sex, days since symptom onset)" (Moore, Gurley). • The milestone should also include both acute and convalescent samples (multiple participants). • Change milestone timeline from 2026 to 2024.

Milestone 2: By 2027, identify sustainable, long-term funding, determine sites for sample storage, and initiate creation of the virtual reference repository in Bangladesh and India, with samples to be collected during future outbreaks.	• CEPI created a reference panel, but its usefulness for validating rapid or POC diagnostics is limited (Preziosi). • Determining repository location should be determined by a governance structure; creating the governance structure will be a hurdle (Gurley, Montgomery, Osterholm). • Repository location will raise issues of biosecurity and security in the home countries and where samples are shared (multiple participants). • A WHO workgroup could be involved with the governance structure (Preziosi) and it would need to be wider than NiV alone (e.g., henipaviruses) (Montgomery). • Repository samples would need to be well-characterized, requiring a minimum set of metadata on samples (e.g., gender, age, onset date) (Gurley, Montgomery). However, excluding samples without that information might discourage sample sharing (Gurley).	• Revise this milestone to be appropriate with changes made to milestone 1 (e.g., remove “virtual”) (Rahman, Gurley, Moore). • “Repository” language should stay in this milestone, since locations is detailed (i.e., Bangladesh, India) (Rahman, Gurley, Moore). • Change milestone timeline from 2026 to 2025.

Diagnostics: Strategic Goal 2 and Aligned Milestones

Strategic Goal or Milestone	Discussion highlights	Suggested actions
Strategic Goal 2: Continue to develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are suitable for use in peripheral settings and that have minimal requirements for biosafety precautions and staff training.	• Milestone should be POC “and” near-patient, not “or” (Gurley). • We want tests that a community worker can perform with minimal biosafety risks (Montgomery). • Pan-henipavirus testing is the ultimate goal, but we need a NiV diagnostic now (Gurley). • In addition to “suitable for use,” we want to specify sustained storage/shelf life of tests (Roth). • Test affordability is the key to POC adoption (Montgomery). • The target product profile (TPP) for these tests is vague, so it is worthwhile to state “extended shelf life” or they might have a short shelf life, which impacts affordability (Roth).	• Change goal language from “point-of-care or near-patient” to “point-of-care and near-patient” (Gurley) and include language on extended shelf life (Roth).
Milestone 1: By 2025, engage appropriate regulatory agencies and	• “Commercialization” is intended to mean test approval and that tests are made commercially available. The	• Change timeline from 2025 to 2024.

NRAs to inform commercialization pathways for NiV diagnostic assays. This effort should include clarifying regulatory pathways for approval (including approval for emergency use) of NiV diagnostics.	milestone describes a discussion with regulators about what is needed to put a product on the market (Gurley, Broder, Moore). • Work towards this milestone can begin with existing samples (i.e., does not need to wait until repositories detailed under Strategic Goal 1 are created) (multiple participants). • NiV diagnostics might qualify for the Expert Review Panel for Diagnostics (ERPD) pathway hosted by WHO. Decisions would be made in light of difficulty to obtain clinical samples (Preziosi, Mazzola). • The existing use cases and TTPs for NiV diagnostics have not yet been finalized, so a new milestone should be added to accomplish this in 2024 (Mazzola).	• Add a new milestone for 2024 to finalize the draft TTPs and use cases for NiV diagnostics.
Milestone 2: By 2026, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP and can be used in peripheral sites.	• “Preclinical evaluation” is a FIND term that means laboratory work, not clinical samples. Consider changing to “analytical evaluation” or other term (Gurley, Montgomery, Moore). • Milestone timeline has to follow after the repository activities detailed under Strategic Goal 1 (multiple participants). • Participants were not aware of promising candidates with potential to seek approval in 2025 or 2026. • Technologies that can be applied to POC testing may advance in the next 2 years (Osterholm). Participants recommended that the milestone timeline stay at 2026, with recognition that there may be technological advancements that could be leveraged for NiV diagnostics by that time (Luby, Montgomery). • A suggestion was made to add a new milestone calling for the acceleration of POC and near-patient testing using existing technologies (Mazzola, Montgomery). • Matchmaking between diagnostic test developers/platforms and available reagents (e.g., mAbs)	• No change to the timeline o wording. • Create a new milestone (which will be a new milestone 2) that calls for the acceleration of POC and near-patient testing using existing technologies.

	could be done (e.g., as FIND did with Duke University) (Montgomery, Mazzola, Roth). • For milestones 2 and 3: WHO sets international reference standards and is creating an antibody standard. Standards have to be completed before the preclinical evaluation, so the order for milestones 2 and 3 should be reversed (standards [milestone 3] by 2025, preclinical work [milestone 2] by 2026) (Mazzola, Gurley, Moore, Montgomery).	
Milestone 3: By 2026, create international reference standards for calibrating and harmonizing NiV diagnostic assays.	• The milestone timeline has to follow after the repository activities detailed under Strategic Goal 1 (multiple participants). • For original milestones 2 and 3: WHO sets international reference standards and is creating an antibody standard for NiV. Standards have to be completed before the preclinical evaluation, so the order for milestones 2 and 3 should be reversed (standards [original milestone 3] by 2025, preclinical work [original milestone 2] by 2026) (Mazzola, Gurley, Moore, Montgomery).	• Change timeline from 2026 to 2025 (this will also change the order of original milestones 2 and 3, as milestone 3 will remain 2026).
Milestone 4: By 2026, develop a minimum protocol or set of best practices (that biosafety committees will accept) for inactivation of clinical samples from humans and animals that are being tested for NiV.	• Remove the word “minimum” from the milestone and change the timeline to 2024 (Rahman, Gurley, Moore). • Questions related to this have likely been addressed before, such as with PCR for suspected NiV patients in Bangladesh (Luby). However, new technologies (e.g., those on the horizon that would be considered “plug-and-play”) require consideration (Montgomery). • Standardized methods for inactivation would be desired (Gurley). • Every inactivation method has to be tested for use in the US. For companies, deactivation information is usually proprietary information and divulged only to regulatory agencies (de Wit).	• Revise the milestone: remove “minimum” and change the timeline to 2024 (Rahman, Gurley, Moore).
Milestone 5: By 2027, complete clinical validation of performance and operational suitability for at least two of	• No comments.	• No change to the timeline or wording.

the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP and can be used in peripheral sites.		
Milestone 6: By 2028, obtain regulatory approval for at least one rapid, point-of-care or near-patient care NiV diagnostic test that can be commercialized and standardized.	• Milestone wording leaves standardization up to the developer (Mazzola). • This is an aspirational milestone (Montgomery). • The company Molbio has emergency use authorization (EUA) in India for its Truenat test, but that test might not be used currently. While an EUA isn't regulatory approval, it can continue to be used for a long time (e.g., Ebola test) (Mazzola, Moore). • Change "obtain" to "promote" and/or "support" (Mazzola, Moore); leave the timeline at 2028 since milestone 5 is for 2027.	• Change "obtain" to "promote" and/or "support" (Mazzola, Moore).

Diagnostics: Strategic Goal 3 and Aligned Milestones		
Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known spillover risk to promote early detection of NiV.	• No comments.	• No edits suggested.
Milestone 1: By 2026, expand national laboratory networks for NiV detection in the primary affected countries (Bangladesh and India) that include plans for enhancing laboratory preparedness to enable earlier and timely detection of NiV infection during future outbreaks.	• As discussed previously, the milestone should refer to leveraging existing platforms and infrastructure, particularly given what was established for COVID (Gurley, de Wit, Rahman). • This needs to happen quickly to keep systems online. One strategy would be to assess post-COVID genomic activities related to materials and personnel, and seek alternative funding to keep this running, not just for NiV but for any potential pandemic threat (Preziosi, multiple participants). *[Note: This was not added as a milestone to the roadmap, as this seems to be more of an implementation issue.]* • Use "at-risk countries" instead of "affected countries" (Rahman).	• Remove "India and Bangladesh" from the milestone (Moore). • Change "affected countries" to "at-risk countries" (Rahman). • Change timeline from 2026 to 2025. • Verify that any related milestones in the cross-cutting section are revised, if needed, per diagnostics discussions.

	• Staffing is the key and there needs to be a system in place to keep places running. It is difficult to get funding for surveillance and detection activities (Roth, multiple participants). • Biosafety can be an issue (Montgomery). • Vigilance, safety, and training are necessary. We want active virus for some applications (Gurley, Montgomery). • Using serology to detect spillovers in humans and inform acute surveillance might be useful in other countries (Montgomery). • Consider adding other countries given need for lab capacity to diagnose NiV (for milestones 1-3) (Moore).	
Milestone 2: By 2027, generate well-characterized and up-to-date proficiency panels for NiV diagnostic testing to be used in selected laboratories in Bangladesh and India.	• This work might be done earlier than 2026 in India and Bangladesh; the timeline represents other at-risk countries. • Consider adding other countries (i.e., at-risk countries) given need for lab capacity to diagnose NiV (for milestones 1-3) (Moore).	• Remove “India and Bangladesh” from the milestone (Moore). • Revise to “…proficiency panels and network quality controls for NiV diagnostic testing.” • Add at-risk countries. • Change timeline from 2027 to 2026.
Milestone 3: By 2029, implement routine EQA monitoring of NiV diagnostic testing at selected laboratories in Bangladesh and India.	• Consider adding other countries given need for lab capacity to diagnose NiV (for milestones 1-3) (Moore).	• Remove “India and Bangladesh” from the milestone (Moore). • Add at-risk countries. • Change timeline from 2029 to 2026.

Joel concluded the diagnostics section with a facilitated discussion on roadmap implementation, including barriers to implementation and ideas for implementation. Key points from the discussion:

- Barriers:
    - Financial support and coordination (Roth)
    - In-country leadership attention/support (Gurley)
    - The lack of a sense of urgency (Gurley)

    - The lack of in-country champions (Rahman)
- Ideas for implementation:
    - WHO's role is critical.
    - A sense of urgency and buy-in is needed, specifically from:
        - Community and the affected/vulnerable populations (Rahman, Lackritz)
        - Political leadership in affected countries (Formenty, Osterholm)
    - Involving clinicians in overcoming barriers to sample sharing (Gurley)
        - Physicians would likely get behind this effort (Gurley); people already give samples to iccdr,b (which has decades of trust/presence in the community) (Rahman, Lackritz).
    - Funding should be internationally sourced by global public-private partnerships; this is a global pandemic prevention issue and we shouldn't ask the countries that are already stretched thin to protect wealthy countries.

Therapeutics in the Nipah R&D Roadmap

Emmie de Wit served as facilitator for discussion on therapeutics in the draft roadmap.

Emmie led the group in a review of important advances, barriers, challenges, and key needs for NiV therapeutics. Key points from that discussion focused on current challenges in the therapeutics field:

- Funding clinical trials is a challenge (multiple participants).
- IV administration of mAbs is also a challenge (Broder, Montgomery).
- NIAID funded grants for NiV antiviral discovery, for which Christopher Broder's group partnered with Novartis.
- In Bangladesh, patients often do not present until day 4 after symptom onset (Gurley). Because of that, a potent anti-NiV monoclonal likely to prevent significant mortality associated with diagnosed disease would be useful (Broder).
- With therapeutics, more patients would likely survive NiV, but would have sequelae unless an agent crosses the blood-brain barrier or cases are treated upon suspicion, like with rabies (de Wit, Broder). Patients would be surviving with neurological deficits (Luby).
- It's not clear whether mRNA therapeutics would be successful unless there is gene therapy (Broder).
- Expense will be a barrier, as was seen with COVID therapeutics (Rahman).
- The few numbers of patients presenting each year is a huge barrier to therapeutics development; this could maybe be overcome with better surveillance, although that is somewhat unlikely (Luby).
- Numerous challenges exist with developing NiV therapeutics; however, we should be considering the fact that the critical target here is to have therapeutics not just to treat current patients, but to have drugs available in case of a catastrophic pandemic caused by NiV or a related virus (Luby).
- Guidelines for supportive care are needed that can apply whether or not clinical trials are successful (Gurley).

Emmie then led a discussion of the draft roadmap strategic goals and milestones for NiV therapeutics. Discussion highlights are detailed in the tables below:

Therapeutics: Strategic Goal 1 and Aligned Milestones		
Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.	• No comments.	• No edits suggested, other than expanding to include related viruses (per earlier discussions).
Milestone 1: By 2024, convene a consortium of key stakeholders—in affected areas and internationally—to address the key challenges with conducting clinical trials of therapeutic agents during future NiV outbreaks. This consortium could potentially be modeled after the West Africa Lassa Fever Consortium (WALC) (ISARIC 2023).	• Clinical trial guidelines for Bangladesh and India should come first, but other affected countries should be included (multiple participants).	• Add related viruses (per earlier discussions). • No change in the timeline.
Milestone 2: By 2025, develop NiV standard of care guidelines to be used in affected countries, disseminate the guidelines, and conduct outreach to clinicians as appropriate.	• Based on Lassa and CCHF experience of developing and implementing training modules for physicians in Africa, consider how to raise awareness of symptoms and standards of care in affected regions. For the time being, consider adapting standards of care for other infectious diseases (Formenty). • We need to develop a network/critical mass of clinicians to adopt these standards of care if we want to enhance NiV detection (Formenty). • Consider adding another milestone on clinician awareness/training on standards of care; infection control could be tied to that (de Wit). Training is needed at the university level (Formenty). • Shahana Parveen noted that a training module that includes infection control was developed; she can share that.	• Revise milestone to add "infection control and standard of care guidelines" (Gurley, Moore). • Change timeline from 2025 to 2024. • Create a new milestone on training clinicians regarding infection control and standards of care guidelines, with a 2025 target date (de Wit, Moore).

	• In Bangladesh, family members provide most of the hands-on care in hospitals. That creates closed loops that limit hospital-wide outbreaks. As health systems develop, there are more opportunities for transmission (Gurley). Family members providing patient care need to be instructed in infection control (Parveen). • Training is easy compared with supply chains and running water for infection control (Gurley).	
Milestone 3: By 2025, complete an agreed-upon generic prepositioned protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates (mAbs and small molecules, including repurposed drugs) to be implemented in NiV-affected areas during outbreaks and develop plans for operationalizing the protocol.	• WHO is working on a rolling protocol (Roth). • COVID has changed the way such activities are approached and can serve as an example; as such, the dates can be moved up (multiple participants). • NIAID developed a generic protocol for mAbs with an intent that it could be used for other therapeutics. This could be reinvigorated. It was written for post-exposure prophylaxis (PEP) but could be modified to include pre-exposure prophylaxis (PREP) (Moore, Broder, de Wit, Gurley). • Semantics of pre- and post-exposure can be tricky: if you have been exposed but it is not detected, it is pre-exposure. • In an outbreak with pandemic potential, both those with known exposure and likely exposure should be treated (Osterholm).	• Change timeline from 2025 to 2024.
Milestone 4: By 2025, complete an agreed-upon generic prepositioned protocol for conducting PEP trials of promising therapeutic candidates (mAbs and small molecules, including repurposed drugs) to be implemented in NiV-affected areas during outbreaks and develop plans for operationalizing the protocol.		• Change timeline from 2025 to 2024. • Add PREP to this milestone.
Milestone 5: By 2026, complete a broader, harmonized regional protocol (to be used across Bangladesh and India) for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during outbreaks or potentially during periods of endemic disease (if comparable data can be generated over	• Milestone 3 and 5 are essentially the same; the group agreed to remove milestone 5.	• Remove this milestone.

time and across different clinical sites) and develop plans for operationalizing the protocol.		
Milestone 6: By 2026, generate a reliable source or stockpile of a mAB (m102.4 or other mAb) to be used in outbreak-related clinical trials for both PEP and early clinical treatment.	• CEPI is working on this, but does not support enough indirect costs to make production feasible. There are people ready to do this work if there was sufficient funding (Broder). • mAbs have long shelf lives (Gurley, Broder).	• No change to timeline or wording.

Therapeutics: Strategic Goal 2 and Aligned Milestones		
Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 2: Develop and evaluate therapeutic agents for the treatment of NiV infection and for PEP to prevent NiV infection.	• No comments.	• No edits suggested.
Milestone 1: By 2025, create and implement a prioritization process for determining which promising NiV therapeutic candidates should be further evaluated in clinical trials, once adequate animal data demonstrating safety and efficacy are available.	• This should be done as quickly as possible to determine qualifying animal data (de Wit). • This timeline works well with the current NIH Antiviral Drug Discovery (AViDD) Centers for Pathogens of Pandemic Concern, which have 2 years of funding left (Freiberg).	• Add a reference to a “governance structure” in the milestone. • No change to timeline.
Milestone 2: By 2026, complete preclinical evaluation in animal models—with administration of the therapeutic agent more than 24 hours after challenge and potentially after symptom onset—of the preliminary safety, tolerability, and efficacy of at least two promising small-molecule therapeutic candidates or combination therapies for the treatment of NiV infection.	• AViDD Centers should be pushing out antivirals; the 2026 timeline is realistic (de Wit).	• No change to the wording or timeline.
Milestone 3: By 2027, further explore in animal models whether two or three of the most promising small-molecule	• This milestone can be removed (de Wit).	• Remove this milestone.

therapeutic candidates are likely suitable for PEP and, therefore, should be assessed as PEP in clinical trials.		
Milestone 4: By 2027, determine the most cost-effective and feasible routes of administration for use in real-world settings for at least two promising small-molecule therapeutic candidates.	• This milestone can be removed (de Wit).	• Remove this milestone.
Milestone 5: By 2027, complete at least one additional clinical trial (phase 2 or 2/3) in a NiV-affected area of m102.4 or other suitable mAb to further assess safety, tolerability, and efficacy (if NiV incidence allows efficacy assessment).	• Revise the milestone so it is not focused on m102.4 specifically (multiple participants). • The group agreed that this milestone should be changed to a phase 1 trial. • Then develop a new milestone on phase 2 or phase 2/3 trials for a mAb or mAb cocktail; this milestone should have a later timeline (de Wit).	• Change the milestone to; “complete one additional phase 1 clinical trial of a monoclonal antibody” (i.e., do not specify 102.4) (Moore). • With language revision, allow for a mAb cocktail, e.g., “mAb(s)” (Broder, Roth). • Change the timeline from 2027 to 2025 (Preziosi). • Create a new milestone on phase 2 or 2/3 trials for mAbs with a timeline of 2027.
Milestone 6: By 2028, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising small-molecule therapeutic candidates or combination therapies for the treatment of NiV infection.	• The group agreed with the language “combination therapies”; there is no need to specify monoclonal antibodies. • Consider a separate milestone for combination therapeutic trials; alternatively, that could be two arms of the same phase 1 trial (Moore, Preziosi, Gurley). This milestone might be maintained with revisions to the language (vs. creating a new milestone).	• Possible revision: “By 2028, complete at least one phase 1 clinical trial of at least two small molecule therapeutic agents or combination therapies …” *[Note: The language was not finalized during the meeting and will be revised based on written feedback from the group].* • No change to the timeline.

Emmie concluded the therapeutics section with a facilitated discussion on roadmap implementation, including barriers to implementation and ideas for implementation. Key points from the discussion:

- Barriers:
    - Money
    - Lack of coordination
    - Lack of urgency
    - Time
    - Delays in ethical clearance; phase 1 trials would mitigate this, given acceptance of results from other countries (clinical trial harmonization).
    - Therapeutics for NiV are not going to make money, they're mainly for pandemic preparedness.
    - The number of detected cases is small, which is a barrier to drug R&D.
- Ideas for implementation:
    - Synergy with other efforts (e.g., JE work) (Preziosi).
    - Use existing capacity for conducting clinical trials in affected countries (Bangladesh and India); leverage existing clinical trial infrastructure (Preziosi).
    - Build capacity for participating hospitals/facilities:
        - Find ways to improve overall quality of care as part of trials, rather than funding high-expense treatment for a few patients (Gurley).
        - There must be continuity for enrolled patients in all circumstances (Hassan).

Vaccines in the Nipah R&D Roadmap

Christopher Broder served as facilitator for discussion.

Chris began the session with a review of important advances, barriers, challenges, and key needs for NiV vaccines. Key points from that discussion focused on recent advancements in the vaccine field:

- The measles NIV construct vaccine from the University of Tokyo is a candidate of note (Luby).
- Another vaccine candidate of note is the ChAdOx1 NiV vaccine, with a phase 1 trial starting by December 2023 (Broder).
- It has been amazing to see a robust set of vaccine candidates in the last 5 years, with much thanks to CEPI (Luby).
- Vaccines currently in phase 1 trials are: the NIH-Moderna mRNA vaccine, an rVSV vaccine that expresses the envelope glycoproteins of both Ebola virus (EBOV) and NiV, and a soluble G protein vaccine (Preziosi, Broder).
- Stocks are available and standardized at this stage (Preziosi).

- Elements still needed for the soluble G program include a correlate of protection (CoP) study, challenge strain selection, and identification of who will conduct the studies (Broder).
- The SG protein candidate is the furthest along; researchers are now characterizing polyclonal immune responses from subjects (Broder).
- A subunit vaccine could be ready quickly, which could help stop an outbreak from spreading, as well as be used for laboratory workers and first responders (Broder).

Chris then led a discussion of the draft roadmap strategic goals and milestones for NiV vaccines. Discussion highlights are detailed in the tables below.

Vaccines: Strategic Goal 1 and Aligned Milestones		
Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 1: Develop the tools and policies necessary for evaluating and potentially approving one or more NiV candidate vaccines through a nontraditional regulatory pathway.	• No comments.	• No edits suggested.
Milestone 1: By 2024, generate a fully characterized and controlled virus challenge stock (or potentially one each for NiV-M and NiV-B) for assessing candidate vaccines in animal models.	• Other than the mRNA vaccine, all candidate vaccines are based on the G protein, which is nearly identical between the Malaysian and Bangladesh strains, and therefore multiple strains are not needed for challenge studies (Broder). • One challenge strain from NiV-B is apparently established (Broder, de Wit). • All NiV strains are 98% identical. Hendra (at 20% divergent) is 100% protective against both NiV-B and NiV-M, which is a proof of concept for using a single challenge strain in an animal model vaccine efficacy trial (Broder). • It would be helpful for this group to share what makes the most sense for the field. NiV-B is responsible for more outbreaks, spillovers, and appears more transmissible and causes more severe disease. As such, the challenge stock should be NiV-B. There might be disagreement on that suggestion, but that choice would be focused and achievable (Luby).	• Remove "(or potentially one each for NiV-M and NiV-B)." • Add (preferably a NiV-B strain). . .

Milestone 2: By 2024, establish benchmark parameters (e.g., route of challenge, timing of challenge, and challenge dose) for testing of NiV candidate vaccines in animal models, with particular focus toward meeting criteria necessary for approval via a nontraditional pathway.	Discussion for milestones 2 and 3 is combined as follows: • This timeline (2024) seems too soon, unless a program funding vaccine development wants this path forward. So far, there are limited experiments in primates with aerosolized devices. The model must be interpretable in a small number of animals (Broder). • An animal challenge model must be coordinated with regulatory authorities because of the expense (de Wit). • Repeated testing has not been performed by many different routes. There is one (intertracheal and internasal) that is accepted; other models on aerosol exposure are being pursued (Broder). • It is possible that the existing serum neutralizing IgG antibody will be the CoP. • We will never be done characterizing animal models (Broder). • There is significant overlap between milestones 2 and 3. They can be merged and given a timeline of 2025. CEPI is aiming for Accelerated Approval, so we need a good surrogate marker; this will be easier than the Animal Rule pathway (de Wit, Broder, Moore). • With accelerated approval, the animal model will most likely be the African green monkey (Broder).	• Change timeline from 2024 to 2025. • Add language that the work in this milestone be done “in conjunction with regulatory authorities” (Moore). • Add language to specify a “well-characterized animal model” (Moore). • Add surrogate markers per discussion of the next milestone.
Milestone 3: By 2024, further characterize as needed at least one animal model suitable for determining surrogate markers that correlate with vaccine efficacy.		• Remove milestone 3 (given overlap with milestone 2) and add determining surrogate markers to the previous milestone (Moore).
Milestone 4: By 2025, define the protective threshold against NiV infection for serum neutralizing IgG antibodies (as a CoP or surrogate marker), which can be used in animal studies and for immunobridging to humans.	• Traditionally, we do not see high neutralizing titers early on. With our intranasal vaccine, we see good antibody response and good protection in hamsters. We are moving to nonhuman primates (NHPs) in September 2023 (Spiropoulou). • The challenge route and the animal model are going to be different each time. But for vaccines used in human trials, antibody response in serum will be measured (Broder). • The group discussed whether neutralizing antibodies should be the sole CoP. • Avoid language in the milestone that locks into serum neutralizing antibodies despite its prominence as a likely	• Change language to include “serum neutralizing antibodies or other functional CoPs” (Broder, Spiropoulou, Moore). • No change to timeline.

	CoP. Include "other" CoPs due to the ability to induce T-cell responses.	
Milestone 5: By 2025, convene a group of key stakeholders to agree on, through a consensus approach, the most suitable assays (e.g., assays that are developed using live NiV vs. assays developed using pseudoviruses) for determining serum neutralizing IgG antibody titers for NiV vaccine R&D and develop a strategy for standardizing those assays.	• A WHO anti-NiV antibody standard is already developed and due for publication in October 2023. This milestone may not be needed (Broder).	• Remove this milestone.
Milestone 6: By 2026, generate standardized assays to measure neutralizing IgG antibodies for NiV vaccine R&D, with a particular focus on assays to be used for regulatory approval via nontraditional pathways.	• This should be antibodies in general, not specifically "neutralizing" (Spiropoulou). • The WHO antibody standard is forthcoming and should be used; therefore, this milestone is essentially done (multiple participants).	• Remove the word "neutralizing" from the milestone. • Change timeline from 2026 to 2024 and reorder the milestones.

Vaccines: Strategic Goal 2 and Aligned Milestones

Strategic Goal or Milestone	**Discussion highlights**	**Suggested actions**
Strategic Goal 2: Continue to move the current NiV vaccine pipeline forward toward licensure.	• Based on group discussion around milestones 4-6, consider revising Strategic Goal 2 to be on moving the pipeline forward (i.e., using language from milestones 1 and 2), and the other milestones remaining in this section would be part of that revised Strategic Goal.	• Revise the Strategic Goal as detailed in the discussion column.
Milestone 1: By 2024, complete current phase 1 clinical trials for at least three promising NiV candidate vaccines.	• This work is underway. Keep this as "at least three" as written currently (Broder, de Wit).	• No changes to timeline or wording.
Milestone 2: By 2024, define use cases for NiV vaccines to inform vaccine deployment and manufacturing plans.	• We need to understand which vaccines will come through phase 2 for this work (Gurley).	• Change timeline from 2024 to 2025.
Milestone 3: By 2026, further assess vaccine safety and immunogenicity through additional phase 1 and initial phase 2 clinical trials (preferably in affect	• The CEPI trial in Bangladesh with a subunit vaccine will likely begin before 2026, as they plan to start phase 2 in 2024 (Broder).	• Revise milestone to "initiate phase 2 trials by 2025" (multiple participants).

areas) for at least two of the most promising NiV candidate vaccines.	• The VSV vaccine might start phase 2 in Bangladesh in 2024, and the subunit vaccine would follow. Not many patients are needed, but approval will take some time. Two clinical phase 2 trials by 2026 is realistic (Luby). • The financial support and commitment are there (Luby). • This milestone is not entirely dependent on CEPI, as NIAID also could do phase 2 trials (de Wit).	
Milestone 4: By 2027, complete immunogenicity and efficacy studies in a well-characterized animal model for at least one cross-protective NiV vaccine candidate and define a surrogate marker that demonstrates likely clinical benefit of candidate vaccines.	• The group agreed that this milestone should be removed because this is covered in Strategic Goal 1 of this section.	• Remove this milestone.
Milestone 5: By 2029, conduct well-controlled clinical trials (preferably in affected areas) to assess a surrogate endpoint in human subjects for at least one NiV candidate vaccine.	• Trials for a surrogate endpoint might be necessary to repeat in affected areas with different populations (Broder, Preziosi). • Going back to Strategic Goal 1, and then following the progression of activities, we have 1) characterizing the virus stock, 2) establishing benchmark parameters for well-characterized animal model, 3) defining protective thresholds to be used in animal studies for immunobridging, 4) generate/use WHO standard NiV assay, and then 5) clinical trials. • This milestone date is currently for completely trials; could be changed to initiating trials (and date moved up) (Moore). Completing phase 2 trials could be done faster than by 2029 if initiated by 2025; they could be done by 2027 (Broder). • We may need to do more than one phase 2 trial (e.g., children in Bangladesh). If these are going to be only human studies with efficacy via bridging, questions will be raised, including by regulators, who will want more than initial phase 2 or a more robustly powered trial. It is hard to anticipate an area that has few precedents, but	• Revise language: for at least two candidates (instead of one) and remove "preferably." Additionally, see discussion highlights for possible other revisions (e.g., additional clinical trials including vulnerable populations). • Ensure that the milestone is clearly for phase 2 (i.e., appropriate phase 2 context). • Keep the current timeline.

	expectations should not be for "normal" vaccine development (Luby, Preziosi). Given that, a 2029 date is appropriate (Moore). • Trials will already include humans, and the language should be expanded to detail certain populations. Specifically, children, pregnant women, and women of childbearing age should be noted (Broder, Roth, Luby, Osterholm). We would be compromised in an outbreak without data on pregnant women (Luby). • Discussion on milestone rewording included a few recommendations, including: keep the 2029 date and add additional clinical trials including children and pregnant women; detail at least two vaccines; "in affected area" should be a requirement, not "preferably".	
Milestone 6: By 2030, complete a regulatory dossier for at least one NiV vaccine candidate based on a suitable animal model with subsequent immunobridging to humans for review via a nontraditional approval pathway.	• By 2030 is reasonable for this milestone, but it is unclear if this is for licensure or EUA (Broder, Preziosi).	• Add language on "(for licensure or emergency use)" to the milestone (Moore).

Chris concluded the vaccines section with a facilitated discussion on roadmap implementation, including barriers to implementation and ideas for implementation. Key points from the discussion:

- Barriers:
    - Money
    - Lack of stakeholder engagement and buy-in
    - Lack of readiness/coordination for trials during outbreaks
        - Consider adding a milestone in the cross-cutting section, applying to vaccines, therapeutics, and diagnostics (e.g., Strategic Goal 3) (Luby)
            - Include research protocol development (Gurley)
            - There are lessons learned from Ebola that apply; consider use of universal protocols beyond currently affected countries for pandemic prevention (Montgomery)
        - *[Note: We added a milestone in the cross-cutting section on developing protocols for emergency use of candidate NiV MCMs during outbreaks.]*
    - Regulatory uncertainty, given alternate pathways (Rahman, Luby)

  - Requirements for stockpiling
- Ideas for implementation:
  - A lot of forward movement currently exists, with huge strides since 2017; keep up the momentum (Gurley)
  - CEPI is committed to take these vaccines through phase 2 (Rahman, Broder)
  - Stockpiling and plans for use
  - Advocacy plans among stakeholders within affected countries
  - Ensure that trials are conducted in affected countries

Roadmap Implementation

Marie-Pierre Preziosi led a facilitated discussion on roadmap implementation, with questions to panelists Shahana Parveen and Mahmudur Rahman and open discussion to all participants.

- Question: Given the discussions from the last 2 days, what are the most important issues for Bangladesh specifically that align with the goals and milestones for moving NiV diagnostics, therapeutics, and vaccines forward?
  - To deal with small outbreaks, we need POC diagnostics, followed by therapeutics and vaccines (Rahman).
  - Vaccines will come first if the situation changes and there is a large outbreak (Rahman).
  - Rapid tests, especially for hospitalized patients, are the most pressing need (Parveen).
  - Vaccine stockpiles are important, but the vaccines shouldn’t remain stockpiled until a major outbreak. It would be better to use the vaccines, even in a research protocol, or offer them to healthcare and laboratory workers for prevention (Gurley).
    - The mistake for mpox (which we can avoid with NiV) is that the vaccine was stockpiled but not released to healthcare workers and laboratory workers who were at risk (Broder). This also happened with Ebola (Roth).
  - Enable voluntary preventive use of vaccines to prevent pandemics; don’t wait until a pandemic starts (Gurley).
  - Countries should have a vaccination strategy that prioritizes who gets vaccines, such as healthcare workers (Rahman).
  - Countries need to improve patient outcomes now with supportive care. There is a need to improve clinical care capacity, along with laboratory and research capacity, and in support of clinical trials (Hassan).
  - WHO is currently revising treatment guidelines and long-term care for people with neurological sequelae and brain infections (Preziosi).
- Question: What are the most critical resources that you need in Bangladesh to support development of NiV diagnostics, therapeutics, and vaccines?
  - Strong collaboration with CDC and WHO; we need a common platform and collaboration with existing efforts at country-level (Parveen).
  - Technology transfer to countries to enable development of MCMs (Rahman).
  - Consider taking advantage of the money that is moving through One Health efforts now, including from the World Bank (Rahman).
  - There should be a mapping initiative (Rahman, Preziosi).
  - Countries need investment in research; WHO is a potential organizer of such support (Lackritz).

  - SEARO is engaged in helping at-risk countries. It is easier for national institutions to push for surveillance and diagnostics. There are many cases going undiagnosed and we could change that (Formenty).
- Question: What role do you see WHO playing in promoting R&D of NiV MCMs and implementing the goals and milestones in the NiV roadmap?
  - WHO should oversee the development and implementation of an action plan to accomplish roadmap milestones, and designate who will accomplish the milestones. WHO can play an important role in initiating/collaborating on regulatory issues. WHO can initiate international collaborations, and specifically between India and Bangladesh (Parveen).
  - WHO must have a coordinating role in Bangladesh for multiple players, including CDC, CEPI, and the EcoHealth Alliance. An example is the Asia Pacific Strategy for Emerging Diseases and Public Health Emergencies (APSED III, formerly APSED) (Rahman).
  - Regarding a follow-up question as to whether other stakeholders (e.g., agriculture, veterinary) should be involved (Preziosi):
    - In Bangladesh, One Health is institutionalized. Other ministries will come forward during outbreaks. World Bank is also an important partner, as are UNICEF, CDC, and the Bill & Melinda Gates Foundation (Rahman).
    - Stakeholder mapping needs to be done (Rahman).
  - We can ask WHO to play a role, but the technical experts on NiV are responsible for advancing this agenda. It is great if Wellcome, the Bill & Melinda Gates Foundation, etc. fund this work generously, but the reality is that those contributions are incremental. We have to continue to push on the value of this agenda throughout our work and networks (Luby).
  - Champions are needed; we can assemble a group to make the investment case for NiV MCMs (Preziosi).
  - SEARO is developing strategy documents to prepare countries in the region for NiV outbreaks, including MCM R&D along with surveillance. We need to push for this beyond SEARO and the Western Pacific Regional Office (WPRO), notably in Africa (Formenty).
  - Governments need to fund NiV R&D, as well as development partners. Within countries, NiV researchers need to be networked (Rahman).
  - While grateful for the work CEPI is doing to push NiV vaccine development, there needs to be similar funding support for therapeutic development (Formenty).
  - From the One Health consideration, we need rapid, field-based diagnostics that can be used in species other than humans (Gurley).
    - This consideration could be added as a TPP-focused milestone (Preziosi).
    - While scientifically a simple issue, the application of such a diagnostic is complex with regard to different agencies involved at the country level (e.g., agriculture, health) (Gurley).
    - *[Note: Diagnostics for animal species was not added to the roadmap, as the taskforce had decided originally (in 2017-18) that issues related to animals were out of scope for the R&D roadmap.]*
  - The roadmap has milestones that include protocols and epidemiological studies, but we should verify that they adequately/explicitly mention continuing epidemiological studies (Rahman, Gurley).
- Question: Who do you think are the most important external partners and what do you think are the most critical roles for those partners in R&D of NiV MCMs?

    - In Bangladesh, the national government, specifically the Ministry of Health, is the central resource for engaging external partners, connecting with in-country experts, and engaging with external groups. This applies to the stakeholder mapping that we hope to accomplish, and other activities, such as those related to One Health (Parveen).
    - A consortium for engagement based on the One Health platform could be created, potentially by early 2024 (Preziosi, Parveen).
- Question: What is the best way to encourage other in-country experts on issues related to NiV MCMs, including WHO regional representatives?
    - Bangladesh has a One Health Forum, initiated in 2007, now with more than 1000 members. It regularly convenes and has an executive committee representing animal, human, and environmental health. The forum acts as a catalyst to push the government to work. There is also a One Health steering committee at the inter-ministerial level; both can be leveraged toward NiV MCMs (Rahman).
- Question: What are the best ways to promote regional coordination between India and Bangladesh?
    - There should be a memorandum of understanding (MOU) between India and Bangladesh regarding validation. We had this in the past for leishmaniasis, and included Nepal. We tried [to have these] for avian influenza and anthrax, but failed. These MOUs must be negotiated at the highest government level and are effective because they become an obligation for each country and work well at the local level. They would be useful for MCMs, regional coordination, and cooperation. There are, however, challenges in getting these set up (Rahman). [Note: Mahmudur Rahman agreed to provide the leishmaniasis MOU to Marie-Pierre Preziosi.]
    - SEARO is a potential forum for engagement between India and Bangladesh, to encourage bilateral exchanges (Luby).
    - Engaging at the community level is challenging, but communities can be leveraged carefully (Rahman).

Publication Process

Kristine Moore shared an overview on next steps for the roadmap and an accompanying manuscript. Key points:

- The roadmap will be revised per this meeting, finalized, and published on the WHO website in early 2024.
- Once the updated draft roadmap is ready (i.e., following revisions from this meeting), it will be shared with the taskforce and select other experts (e.g., CEPI) to sign off.
- A manuscript will also be developed. Group comments on the manuscript:
    - A journal for publishing should be selected with policymakers and funders in mind; we want to make it easy for them to recognize the roadmap's purpose and goals (Golding).
    - We should consider more than one publication, with different objectives for different target audiences (e.g., One Health community, development world/development banks beyond World Bank, etc.). Many groups would take a wider interest in short, clear, nontechnical publications that lay out problems and benefits relevant to the roadmap. This is a promotion, not just a single publication (Roth). This could include WHO promotion two-pager (Preziosi) and/or policy briefs (Roth).
    - A full communication package, including sizeable, usable versions of the roadmap that is useful for graphics and seminar presentations, could be shared with stakeholders, via targeted webinars, etc. This would be part of a one-year communication that WHO would develop (Golding).

  - Journals suggested by group members for manuscript publication consideration included: PLOS Neglected Tropical Diseases, BMJ Global Health, and Emerging Infectious Diseases.

APPENDIX: Nipah R&D Roadmap Taskforce Meeting Agenda

Nipah R&D Roadmap Taskforce Meeting
July 31 & August 1, 2023
London, UK

Agenda times listed in British Standard Time (BST)

Day 1: Monday, July 31

8:45 am *Registration, tea, coffee, and light breakfast*

Session 1: Welcome, Introductions, and Overview

9:15 am Welcome (Josie Golding, Wellcome)

9:20 am Introductions (Michael Osterholm, Center for Infectious Disease Research and Policy [CIDRAP])

9:30 am Update on the WHO Blueprint Initiative (Marie-Pierre Preziosi, WHO)

9:40 am Overview and meeting objectives (Kristine Moore, CIDRAP)

Session 2: Nipah Cross-Cutting Issues
Session Facilitator: Emily Gurley, Johns Hopkins Bloomberg School of Public Health

9:50 am General comments on Nipah cross-cutting issues in the current roadmap draft
- Key progress in the past 5 years
- Critical areas where additional efforts are needed

10:10 am Review the Nipah cross-cutting goals and milestones

10:30 am *Break*

10:45 am Review the Nipah cross-cutting goals and milestones (continued)

11:45 am Concluding comments for Nipah cross-cutting issues

- Barriers to implementing the goals and milestones
- Ideas for Roadmap implementation for this section

Session 3: Nipah Diagnostics
Session Facilitator: Joel Montgomery, US Centers for Disease Control and Prevention (US CDC)

12:05 pm General comments on Nipah diagnostics in the current roadmap draft
- Key progress in the past 5 years
- Critical areas where additional efforts are needed

12:30 pm *Lunch*

1:30 pm Review the Nipah diagnostics goals and milestones

2:50 pm *Break*

3:05 pm Concluding comments for Nipah diagnostics
- Barriers to implementing the goals and milestones
- Ideas for Roadmap implementation for this section

Session 4: Nipah Therapeutics
Session Facilitator: Emmie de Wit, US National Institute of Allergy and Infectious Diseases (NIAID)

3:25 pm General comments on Nipah therapeutics in the current roadmap draft
- Key progress in the past 5 years
- Critical areas where additional efforts are needed

3:45 pm Review the Nipah therapeutics goals and milestones

5:10 pm Concluding comments for Nipah therapeutics
- Barriers to implementing the goals and milestones
- Ideas for Roadmap implementation for this section

5:30 pm *Adjourn Day 1*
Drinks and reception

6:00 pm Dinner

Day 2: Tuesday, August 1

8:45 am *Registration, tea, coffee, and light breakfast*

9:15 am Welcome for Day 2 (Michael Osterholm, CIDRAP)

Session 5: Nipah Vaccines

Session Facilitator: Christopher Broder, Uniformed Services University of the Health Sciences

9:20 am	General comments on Nipah vaccines in the current roadmap draft • Key progress in the past 5 years • Critical areas where additional efforts are needed
9:40 am	Review the Nipah vaccines goals and milestones
10:15 am	*Break*
10:30 am	Review the Nipah vaccines goals and milestones (continued)
11:10 am	Concluding comments for Nipah vaccines • Barriers to implementing the goals and milestones • Ideas for Roadmap implementation for this section

Session 6: Nipah R&D Roadmap Implementation and Next Steps

11:30 am	Discussion: Roadmap implementation (Marie-Pierre Preziosi, WHO) Discussion panelists: • Shahana Parveen, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) • Mahmudur Rahman, Eastern Mediterranean Public Health Network (EMPHNET)
12:15 pm	Discussion: Publication process (Kristine Moore, CIDRAP)
12:25 pm	Wrap up and next steps (Michael Osterholm, CIDRAP)
12:30 pm	*Meeting close*
12:30 pm	*Lunch*

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 6-year framework beginning in 2024 for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs)—diagnostics, therapeutics, and vaccines—against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999 (Chew 2000, Chua 1999, Chua 2010, Parashar 2000, Paton 1999). As part of that outbreak, 265 human cases of NiV disease (NiVD) were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiVD was subsequently recognized, however, in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since (Hsu 2004, WHO 2018, Agrawal 2023). NiVD has also been identified periodically in eastern India (in 2001 and 2007) and, in 2018, an outbreak occurred for the first time in southern India (Arunkumar 2019, Chadha 2006, Chattu 2018, Sharma 2018, Soman Pillai 2020). Additionally, one case was reported from Kerala state in 2019 (Sudeep 2021). Case-fatality rates during outbreaks in Bangladesh and India have generally ranged from 50% to 100% (Sharma 2018). NiV infection in humans results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae (Goh 2000, Hossain 2008, Tan 2002). A summary of 14 years of NiVD investigations in Bangladesh found no evidence of asymptomatic infection (Nikolay 2019).

The primary natural reservoir host for NiV in South Asia, where cases continue to occur, is *Pteropus* bats. A recent study suggests that discrete multiannual local epizootics in bat populations contribute to the ongoing sporadic nature of human NiV outbreaks in South Asia (Epstein 2020). The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock and other domestic animals, which can serve as a source of exposure to humans (Islam 2023). Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in *Pteropus* bats and several other related bat species in Southeast Asia, the Western Pacific, and Africa (Anderson 2019, Breed 2010, Epstein 2008, Hasebe 2012, Iehlé 2007, Plowright 2019, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, Yob 2001). In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, dogs were found to be infected with NiV on the farms involved in the outbreak (Field 2001). In outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role to date, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission (Arunkumar 2019, Gurley 2007, Islam

2016, Luby 2009, Nikolay 2019, Rahman 2008). However, a recent study in Bangladesh found NiV antibodies in cattle, dogs, and cats from six sites where spillover human NiV infection cases occurred during 2013–2015 (Islam 2023), suggesting the potential of zoonotic spread via intermediary hosts. Respiratory transmission via droplet spread may play an important role in propagating outbreaks (Nikolay 2019, Spiropoulou 2019). The virus could have pandemic potential if a more human-adapted strain, with greater person-to-person transmission emerges (Luby 2013).
~~The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock and other domestic animals, which can serve as a source of exposure to humans (Islam 2023).~~ NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen—Hendra virus (HeV)—which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia (Murray 1995, Selvey 1995). To date, at least 66 spillover events—all in Australia—involving more than 100 horses and seven humans have been identified (Wang 2023). Changes in bat behavior related to habitat loss and climate change appear to have increased the spillover risk of HeV from bats to horses (Eby 2023). Another henipavirus (Langya virus) was recently identified as the probable cause of febrile illness among a group of people in China (Zhang 2022). An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014 (Ching 2015). This outbreak likely involved spillover of the virus into horses and subsequent disease in humans following consumption of contaminated horsemeat; disease also occurred in healthcare workers who cared for infected patients. Detailed genomic information for this virus is limited.

Several other henipaviruses have been identified, although the zoonotic and pathogenic potential of such viruses remains unknown (Li H 2023) and additional research is needed to better understand the global health threat of these viruses. Since additional bat species (particularly in Africa) and potentially other animals may serve as reservoir hosts for henipaviruses, the risk of spillover to humans may encompass up to three-fourths of the world's population. Although this roadmap is primarily focused on NiV, many of the issues identified also apply to other henipaviruses. While the current incidence of detected disease caused by henipaviruses is low, the COVID-19 pandemic clearly demonstrated that viruses can transform into serious global threats without significant warning; therefore, vigilance is needed to better understand the epidemiology of henipaviruses and to monitor their global occurrence.

Genomic sequencing has demonstrated that there are two main clades of NiV: the M genotype, which comprises the Malaysian NiV isolates (NiV-M), and the B genotype, which includes Bangladesh (NiV-B) and India NiV isolates (NiV-I) (Liew 2022, Yadav 2019). These three strains share a high percentage of homology (NiV-M and NiV-B strains share 91.8% homology, and NiV-I strains share 85.14to 96.15% homology with both NiV-M and NiV-B). Some strain-related differences, however, have been noted in the clinical features of infection in humans and experimentally infected non-human primates, with strains of the B clade appearing to be more pathogenic than those of the M clade (Mire 2016). Recent data indicate that strains from Bangladesh are segregated into two additional distinct sublineages that have intermingled geographically and temporally in that area over time (Rahman 2021, Whitmer 2020). At this time, however, the molecular epidemiology of NiV remains somewhat unclear and issues around strain diversity and strain evolution require further elucidation (Rahman 2021). One recent summary involving the most comprehensive analysis of available genomic data to date suggests that only about 15% of the overall NiV genetic diversity has been uncovered. Moreover, findings from that analysis demonstrated co-circulation of distinct lineages among bats, coupled with slow migration over large spatial areas (Cortés Azuero 2023).

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of MCMs to enable effective and timely emergency response to infectious disease epidemics. NiVD is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist) (WHO 2023). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this roadmap ranges from basic research to late-stage development of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply broadly to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) Each section includes barriers (inherent obstacles or technical challenges that may influence the likelihood of success for development of NiV MCMs) and gaps (key needs or unresolved limitations in knowledge that are critical to the development of new NiV MCMs). These are followed by strategic goals and milestones, which build on the gaps and barriers and are focused on achievements for the next 6 years (beginning in 2024) that are necessary for moving NiV MCMs forward. The roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed. Each section also includes additional ongoing priorities that should be considered for NiV MCM R&D.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include minimizing zoonotic NiV transmission, improving use of personal protective equipment (PPE), ensuring adequate hand hygiene and environmental hygiene, promoting effective community engagement, implementing adequate infection prevention and control practices, developing adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and promoting workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of this R&D roadmap, but need to be addressed as part of a broader public health control strategy. Further research of NiV and related henipaviruses in animal species, including development of appropriate MCMs targeted to animal populations (such as vaccines [McLean 2019]), also is needed, since disease in animals may amplify occurrence of NiV (or a related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

VISION

Robust MCMs to detect, prevent, treat, and control human outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or

potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care or near patient diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Barriers and Gaps

Barriers

- Securing funding for NiV/henipavirus research represents a substantial challenge, since economic incentives to invest in NiV research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with small and sporadic outbreaks (Gómez Román 2020). The development of a sustainable value proposition for industry and international philanthropic public-private partnerships is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition should be informed by a robust assessment of the risk of future outbreaks of NiV and related henipaviruses and the economic, societal, and health impacts that such outbreaks could generate.
- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand (Gouglas 2018). In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- National regulatory authorities in countries where NiV/henipavirus outbreaks are likely to occur have different regulatory requirements for authorization, licensure, or emergency use of NiV medical countermeasures, which complicates the approval process, particularly for candidate vaccines and therapeutics (Gómez Román 2022). Engagement of international regulators ~~An international, NiV-focused regulatory group has been created and~~ will be an important mechanism for coordinating regulatory issues as NiV MCMs are moved forward (Gómez Román 2020).
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (biosafety level 4 [BSL-4]) conditions (Geisbert 2020, Gómez Román 2022), which can increase the cost and complexity of MCM development.
- To date, NiV spillovers to human communities have been identified most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The primary natural reservoir for NiV and henipaviruses is fruit bats of the *Pteropus* genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar (Bruno 2022). Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV or related viruses; such bats can be found across Africa and parts of the Middle East (Bruno 2022). This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where

the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to the lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.

- The development and accessibility of animal models that recapitulate human NiV disease are critical for NiV MCM development, given the limitations on clinical samples and the infeasibility of traditional clinical efficacy trials. While ferrets and Syrian golden hamsters are well-established animal models for NiV research, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans (Foster 2022, Geisbert 2010, Geisbert 2020, Geisbert 2021, Johnston 2015, Price 2021, van Doremalen 2022, Woolsey 2023). Studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways; however, costs, space requirements (particularly in BSL-4 containment facilities) and ethical concerns constrain their use (Arnason 2020, Bossart 2012, Geisbert 2010, Johnston 2015).
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection occurs in relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power (Gómez Román 2020). It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks; however, it is likely that at least vaccines will need to be licensed via nontraditional regulatory pathways.
- Patients usually present late in the clinical course of disease and disease progression is often rapid, making it difficult to collect clinical samples before patients succumb to the disease. Additionally, autopsies are often not the standard of care in affected areas, which further reduces opportunities to collect clinical specimens. These barriers hinder the ability to understand disease pathogenesis and immunologic responses to infection, which are important for MCM R&D (Amaya 2020, Arunkumar 2019, Gurley 2020, Liew 2022, Mazzola 2019).
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV vaccines and therapeutics.

Gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of NiV/henipavirus MCMs, as well as other preventive measures, depend on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of human disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas (Bruno 2022, Singhai 2021). Improved surveillance might also support the business case for investment in NiV MCMs by identifying a higher incidence of disease than previously demonstrated and revealing a broader geographic range of risk.

- Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of *Pteropus* bats and potentially other bat species around the globe (Gómez Román 2020, Plowright 2019).
- Additional research is needed to optimize and further characterize relevant animal challenge models (particularly ferret, Syrian golden hamster, and AGM models) for promoting development and evaluation of NiV MCMs (Geisbert 2020, Gómez Román 2020, Price 2021, Rockx 2014). Examples of additional issues involving animal models include the following (Dhondt 2013, Gómez Román 2020, Johnston 2015, Mathieu 2015, Mire 2019, Price 2021):
    - Determine the appropriate animal model(s) for screening assay development.
    - Standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development.
    - Determine when after-challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans.
    - Identify the best models for studying chronic (relapsing) infection.
    - Determine if different animal models are needed for different clinical endpoints such as infection, disease, and transmission.
    - Determine if models perform differently based on the route of virus administration.
    - Improve overall standardization of the models.
- Other research needs include the following:
    - Improved understanding of the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs (Gurley 2020, Liew 2022). This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunologic clearance and cause delayed onset or recurrent encephalitis, and identifying factors influencing the development of permanent neurologic sequelae.
    - Ongoing phylogenetic and evolutionary analyses of NiV strains to monitor viral heterogeneity and antigenic changes that may affect the epidemiologic and clinical features of disease over time and thereby influence MCM development (Cortés Azuero 2023, Gurley 2020, McKee 2022, Rahman 2021).
    - Additional research to determine if: (1) NiV strain variations influence the ability to detect NiV infections; (2) different strains have different phenotypic characteristics, such as different clinical manifestations or transmission dynamics, and (3) if strain variability impacts efficacy of vaccines or therapeutics (CEPI 2023a, Gurley 2020, Whitmer 2020).
    - Whole-genome sequencing of NiV isolates to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses (CEPI 2023a).
    - Sociological and anthropological research to understand how to best engage populations at high risk of exposure (such as persons who consume date palm sap, healthcare workers, and workers at the human-animal interface) and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially

if therapeutics and vaccines do not consistently prevent disease (Singhai 2021, Lancet 2018).
    - Prospective serosurveillance of henipavirus exposure from susceptible animal species and proximate human populations in areas of predicted risk to assess the potential of human spillover and to build preparedness for detection of human cases and for limiting exposure (Daszak 2020, Deka 2018, Gómez Román 2020, Plowright 2019).
    - Expanded use of machine-learning approaches can facilitate an improved understanding of the risk of NiV/henipavirus spillover events (Plowright 2019). Ecological studies are also needed to enhance understanding of the dynamics governing prevalence and shedding of NiV and other henipaviruses in bats (Plowright 2019).
- Other important needs include the following:
    - Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop and manufacture NiV MCMs (Gómez Román 2020).
    - Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control (Bruno 2022).
    - Advocacy to policy makers in affected countries and to global stakeholders to ensure they understand the potential health, societal, and economic benefits of devoting resources to improving NiV surveillance, detection, prevention, and control measures (Gómez Román 2020).
    - Scenario planning to clarify regulatory pathways for product approval in countries where NiV/henipavirus outbreaks are likely to occur, including determining whether or not efficacy data from animal models is sufficient for regulatory approval. Such planning should take into consideration the local epidemiology of NiV infection and the different requirements of local national regulatory authorities for product approval and emergency use authorization (Gómez Román 2022). Regulatory pathways and NRA capabilities vary between countries; therefore, early engagement, potentially with support from WHO and other key international stakeholders, is essential to identify country-specific considerations. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.
    - Standardized and well-characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection (Rampling 2019, Satterfield 2016). Assays that can be used at lower biosafety levels are an important priority. WHO international standards should be used (when available) as calibrators and reported in units/ml to harmonize assay results.
    - Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical

management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection (Singhai 2021).

- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and strengthen disease prevention and preparedness activities. This effort could potentially involve linking NiV surveillance with other surveillance efforts, such as surveillance for Japanese encephalitis (JE). Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.
- Efforts to reduce the cost of medical countermeasure development and ongoing production.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify additional and ongoing sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2024~~5~~, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the ~~potential~~ global threat of NiV and related henipaviruses (including the risk of gain-of-function research involving such viruses), (2) demonstrates the need for global engagement to address the threat, and (3) outlines the global social and economic benefits of generating accessible and affordable NiV MCMs.~~, and (3) details the positive impact on the health systems in affected areas.~~.
2. By 2025~~6~~, create a funding plan based on the global value proposition for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.
3. By 2025~~6~~, develop a coordinated strategy (to include addressing indirect costs and shortfalls) for promoting and incentivizing greater industry engagement in R&D for NiV MCMs, particularly in affected countries.

Strategic Goal 2: Improve understanding of ~~NiV~~ the epidemiology and ecology of NiV and related henipaviruses to better define the disease burden, risk factors for infection, reservoir hosts, and risk of spillover events in ~~affected~~ at-risk countries.

Milestones:

1. By 2024~~6~~, generate and implement standardized protocols for case investigation in at-risk countries, using a One Health approach, that are aimed at identifying risk factors for primary NiV

infection and at conducting case-contact studies to better understand chains of transmission (Hedge 2023).

2. By 2025, assess country-level capacity in at-risk countries for implementing standardized clinical protocols for case investigation, and develop plans for addressing key gaps in implementation.

~~1.~~3. By 2025, develop and initiate a plan for enhancing human ~~NiV~~ surveillance for NiV and related henipaviruses in India and Bangladesh outside of existing surveillance areas (i.e., where cases previously have been identified). This should include securing funding, identifying additional surveillance catchment areas, engaging key partners in those areas, generating standardized surveillance protocols, ~~and~~ conducting training for implementation, and leveraging other surveillance activities (such as surveillance for JE).

~~2. By 2026, initiate enhanced human NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), promote early case detection, and better define the disease burden in India and Bangladesh, particularly outside of areas where cases previously have been identified.~~

~~3.~~4. By 2025, develop plans for conducting human surveillance for NiV and related henipaviruses in at-risk countries other than India and Bangladesh.

5. By 2025, develop plans for conducting animal surveillance for NiV and related henipaviruses in at-risk countries other than India and Bangladesh.

6. By 2025~~6~~, develop plans for conducting additional research in at-risk countries ~~India and Bangladesh~~ to identify the potential for and drivers of spillover events for NiV and related henipaviruses, using a One Health approach, particularly in areas where NiV cases have not yet been identified.

~~4.~~7. By 2026, establish capacity at selected surveillance sites in India and Bangladesh to investigate NiV-related deaths by using post-mortem minimally invasive tissue sampling to enhance understanding of NiV disease pathogenesis (Bassat 2021).

Strategic Goal 3: Determine the requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure or approval of NiV MCMs by engaging ~~an~~ international ~~regulatory working group~~ regulators representing NRAs in affected areas and other key international stakeholders.

Milestones:

1. By 2024, conduct scenario planning to identify gaps, clarify ~~the~~ regulatory procedures, and determine the acceptable pathways for approval and emergency use authorization of NiV MCMs (including vaccines, novel candidate therapeutics, repurposed therapeutics, and diagnostics) in countries at highest risk for NiV outbreaks ~~(i.e., Bangladesh and India)~~ through an international ~~NiV-focused regulatory group.~~ forum of regulators.

~~2.~~ By 2025, ~~determine if any key gaps exist for regulatory approval and emergency use authorization of candidate NiV MCMs in affected countries and~~ develop strategies to address

~~those~~ gaps in regulatory approval and emergency use authorization of candidate NiV MCMs, with specified timelines for completion.

2. By 2025, assess capacity in affected countries to conduct clinical trials and field studies of MCMs, particularly during outbreaks.

3. By 2026, create a system for monitoring regulatory issues related to licensure or use of candidate NiV MCMs over time. ~~ensure that any issues affecting approval and emergency use of candidate MCMs in affected countries have been resolved and that consensus has been achieved on the necessary regulatory steps and procedures.~~

~~3.~~ By 2026, develop protocols for emergency use of candidate NiV MCMs during outbreaks.

Strategic Goal 4: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection~~ in humans and animal models~~.

Milestones:

1. By 2024~~5~~, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
2. By 2025, conduct additional research to further optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs.

~~3.1. By 2026, conduct sequencing of NiV strains from existing clinical samples obtained from past NiV cases to assess variability of NiV strains in India and Bangladesh (CEPI 2023a).~~

3. ~~By 2026, establish capacity at selected surveillance sites in India and Bangladesh to investigate NiV-related deaths by using post-mortem minimally invasive tissue sampling to enhance understanding of NiV disease pathogenesis (Bassat 2021).~~

4. By 2026, develop a standardized human pathology investigation protocol for improving understanding of NiV pathogenesis.

5. By 2027~~8~~, conduct research ~~in animal models~~ to determine if strain variability impacts efficacy of promising NiV MCMs or the accuracy of diagnostic tests.

Additional Priority Areas/Activities

Research

- **Continue to expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach. Such research should include serosurveys in different animal species and ecologic studies in bats, and should utilize computational approaches, such as machine-learning.
- **Continue to perform** phylogenetic and evolutionary analyses, using whole-genome sequencing, of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- ~~By 2026,~~ **Continue to conduct** sequencing of NiV strains from existing clinical samples obtained from past NiV cases to assess variability of NiV strains in India and Bangladesh (CEPI 2023a).

- **Incorporate**, on an ongoing basis, additional NiV strains into preclinical research as newer strains become available.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Continue to explore** alternative strategies to using animal models for research (e.g., use of organoids, other in-vitro approaches, computational modeling).
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability of henipaviruses to improve understanding of their global distribution.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection, including potential regulatory pathways for MCM licensure and approval.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** an open-access shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in ~~endemic~~ affected and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas. These should include efforts to reduce production costs and ensure equitable global access as needed.

- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an “affordable and fair” price is one that can reasonably be paid by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

DIAGNOSTICS

Barriers and Gaps

Barriers

- Initial signs and symptoms of NiV infection are nonspecific, and infection is often not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent infection can last for months to years after initial exposure, which can complicate epidemiologic investigation (CDC 2020).
- The accuracy of laboratory results can be affected by a variety of factors, such as clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory (WHO 2019, Mazzola 2019).
- The time required to perform diagnostic testing using conventional laboratory methods is problematic, given the potential for rapid disease progression of NiV infection (Sayed 2019).
- Diagnostic needs vary across the latent, acute, and convalescent phases of NiV infection (Bruno 2023).
- Limited laboratory infrastructure and diagnostic capabilities in peripheral settings can lead to delays in diagnosis and in outbreak investigation and response (Berge 2019, Bruno 2022, Chua 2013, Wang 2012, WHO 2019).
- Owing to the high biosafety precautions necessary when working with NiV, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis (Watanabe 2020, Widerspick 2022).
- Limited NiV-positive clinical samples are available, which are important for validation of diagnostic tests (Mazzola 2019).
- *Pteropus* species (and likely other bat species) may carry other henipaviruses in addition to NiV and HeV, some of which could be pathogenic in humans and livestock. Antibodies to different henipaviruses appear to be highly cross-reactive, making it difficult to discriminate the particular henipaviruses that are in circulation using serologic assays, which is critical to ensuring diagnostic preparedness to respond to future outbreaks (Mazzola 2019, Wang 2012, WHO 2019, Yang 2022).

Gaps

- Further research is needed to:

  - Improve understanding of the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease (Arunkumar 2019, Berge 2019, Mazzola 2019, Thakur 2019).
  - Determine criteria for test performance and further evaluate performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as rapid diagnostic tests) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
  - Continue to develop commercialized or standardized rapid nucleic acid tests that can quickly confirm active NiV infection at the point of care or at the level of near-patient care (Berge 2019, Pollack 2023, WHO 2019). This effort may require clarification of the regulatory pathways for commercialization of NiV diagnostic tests. To date, one rapid test has been approved for emergency use in India during an outbreak that occurred in 2018 (Yadav 2021).
  - Generate international reference standards to calibrate diagnostic assays to ensure proficiency testing of new diagnostics (Berge 2019, WHO 2019).
  - Clinically validate the performance and operational suitability of new promising diagnostics, particularly rapid diagnostic tests, in endemic geographic regions (Berge 2019, Pollack 2023).
  - Continue to assess and operationally validate safe and simple methods of sample inactivation that do not interfere with diagnosis and that can be used at peripheral sites (Pollack 2023, WHO 2019, Watanabe 2020, Widerspick 2022, Yadav 2021).
  - Establish operational suitability at the point of care (i.e., at peripheral community settings) for NiV diagnostic tests by developing an integrated approach to facilitate rapid, accurate, and safe testing procedures (Pollack 2023).This could include a minimal protocol or best practices approach for sample inactivation before testing (WHO 2019).
- Other needs include the following:
  - Development of a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples to assess and validate diagnostic tests (Berge 2019, WHO 2019). As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6) identify who would have access to the samples; (7) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)

  - Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection (Berge 2019, WHO 2019).
  - In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
  - Systems for external quality assessment (EQA) monitoring of tests using up-to-date clinical specimen panels and reference standards (Mazzola 2019).
  - A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for NiV diagnostics, particularly given the costs of regulatory approval.
  - Improvement of diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses as they arise (Wang 2012).
  - Ongoing efforts to develop affordable and easy-to-use multiplex panels for detection of a range of pathogens using a syndromic approach, if such panels can be deemed cost-saving (Mazzola 2019).
- Use cases and target product profiles (TPPs) have been ~~developed~~ drafted for NiV diagnostics (WHO 2019); however, these need to be finalized and the criteria may need to be modified over time as new lineages are identified (Mazzola 2019).
- Since NiV strains are continuing to evolve, it's possible that current diagnostic tests could fail to detect an emergent variant of NiV. Recently, a novel strain of HeV was identified in Australia that was not detected through conventional PCR testing owing to gene sequence mismatches (Annand 2022). Diagnostic tools that can detect a broader range of NiV, HeV, or related viruses capable of spillover may be needed to advance the ability to forecast spillover risks and to detect emergent viruses (Peel 2022).

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of ~~reference repository~~ repositories of clinical samples from NiV-infected patients.

Milestones:

1. By 2024~~6~~, develop and standardize plans and protocols (including the governance structure) for creating ~~a virtual reference repository~~repositories of well-characterized acute and convalescent clinical samples to include a recommended set of metadata (i.e., age, sex, days since symptom onset), as feasible, and to be maintained in the two primary NiV-affected countries (Bangladesh and India).

2. By 2025~~7~~, identify sustainable, long-term funding, determine sites for sample storage, and initiate creation of the ~~virtual reference repository~~repositories in Bangladesh and India, with ~~samples~~ samples and associated metadata to be collected during future outbreaks.

Strategic Goal 2: Continue to develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care and~~or~~ near-patient NiV diagnostic tests that are suitable for use in peripheral settings, have extended shelf lives, and ~~that~~ have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2024, finalize the draft use cases and TPPs for NiV diagnostics.

2. By 2024~~5~~, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays. This effort should include clarifying regulatory pathways for approval (including approval for emergency use) of NiV diagnostics.

~~1.~~3. By 2024, generate a call to accelerate the development of point-of-care and near-patient diagnostic testing for NiV.

4. By 2024, develop a protocol or set of best practices (that biosafety committees will accept) for inactivation of clinical samples from humans and animals that are being tested for NiV.

5. By 2025, create international reference standards for calibrating and harmonizing NiV diagnostic assays.

~~2.~~6. By 2026, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP and can be used in peripheral sites.

~~3. By 2026, create international reference standards for calibrating and harmonizing NiV diagnostic assays.~~

~~4. By 2026, develop a minimum protocol or set of best practices (that biosafety committees will accept) for inactivation of clinical samples from humans and animals that are being tested for NiV.~~

~~5.~~7. By 2027, complete clinical validation of performance and operational suitability for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP and can be used in peripheral sites.

~~6.~~8. By 2028, ~~obtain~~ promote regulatory approval for at least one rapid, point-of-care or near-patient~~-~~care NiV diagnostic test that can be commercialized and standardized.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known spillover risk to promote early detection of NiV.

Milestones:

1. By 2025~~6~~, continue to expand national laboratory networks for NiV detection in ~~the primary affected~~ at-risk countries ~~(Bangladesh and India)~~ that include plans for enhancing laboratory preparedness to enable earlier and timely detection of NiV infection during future outbreaks.

2. By 2026~~7~~, generate well-characterized and up-to-date proficiency panels and network quality controls for NiV diagnostic testing to be used in selected laboratories in ~~Bangladesh and India~~at-risk countries.

3. By 2026~~9~~, implement routine EQA monitoring of NiV diagnostic testing at selected laboratories in ~~Bangladesh and India.~~at-risk countries.

Additional Priority Areas/Activities

Research

- **Continue to explore** new diagnostic approaches that may allow earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** criteria for test performance and **continue to evaluate** performance characteristics for promising new assays for diagnosis of NiV infection.
- **Continue to conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection as they become available.
- **Continue to research** methods of diagnostic testing that are able to differentiate between various pathogenic henipaviruses.
- **Continue to develop** affordable and easy-to-use multiplex panels for detection of a range of pathogens using a syndromic approach.
- **Consider** development of diagnostic tools that can detect a broader range of NiV, HeV, or related viruses capable of spillover.

Product development

- **Refine** over time, as needed, criteria in the existing TPPs to include identification of different NiV lineages/strains.
- **Continue to develop and evaluate** point-of-care and~~or~~ near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), can capture antigenically diverse strains of the virus, and can be performed accurately and safely in peripheral settings under a variety of circumstances.
- **Expand** diagnostic test development for other henipaviruses over time.

Key capacities

- **Establish** operational suitability in peripheral laboratories of rapid diagnostic tests over time, as new tests become available.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed.

Policy and commercialization

- **Develop** guidance on optimal strategies for the deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

THERAPEUTICS

Barriers and Gaps

Barriers

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.

- A limiting constraint to assessing the effectiveness of promising therapeutics is the number of patients with NiV infection who can be enrolled in clinical trials over time, given the small number of cases that are identified annually.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment of patients and PEP for exposed persons, which can significantly impact clinical evaluation of therapeutic candidates.
- NiV can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in affected countries often do not have adequate infection control–programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.
- One promising therapy is the monoclonal antibody (mAb) m102.4; however, the projected cost per patient for this agent is expected to be more than $1,000 (Gómez Román 2022); this high cost poses an important barrier to its use.

Gaps

- Patients may benefit from optimal supportive care, independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials to inform best-practice guidelines and evidence-based policy decisions. Standard of care guidelines will be important for conducting clinical efficacy trials of therapeutic agents.
- During the 1998-99 NiV outbreak in Malaysia, clinicians used ribavirin to treat 140 patients. Outcomes data revealed a lower mortality rate among treated patients (Chong 2001), but the findings may have been biased by the use of historical controls. No additional clinical studies of ribavirin have been conducted, and limited studies in animals have not demonstrated efficacy of ribavirin following NiV or Hendra virus challenge (Georges-Courbot 2006, Rockx 2010). Ribavirin, however, may prove useful for PEP (Banerjee 2019); therefore, further challenge studies in animal models should considered to explore this possibility.
- Studies in animals have evaluated the usefulness of several agents (including remdesivir, favipiravir, and fusion inhibitory peptides) when delivered prior to disease onset or early during the disease course (Dawes 2018, Lo 2019, Mathieu 2018). One recent study showed that AGMs were only partially protected when remdesivir was administered 3 days post-inoculation; therefore, early administration seems critical for effective treatment (de Wit 2023). Patients with NiV infection are often detected late in the clinical course, which creates challenges for predicting how well a therapeutic agent will work in the field. Additional challenge studies in animals, therefore, are needed to: (1) assess the clinical benefit of these therapeutics as treatment options when administered after symptom onset or at least more than 24 hours after initial exposure, (2) determine whether these agents may be appropriate for PEP, and (3) clarify the most feasible and cost-effective route of administration (e.g., oral, intranasal, intravenous)

appropriate for real-world conditions, particularly if being considered for mass prophylaxis in an outbreak setting (Gómez Román 2022).

- m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV (Broder 2012, Guillaume 2004, Playford 2020). Several other mAbs have also been assessed in animal models and appear promising (Gómez Román 2022). A phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia during 2015 and 2016 (Playford 2020). In that trial, m102.4 was well tolerated and safe, with no evidence of an immunogenic response.
- Additional research needs for mAbs as treatment or PEP for NiV infection include:
    - Additional clinical trials in endemic areas to further assess the safety, tolerability, efficacy, and pharmacokinetic parameters of m102.4 (and possibly other mAbs with adequate preclinical data) for PEP and potentially early treatment of clinical disease (CEPI 2023b).
    - Additional research to determine the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with m102.4, it may be necessary to consider mAb cocktails (Borisevich 2016, Playford 2020).
    - Animal studies to determine if mAb cocktails that combine several different mAbs into one formulation are more efficacious than administering one mAb alone (Dang 2021).
    - Future studies to ascertain the efficacy of m102.4 for treatment and prophylaxis against different viral strains of NiV and Hendra viruses, particularly among populations living in settings where there is the potential for an outbreak.
    - Adequate stockpiles of m102.4 (or potentially other mAbs) to ensure urgent access at the onset of a NiV outbreak.
- Given the limited number of NiV cases identified each year, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how best to allocate scarce resources for conducting such trials.
- A prepositioned, agreed-upon protocol for conducting clinical trials of promising therapeutics during NiV outbreaks would be of value in advancing clinical evaluation of such agents (Spiropoulou 2019).
- Diagnostic criteria and standardized testing are needed for including patients in clinical trials of therapeutics.
- Additional research needs include the following:
    - Further research to broaden the number of novel antiviral candidates (including repurposed drugs) for treatment of NiV infection and strengthen the therapeutic pipeline. Computational aided drug design is one tool that can be useful for this discovery (Yang 2023).
    - Additional data to establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship of promising therapeutic candidates.
    - Additional data to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP stockpiling and

distribution in both affected and at-risk areas, particularly Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.

- Additional information to determine whether or not strain differences will affect the response to therapeutic candidates and results from clinical trials.
- Additional data to determine the therapeutic windows for promising therapeutics for the different NiV strains, as highlighted by a recent study in AGMs that showed that the therapeutic window for m102.4 against a strain from Bangladesh/India was shorter than for a strain from Malaysia (Mire 2016).

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future outbreaks of NiV or related viruses. ~~outbreaks.~~

Milestones:

1. By 2024, convene a consortium of key stakeholders—in affected areas and internationally—to address the key challenges with conducting clinical trials of therapeutic agents during future ~~NiV~~ outbreaks of NiV or related viruses. This consortium could potentially be modeled after the West Africa Lassa Fever Consortium (WALC) (ISARIC 2023).
2. By 2024~~5~~, develop NiV infection control and standard of care guidelines to be used in affected countries and ~~,~~disseminate the guidelines. ~~, and conduct outreach to clinicians as appropriate~~.
3. By 2024~~5~~, complete an agreed-upon generic prepositioned protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates (mAbs and small molecules, including repurposed drugs) to be implemented in NiV-affected areas during outbreaks and develop plans for operationalizing the protocol.
4. By 2024~~5~~, complete an agreed-upon generic prepositioned protocol for conducting PEP (and potentially pre-exposure prophylaxis [PREP]) trials of promising therapeutic candidates (mAbs and small molecules, including repurposed drugs) to be implemented in NiV-affected areas during outbreaks and develop plans for operationalizing the protocol.
5. By 2025, conduct outreach and training to clinicians on the NiV infection control and standard of care guidelines in India and Bangladesh.

~~1. By 2026, complete a broader, harmonized regional protocol (to be used across Bangladesh and India) for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during outbreaks or potentially during periods of endemic disease (if comparable data can be generated over time and across different clinical sites) and develop plans for operationalizing the protocol.~~

6. By 2026, generate a reliable source or stockpile of a mAB (m102.4 or other mAb) to be used in outbreak-related clinical trials for ~~both~~ PEP, possibly PREP, and early clinical treatment.

Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2025, create and implement a prioritization process, with a governance structure, for determining which promising NiV therapeutic candidates should be further evaluated in clinical trials, once adequate animal data demonstrating safety and efficacy are available.

2. By 2026, complete preclinical evaluation in animal models—with administration of the therapeutic agent more than 24 hours after challenge and potentially after symptom onset—of the preliminary safety, tolerability, and efficacy of at least two promising small-molecule therapeutic candidates or combination therapies for the treatment of NiV infection.

~~3. By 2027, further explore in animal models whether two or three of the most promising small-molecule therapeutic candidates are likely suitable for PEP and, therefore, should be assessed as PEP in clinical trials.~~

~~4. By 2027, determine the most cost-effective and feasible routes of administration for use in real-world settings for at least two promising small-molecule therapeutic candidates.~~

3. By 2025~~7~~, complete at least one additional phase 1 clinical trial ~~(phase 2 or 2/3)~~ in a NiV-affected area of ~~m102.4 or other suitable~~ a promising mAb or mAb cocktail to ~~further~~ assess safety and ~~,~~ tolerability. ~~, and efficacy (if NiV incidence allows efficacy assessment).~~

~~5.~~4. By 2027, complete at least one phase 2/3 clinical trial in a NiV-affected area of a promising mAb or mAb cocktail to further assess safety, tolerability, and potentially efficacy (if NiV incidence allows efficacy assessment).

~~6.~~5. By 2028, complete at least one phase 1 clinical ~~evaluation~~ trial of the preliminary safety~~,~~ and tolerability ~~, and (possibly) efficacy~~ of at least two promising small-molecule therapeutic candidates or combination therapies for the treatment of NiV infection.

Additional Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as m102.4, other mAbs, remdesivir, and favipiravir) for treating and preventing NiV infection, including conducting additional studies in animal models and clinical trials as appropriate and feasible. This should include determining the therapeutic windows for use of therapeutic agents as treatment or PEP.
- **Clarify,** in animal models, the potential for development of escape mutants from use of mAbs.
- **Continue to conduct** preclinical research on mAbs other than m102.4 and on mAb cocktails to assess safety, tolerability, and efficacy for treating NiV infection
- **Continue to expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation, potentially using pseudotyped viruses for initial screening of compounds (Li T 2023).
- **Consider conducting** additional challenge studies in animal models to assess whether or not ribavirin may be suitable for PEP following NiV exposure.

- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Continue to develop, evaluate, and license** safe and effective therapeutic agents for the treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized in advance of outbreaks, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in affected areas to improve clinical management and supportive care of patients with NiV infection and to improve infection control practices to limit person-to-person spread.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- **Explore** strategies for decreasing the costs associated with m102.4 or other mAbs, such as exploring the possibility of administering mAbs subcutaneously rather than intravenously.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

VACCINES

Barriers and Gaps

Barriers

- Large clinical efficacy trials, which typically are required for vaccine licensure, will likely not be feasible for NiV vaccines, owing to the sporadic and unpredictable nature of NiV outbreaks and the low case numbers usually involved (Gómez Román 2022, Nikolay 2021, Satterfield 2016).
- In the absence of large clinical efficacy trials, authorization and licensure will likely involve nontraditional regulatory pathways to guide the evaluation of safety and efficacy (Gómez Román 2022). However, experience is limited with these routes (such as the US FDA's Animal Rule or Accelerated Approval Program, the EMA's conditional market authorization, and authorization under exceptional circumstances) and there are few successful models for vaccine authorization and approval.
- The limited commercial value of NiV vaccines may impede industry's involvement in developing and producing NiV vaccines without significant financial support (e.g., through partnerships with organizations such as CEPI, PATH, and high-income country government agencies) (Gómez Román 2022).

- The affordability of creating and maintaining a NiV vaccine stockpile and deploying vaccines during outbreaks is a key issue for low- and middle-income countries; supplementary funding will likely be required to ensure vaccine preparedness for NiV outbreaks (Gómez Román 2022).
- The absence of improved diagnostic assays for the timely diagnosis of infection creates an important challenge by delaying implementation of a rapid reactive vaccination strategy for NiV outbreak control.

Gaps

- NiV vaccines are needed that: (1) are readily accessible with adequate supply chains, particularly in low-resourced areas, (2) can protect against different NiV strains, and (3) provide rapid onset of immunity to adequately prevent and control outbreaks in a timely manner.
- Use cases for NiV vaccines need to be better defined, as how vaccines are to be used will affect vaccine deployment and manufacturing plans.
- Reference standards for NiV antibodies are needed to evaluate candidate NiV vaccines.
- Expanded partnerships among researchers, funders, and regulators are needed to advance the development of promising NiV vaccine candidates (Amaya 2020, Gómez Román 2022). NiV vaccine candidates in preclinical development target the F/G glycoproteins and a variety of platform technologies are being considered (e.g., virus vectors, protein subunits, mRNA, and virus-like particles) (Amaya 2020, Geisbert 2021, Gómez Román 2022, Loomis 2021, Monath 2022). NiV vaccine candidates based on three different platforms (protein subunits, mRNA, and viral vectors) are currently in phase 1 clinical trials (Auro Vaccines 2022, NIAID 2023, Public Health Vaccines 2023).
- Demonstrating vaccine-induced protection against NiV infection or disease in an animal model will require an immune correlate of protection (CoP) or immune surrogate that can predict the likelihood of protective efficacy and that reflects the protective immune responses generated in humans (Amaya 2020, Escudero-Pérez 2023, Price 2021).
    - Accurate and reliable CoPs for determining the protective efficacy of NiV vaccines have not yet been identified (Loomis 2021). Neutralizing IgG is generally used as a CoP, although the protective threshold still needs to be defined to allow additional vaccine testing in animal challenge models and eventual immunobridging of antibody responses to humans, through phase 1/2 clinical studies (Escudero-Pérez 2023, Price 2021).
    - Once the protective threshold for neutralizing IgG antibodies is determined, the most appropriate and feasible assays (e.g., assays that are developed using live NiV vs. assays developed using pseudoviruses expressing the NiV F and G glycoproteins [Luo 2023]) need to be identified and standardized for use in animal models (Price 2021).
    - Different types or titers of CoPs may ultimately be needed for different vaccine platforms, antigens, clinical outcomes (e.g., protection against infection, severe disease, chronic disease, or death) and potentially host or population characteristics to support the assessment of candidate NiV vaccines using immunogenicity and efficacy data from preclinical studies.
    - Other humoral immune responses that may be relevant as COPs include specific titers of IgM, antibodies and numbers of plasmablasts and activated B cells (Escudero-Pérez

  2023). With regard to cellular immune responses, CD8+ T cell measurements may be useful as a CoP (Escudero-Pérez 2023). Additional research is needed to better define these potential CoPs, which may be particularly important for next-generation vaccines.

- Most current NiV vaccine candidates target the immunodominant fusion (F) and attachment (G) glycoproteins, which elicit potent neutralizing antibody responses (Byrne 2023, Geisbert 2021, Ithinji 2022, Loomis 2020, Loomis 2021, van Doremalen 2022, Wang 2022, Woolsey 2023). Data are needed on the potential role of additional immunogens, such as nucleoproteins and other non-enveloped proteins, in stimulating B and T cell responses that contribute to viral clearance, cross-protection, or immune memory. Robust vaccine-induced humoral and cell-mediated immunity to NiV might include protective antibodies with durable immunologic memory and rapid and efficient effector functions (Escudero-Pérez 2023).
- Additional immunologic research is needed to assess the following key elements of protective immunity against NiV infection and disease:
    - The relative contributions of innate, cell-mediated, and humoral immune responses that lead to protective immunity against NiV.
    - Specific cell types and interactions between different immune compartments in achieving viral clearance, surviving acute disease, and modulating chronic infection (Escudero-Pérez 2023, Liew 2022).
    - The roles of neutralizing and non-neutralizing or binding antibodies in protection against NiV (Liew 2022).
    - Mechanisms and cell subsets of cellular immune responses (e.g., CD8 T cell activation) that play a role in cross-neutralizing (heterologous) protection against co-circulating NiV strains (e.g., NiV-M, NiV-B, and NiV-I) (Amaya 2020, Arunkumar 2019, Escudero-Pérez 2023, Liew 2022).
- If researchers and regulators agree that a nontraditional regulatory pathway is appropriate for licensure of NiV vaccines, then a number of issues need to be addressed, such as the following (Price 2021):
    - Generate a fully characterized and controlled virus challenge stock (or potentially one each for NiV-M and NiV-B) for assessing candidate vaccines in animal models.
    - Further characterize, as needed, at least one animal model suitable for vaccine efficacy evaluation.
    - Standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development. Challenge strains used in experimental research also need to be compared against any circulating strains in humans (CEPI 2023a).
    - Determine the most appropriate CoP or surrogate marker (e.g., IgG neutralizing antibodies) for measuring protection and generate standardized assays for measurement.
    - Bridge NiV vaccine efficacy data from animal models to humans, including identifying thresholds of vaccine protection, to determine appropriate human vaccine doses.

  - If the accelerated approval process is deemed an acceptable regulatory approval pathway for NiV vaccines, then plans will be needed to conduct well-controlled clinical trials to establish that vaccines have an effect on an appropriate surrogate endpoint that is likely to predict clinical benefit against NiVD.
  - Post-licensure clinical trials will also be needed to confirm the clinical benefit of any NiV vaccines that are approved via nontraditional pathways.
- Additional research is also needed to address the following areas:
  - Clarification of vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
  - Alternative vaccine delivery approaches, such as oral tablets or transdermal patches, to facilitate rapid NiV vaccine deployment in response to NiV outbreaks in low-resource settings.
  - Further evaluation of optimal NiV antigen combinations (e.g., including stabilized prefusion F protein trimers, multimeric G constructs, and chimeric proteins containing both pre-F and G glycoproteins), and antigen/vaccine platform combinations for generating rapid and durable protective responses to NiV infection (Byrne 2023, Loomis 2020, Loomis 2021, Srivastava 2023).
  - Research in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- Mathematical modelling and forecasting may be useful in (Nikolay 2021): (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- Researchers should consider efforts toward developing pan-henipavirus vaccines to maximize potential benefit, similar to projects aimed at developing pan-coronavirus vaccines or universal influenza vaccines (Tan 2023). One strategy for developing broadly protective henipavirus vaccines involves identifying conserved epitopes or cross-reactive antibodies targeting Henipavirus F proteins (Byrne 2023, Ithinji 2022). Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, the following will be needed:
  - Guidance on the use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
  - Enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

  - Strategic planning for stockpiling and deploying NiV vaccines.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Develop the tools and policies necessary for evaluating and potentially approving one or more NiV candidate vaccines through a nontraditional regulatory pathway.

Milestones:

1. By 2024, generate a fully characterized and controlled virus challenge stock (preferably using a NiV-B strain) ~~(or potentially one each for NiV-M and NiV-B)~~ for assessing candidate vaccines in animal models.

2. By 2024~~6~~, generate standardized assays to measure ~~neutralizing~~ IgG antibodies for NiV vaccine R&D, with a particular focus on assays to be used for regulatory approval via nontraditional pathways.

~~2.~~3. By 2025~~4~~, in conjunction with regulatory authorities, establish benchmark parameters (e.g., route of challenge, timing of challenge, and challenge dose) for testing of NiV candidate vaccines in well-characterized animal models, with particular focus toward meeting criteria necessary for approval via a nontraditional pathway, including identifying surrogate markers that correlate with vaccine efficacy.

~~3. By 2024, further characterize as needed at least one animal model suitable for determining surrogate markers that correlate with vaccine efficacy.~~

4. By 2025, define the protective threshold against NiV infection for serum neutralizing IgG antibodies or other functional CoPs, ~~(as a CoP or surrogate marker),~~ which can be used in animal studies and for immunobridging to humans.

~~5. By 2025, convene a group of key stakeholders to agree on, through a consensus approach, the most suitable assays (e.g., assays that are developed using live NiV vs. assays developed using pseudoviruses) for determining serum neutralizing IgG antibody titers for NiV vaccine R&D and develop a strategy for standardizing those assays.~~

~~6.~~1. ~~By 2026, generate standardized assays to measure neutralizing IgG antibodies for NiV vaccine R&D, with a particular focus on assays to be used for regulatory approval via nontraditional pathways.~~

Strategic Goal 2: Continue to move the current NiV vaccine pipeline forward through clinical trials. ~~toward licensure.~~

Milestones:

1. By 2024, complete current phase 1 clinical trials for at least three promising NiV candidate vaccines.

2. By 2025~~4~~, define use cases for NiV vaccines to inform vaccine deployment and manufacturing plans.

3. By 2025~~6~~, ~~further assess vaccine safety and immunogenicity through~~initiate ~~additional phase 1 and initial~~ phase 2 clinical trials (preferably in affect areas) to further assess immunogenicity and safety for at least two of the most promising NiV candidate vaccines.

~~4. By 2027, complete immunogenicity and efficacy studies in a well-characterized animal model for at least one cross-protective NiV vaccine candidate and define a surrogate marker that demonstrates likely clinical benefit of candidate vaccines.~~

~~5.~~4. By 2029, conduct additional well-controlled phase 2 clinical trials ~~(preferably~~ in affected areas~~)~~ to assess a surrogate endpoint in human subjects (to include children and pregnant women) for at least two~~one~~ NiV candidate vaccines.

~~6.~~5. By 2030, complete a regulatory dossier (for licensure or emergency use) for at least one NiV vaccine candidate based on a suitable animal model with subsequent immunobridging to humans for review via a nontraditional approval pathway.

Additional Priority Areas/Activities

Research

- **Improve understanding** of humoral (e.g., NiV-specific IgG and IgM antibodies) and cellular (e.g., CD8 T cell) immune responses to NiV infection in animal models to inform the design of vaccines and the identification of correlates of protection (Arunkumar 2019, Escudero-Pérez 2023).
- **Improve understanding** of innate immune responses (e.g., involving interferon impairment and pro-inflammatory cytokine release) in relation to humoral and cellular immune responses to NiV infection (Escudero-Pérez 2023).
- **Continue research** on identifying the key NiV antigens (including surface glycoproteins and internal proteins) that modulate the host immune response to NiV infection to inform future vaccine design (Escudero-Pérez 2023).
- **Continue to research** different types of CoPs (both humoral and cell-mediated) for NiV vaccines that are currently in the R&D pipeline and next-generation vaccines, taking into consideration different vaccine platforms, antigens, and clinical outcomes.
- **Generate** international reference standards to calibrate serologic assays for vaccine potency analyses.
- **Continue to conduct** preclinical evaluation of promising candidate NiV vaccines (current and future vaccines) for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** the possibility of creating pan-henipavirus vaccines that will protect against NiV, HeV, and other henipaviruses.

Product development

- **Continue to develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans.
- **Expand** partnerships among researchers, government agencies, and industry to provide the resources necessary for ongoing R&D of NiV vaccines.
- **Define** vaccine attributes (such as time from administration to immune protection, durability of protection, and the need for booster doses) for the most promising candidate vaccines.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Support** plans for adequate manufacturing and stockpiling of NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptance and promotion within the community.
- **Consider** developing a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if a NiV strain emerges with increased capacity for person-to-person transmission and potential for faster spread.

REFERENCES

Agrawal R, Murmu J, Pattnaik S, Kanungo S, Pati S. Bangladesh sees spike in Nipah virus cases: A matter of public health concern? New Microbes New Infect 2023 Mar 30;53:101119 [Full text]

Amaya M, Broder CC. Vaccines to emerging viruses: Nipah and Hendra. Annu Rev Virol 2020 Sep 29;7(1):447-473 [Full text]

Anderson DE, Islam A, Crameri G, et al. Isolation and full-genome characterization of Nipah viruses from bats, Bangladesh. Emerg Infect Dis 2019;25:166-70 [Full text]

Annand EJ, Horsburgh BA, Xu K, et al. Novel Hendra virus variant detected by sentinel surveillance of horses in Australia. Emerg Infect Dis 2022 Mar;28(3):693-704 [Full text]

Arnason G. The emergence and development of animal research ethics: A review with a focus on nonhuman primates. Sci Eng Ethics 2020 Aug;26(4):2277-2293 [Full text]

Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2019 May 24;219(12):1867-1878 [Full text]

Arunkumar G, Devadiga S, McElroy AK, et al. Adaptive immune responses in humans during Nipah virus acute and convalescent phases of infection. Clin Infect Dis 2019 Oct 30;69(10):1752-1756 [Full text]

Auro Vaccines LLC. Safety and immunogenicity of a Nipah virus vaccine. ClinicalTrials.gov Identifier: NCT04199169. First posted 2019 Dec 12; most recent update 2022 Nov 16 [Trial record]

Banerjee S, Niyas VKM, Soneja M, et al. First experience of ribavirin postexposure prophylaxis for Nipah virus, tried during the 2018 outbreak in Kerala, India. J Infect 2019;78:491-503 [PubMed]

Bassat Q, Varo R, Hurtado JC, et al. Minimally invasive tissue sampling as an alternative to complete diagnostic autopsies in the context of epidemic outbreaks and pandemics: The example of coronavirus disease 2019 (COVID-19). Clin Infect Dis 2021 Dec 15;73(Suppl_5):S472-S479 [Full text]

Berge C and Torheim E. Nipah@20: Nipah virus international conference. Conference proceedings. 2019 December 9 and 10 [Full text]

Borisevich V, Lee B, Hickey A, et al. Escape from monoclonal antibody neutralization affects henipavirus fitness in vitro and in vivo. J Infect Dis 2016 Feb 1;213(3):448-55 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua New Guinea. Emerg Infect Dis 2010;16:1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012;2:176-87 [Full text]

Bruno L, Nappo MA, Ferrari L, et al. Nipah virus disease: Epidemiological, clinical, diagnostic and legislative aspects of this unpredictable emerging zoonosis. Animals (Basel) 2022 Dec 31;13(1):159 [Full text]

Byrne PO, Fisher BE, Ambrozak DR, et al. Structural basis for antibody recognition of vulnerable epitopes on Nipah virus F protein. Nat Commun 2023 Mar 17;14(1):1494 [Full text]

CDC (US Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 6 Oct 2020 [Web page]

CEPI (Coalition for Epidemic Preparedness Innovations). CEPI-funded project to enhance scientific understanding of deadly Nipah virus strains. 19 June 2023 [News Release] CEPI 2023a

CEPI. Call for proposals: Clinical development of a Nipah monoclonal antibody (mAb) to protect against Nipah virus (NiV) disease. 6 June 2023 [Full text] CEPI 2023b

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2):235–240 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-283 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Full text]

Ching PKG, de los Reyes V, Sucaldito M, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015 Feb;21(2):328-31 [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. Malaysian J Pathol 2010; 32(2):69-73 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microb Infect 2013 Jun;2(6):e37 [Full text]

Cortés Azuero O, Lefrancq N, Nikolay B, et al. The genetic diversity of Nipah virus across spatial scales. Preprint on MedRxiv. doi: https://doi.org/10.1101/2023.07.14.23292668. Posted July 16, 2023 [Full text]

Dang HV, Cross RW, Borisevich V, et al. Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. Nat Struct Mol Biol 2021 May;28(5):426-434 [Full text]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8:7604 [Full text]

Daszak P, Olival KJ, Li H. A strategy to prevent future epidemics similar to the 2019-nCoV outbreak. Biosaf Health 2020 Mar;2(1):6-8 [Full text]

Deka MA, Morshed N. Mapping disease transmission risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2).pii:E57 [Full text]

de Wit E, Williamson BN, Feldmann F, et al. Late remdesivir treatment initiation partially protects African green monkeys from lethal Nipah virus infection. Antiviral Res 2023 Jun 23;216:105658 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Eby P, Peel AJ, Hoegh A, Madden W, Giles JR, Hudson PJ, Plowright RK. Pathogen spillover driven by rapid changes in bat ecology. Nature. 2023 Jan;613(7943):340-344 [Full text]

Epstein JH, Anthony SJ, Islam A, et al. Nipah virus dynamics in bats and implications for spillover to humans. Proc Natl Acad Sci USA 2020 Nov 17;117(46):29190-29201 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug;14(8):1309–1311 [Full text]

Escudero-Pérez B, Lawrence P, Castillo-Olivares J. Immune correlates of protection for SARS-CoV-2, Ebola and Nipah virus infection. Front Immunol 2023 Apr 17;14:1156758 [Full text]

Field H, Young P, Yob JM, et al. The natural history of Hendra and Nipah viruses. Microbes Infect 2001 Apr;3(4):307-14 [Abstract]

Foster SL, Woolsey C, Borisevich V, et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. Proc Natl Acad Sci USA 2022 Mar 22;119(12):e2200065119 [Full text]

Geisbert JB, Borisevich V, Prasad AN, et al. An intranasal exposure model of lethal Nipah virus infection in African green monkeys. J Infect Dis 2020 May 11;221(Suppl 4):S414-S418 [Full text]

Geisbert TW, Bobb K, Borisevich V, et al. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. NPJ Vaccines 2021 Feb 8;6(1):23 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010 May 18;5(5):e0010690 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-7 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med 2000;342:1229-35 [Full text]

Gómez Román R, Tornieporth N, Cherian NG, et al. Medical countermeasures against henipaviruses: a review and public health perspective. Lancet Infect Dis 2022 Jan;22(1):e13-e27 [Full text]

Gómez Román R, Wang LF, Lee B, et al. Nipah@20: Lessons learned from another virus with pandemic potential. mSphere 2020 Jul 8;5(4):e00602-20 [Full text]

Gouglas D, Thanh Le T, Henderson K, et al. Estimating the cost of vaccine development against epidemic infectious diseases: A cost minimisation study. Lancet Glob Health 2018 Dec;6(12):e1386-e1396 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Gurley ES, Spiropoulou CF, de Wit E. Twenty years of Nipah virus research: Where do we go from here? J Infect Dis 2020 May 11;221(Suppl 4):S359-S362 [Full text]

Hasebe F, Thuy NTT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012;18:536-7 [Full text]

Hedge S, Lee KH, Styczynski A, et al. Potential for person-to-person transmission of henipaviruses: A systematic review of the literature. Preprint on Medrxiv. doi: https://doi.org/10.1101/2023.02.26.23286473 [Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of Nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium). West Africa Lassa Fever Consortium. Accessed June 2023 [Webpage]

Islam A, Cannon DL, Rahman MZ,et al. Nipah virus exposure in domestic and peridomestic animals living in human outbreak sites, Bangladesh, 2013-2015. Emerg Infect Dis 2023 Feb;29(2):393-396 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Ithinji DG, Buchholz DW, Ezzatpour S, et al. Multivalent viral particles elicit safe and efficient immunoprotection against Nipah Hendra and Ebola viruses. NPJ Vaccines 2022 Dec 17;7(1):166 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2):e0117817 [Full text]

Lancet. Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137):2295 [Full text]

Li H, Kim JV, Pickering BS. Henipavirus zoonosis: outbreaks, animal hosts and potential new emergence. Front Microbiol. 2023 Jul 17;14:1167085 [Full text]

Li T, Liang Z, Huang W, Wang Y. Pseudotyped virus for Henipavirus. Adv Exp Med Biol 2023;1407:175-190 [Abstract]

Liew YJM, Ibrahim PAS, Ong HM, et al. The immunobiology of Nipah virus. Microorganisms 2022 Jun 6;10(6):1162 [Full text]

Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med 2019 May 29;11(494):eaau9242 [Full text]

Loomis RJ, DiPiazza AT, Falcone S, et al. Chimeric fusion (F) and attachment (G) glycoprotein antigen delivery by mRNA as a candidate Nipah vaccine. Front Immunol 2021 Dec 8;12:772864 [Full text]

Loomis RJ, Stewart-Jones GBE, Tsybovsky Y, et al. Structure-based design of Nipah virus vaccines: a generalizable approach to paramyxovirus immunogen development. Front Immunol 2020 Jun 11;11:842 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013 Oct;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug; 15(8): 1229–1235 [Full text]

Luo X, Wang C, Huang Y, Cong S, Tan J, Hou W, Ma F, Zheng L. Establishment of a neutralization assay for Nipah virus using a high-titer pseudovirus system. Biotechnol Lett 2023 Apr;45(4):489-498 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015 Mar;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-227 [Full text]

Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. BMJ Global Health 2019;4:e001118 [Full text]

McKee CD, Islam A, Rahman MZ, et al. Nipah virus detection at bat roosts after spillover events, Bangladesh, 2012-2019. Emerg Infect Dis 2022 Jul;28(7):1384-1392 [Full text]

McLean RK, Graham SP. Vaccine Development for Nipah Virus Infection in Pigs. Front Vet Sci 2019 Feb 4;6:16 [Full text]

Mire CE, Geisbert JB, Agans KN, et al. Use of single-injection recombinant vesicular stomatitis virus vaccine to protect nonhuman primates against lethal Nipah virus disease. Emerg Infect Dis 2019;25:1144-52 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;6:30916 [Full text]

Monath TP, Nichols R, Tussey L, et al. Recombinant vesicular stomatitis vaccine against Nipah virus has a favorable safety profile: Model for assessment of live vaccines with neurotropic potential. PLoS Pathog 2022 Jun 27;18(6):e1010658 [Full text]

Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. Science 1995 Apr 7;268(5207):94-7 [Abstract]

NIAID. Dose Escalation, Open-label clinical trial to evaluate safety, tolerability and immunogenicity of a Nipah virus (NiV) mRNA vaccine, mRNA-1215, in healthy adults. ClinicalTrials.gov Identifier: NCT05398796. First Posted 2022 May 31; most recent update 2023 Apr 15 [Trial record]

Nikolay B, Ribeiro Dos Santos G, et al. Assessing the feasibility of Nipah vaccine efficacy trials based on previous outbreaks in Bangladesh. Vaccine 2021 Sep 15;39(39):5600-5606 [Abstract]

Nikolay B, Salje H, Hossain MJ, et al. Transmission of Nipah virus — 14 years of investigations in Bangladesh. N Engl J Med 2019;380:1804-14 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Full text]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Peel AJ, Yinda CK, Annand EJ, et al. Novel Hendra virus variant circulating in black flying foxes and grey-headed flying foxes, Australia. Emerg Infect Dis 2022 May;28(5):1043-1047 [Full text]

Playford EG, Munro T, Mahler SM, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: A first-in-human, randomised, controlled, phase 1 study. Lancet Infect Dis 2020 Apr;20(4):445-454 [Abstract]

Plowright RK, Becker DJ, Crowley DE, et al. Prioritizing surveillance of Nipah virus in India. PloS Negl Trop Dis 2019 Jun 27;13(6):e0007393 [Full text]

Pollak NM, Olsson M, et al. Evaluation of three rapid low-resource molecular tests for Nipah virus. Front Microbiol 2023;13:1101914 [Full text]

Price A, Sizemore D, Hassell T, Gómez Román R, Holst J, Kristiansen P. Nipah virus assays and animal models for vaccine development. Landscape Analysis, January 2021. Coalition for Epidemic Preparedness Innovations [Full text]

Public Health Vaccines LLC. A phase 1 study to evaluate safety & immunogenicity of rVSV-Nipah virus vaccine candidate PHV02 in healthy adult subjects. ClinicalTrials.gov Identifier: NCT05178901. First Posted 2022 Jan 4; most recent update 2023 Mar 10 [Trial record]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Rahman MZ, Islam MM, Hossain ME, et al. Genetic diversity of Nipah virus in Bangladesh. Int J Infect Dis 2021 Jan;102:144-151 [Full text]

Rampling T, Page M, Horby P. International biological reference preparations for epidemic infectious diseases. Emerg Infect Dis 2019;25:205-11 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Full text]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-4 [Abstract]

Sayed A, Bottu A, Qaisar M, Mane MP, Acharya Y. Nipah virus: a narrative review of viral characteristics and epidemiological determinants. Public Health 2019;173:97-104 [Abstract]

Selvey LA, Wells RM, McCormack JG, et al. Infection of humans and horses by a newly described morbillivirus. Med J Aust 1995 Jun 19;162(12):642-5 [Abstract]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat Pteropus vampyrus in Sumatera, Indonesia PLoS One 2013; 8(7): e69544. Published online 2013 Jul 22 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Full text]

Singhai M, Jain R, Jain S, et al. Nipah virus: Recent perspective and One Health approach. Ann Glob Health 2021 Oct 12;87(1):102 [Full text]

Soman Pillai V, Krishna G, Valiya Veettil M. Nipah virus: Past outbreaks and future containment. Viruses 2020 Apr 20;12(4):465 [Full text]

Spiropoulou CF. Nipah virus outbreaks: Still small but extremely lethal. J Infect Dis 2019;219:1855-7 [Full text]

Srivastava S, Verma S, Kamthania M, et al. Exploring the structural basis to develop efficient multi-epitope vaccines displaying interaction with HLA and TAP and TLR3 molecules to prevent NIPAH infection, a global threat to human health. PLoS One 2023 Mar 15;18(3):e0282580 [Full text]

Sudeep AB, Yadav PD, Gokhale MD, et al. Detection of Nipah virus in Pteropus medius in 2019 outbreak from Ernakulam district, Kerala, India. BMC Infect Dis 2021 Feb 9;21(1):162 [Full text]

Tan CW, Valkenburg SA, Poon LLM, Wang LF. Broad-spectrum pan-genus and pan-family virus vaccines. Cell Host Microbe 2023 Jun 14;31(6):902-916 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Thakur N, Bailey D. Advances in diagnostics, vaccines and therapeutics for Nipah virus. Microbes Infect 2019;21(7):278-286 [Abstract]

van Doremalen N, Avanzato VA, Goldin K, et al. ChAdOx1 NiV vaccination protects against lethal Nipah Bangladesh virus infection in African green monkeys. NPJ Vaccines 2022 Dec 21;7(1):171 [Full text]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec; 11(12):1949–1951 [Full text]

Wang Z, Amaya M, Addetia A, et al. Architecture and antigenicity of the Nipah virus attachment glycoprotein. Science 2022 Mar 25;375(6587):1373-1378 [Abstract]

Wang LF, Daniels P. Diagnosis of henipavirus infection: Current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Wang X, Wise JC, Stewart AJ. Hendra Virus: An update on diagnosis, vaccination, and biosecurity protocols for horses. Vet Clin North Am Equine Pract 2023 Apr;39(1):89-98 [Abstract]

Watanabe S, Fukushi S, et al. Effective inactivation of Nipah virus in serum samples for safe processing in low-containment laboratories. Virol J 2020 Oct 9;17(1):151 [Full text]

Whitmer SLM, Lo MK, Sazzad HMS, et al. Inference of Nipah virus evolution, 1999-2015. Virus Evol 2020 Aug 19;7(1):veaa062 [Full text]

WHO (World Health Organization). Nipah Virus fact sheet. 2018 May 30 [Web page]

WHO. WHO R&D Blueprint. Accessed June 2023 [Webpage]

WHO. WHO R&D Blueprint: Priority diagnostics for Nipah use cases and target product profiles. Draft v0.3. 2019 [Full text]

Widerspick L, Vázquez CA, et al. Inactivation methods for experimental Nipah virus infection. Viruses 2022;14(5):1052 [Full text]

Woolsey C, Borisevich V, Fears AC, et al. Recombinant vesicular stomatitis virus-vectored vaccine induces long-lasting immunity against Nipah virus disease. J Clin Invest 2023 Feb 1;133(3):e164946 [Full text]

Yadav PD, Majumdar T, Gupta N, et al. Standardization & validation of Truenat™ point-of-care test for rapid diagnosis of Nipah. Indian J Med Res 2021 Apr;154(4):645-649 [Full text]

Yadav PD, Shete AM, Kumar GA, et al. Nipah virus sequences from humans and bats during Nipah Outbreak, Kerala, India, 2018. Emerg Infect Dis 2019 May;25(5):1003-1006 [Full text]

Yang S, Kar S. Are we ready to fight the Nipah virus pandemic? An overview of drug targets, current medications, and potential leads. Struct Chem 2023 Mar 8:1-19 [Full text]

Yang M, Zhu W, Truong T, et al. Detection of Nipah and Hendra viruses using recombinant human ephrin B2 capture virus in immunoassays. Viruses 2022 Jul 28;14(8):1657 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Zhang XA, Li H, Jiang FC, et al. A zoonotic henipavirus in febrile patients in China. N Engl J Med 2022 Aug 4;387(5):470-472 [Full text]

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Date: Tuesday, August 29, 2023 5:43:08 PM
Attachments: Nipah Roadmap TF Meeting Summary.Final.docx
Nipah RD Roadmap.PostMeetingRevision.August2023.docx

Dear colleagues: First, I want to thank all of you who participated in the Nipah R&D Roadmap Taskforce meeting on July 31 and August 1, 2023. The discussions were highly productive and your input was extremely valuable. Also, it was great to see so many of you in person.

I would also like to again thank those of you who facilitated the meeting: Emily Gurley, Joel Montgomery, Emmie de Wit, Chris Broder, and Marie-Pierre Preziosi—you all did a great job!

Attached please find a summary report of the meeting and a revised version of the Nipah Virus R&D Roadmap that includes all of the changes that were made based on discussions at the meeting (track changes are included). We also added a couple of additional references.

We would very much appreciate your review of the attached roadmap and any edits or comments that you may have. During the meeting, we mainly focused on the goals and milestones, and that's where the majority of the edits are, but we would very much appreciate your review of the whole document at this point in the process. **Please send any feedback on the document no later than September 15, 2023.**

Also, if you see any glaring omissions or inaccuracies in the summary report, please let me know (although we certainly don't expect you to review and comment on the meeting summary—we are providing it to you for your information only).

Over the coming weeks, I'll be preparing a manuscript about the roadmap and will be sending that to you for your review—tentatively in early October.

Again, thank you all for your support of this important project and for sharing your extensive expertise with us.

Kind regards,

Kris

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Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 6-year framework beginning in 2024 for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs)—diagnostics, therapeutics, and vaccines—against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999 (Chew 2000, Chua 1999, Chua 2010, Parashar 2000, Paton 1999). As part of that outbreak, 265 human cases of NiV disease (NiVD) were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiVD was subsequently recognized, however, in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since (Hsu 2004, WHO 2018, Agrawal 2023). NiVD has also been identified periodically in eastern India (in 2001 and 2007) and, in 2018, an outbreak occurred for the first time in southern India (Arunkumar 2019, Chadha 2006, Chattu 2018, Sharma 2018, Soman Pillai 2020). Additionally, one case was reported from Kerala state in 2019 (Sudeep 2021). Case-fatality rates during outbreaks in Bangladesh and India have generally ranged from 50% to 100% (Sharma 2018). NiV infection in humans results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae (Goh 2000, Hossain 2008, Tan 2002). A summary of 14 years of NiVD investigations in Bangladesh found no evidence of asymptomatic infection (Nikolay 2019).

The primary natural reservoir host for NiV in South Asia, where cases continue to occur, is *Pteropus* bats. A recent study suggests that discrete multiannual local epizootics in bat populations contribute to the ongoing sporadic nature of human NiV outbreaks in South Asia (Epstein 2020). Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in *Pteropus* bats and several other related bat species in Southeast Asia, the Western Pacific, and Africa (Anderson 2019, Breed 2010, Epstein 2008, Hasebe 2012, Iehlé 2007, Plowright 2019, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, Yob 2001). In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, dogs were found to be infected with NiV on the farms involved in the outbreak (Field 2001). In outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role to date, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission (Arunkumar 2019, Gurley 2007, Islam 2016, Luby 2009, Nikolay 2019, Rahman 2008). However, a recent study in Bangladesh found NiV antibodies in cattle, dogs, and cats from six sites where spillover human NiV infection cases occurred during 2013–2015 (Islam 2023), suggesting the potential of zoonotic spread via intermediary hosts. Respiratory transmission via droplet spread may play an important role in propagating outbreaks (Nikolay 2019, Spiropoulou 2019). The virus could have pandemic potential if a more human-adapted strain, with greater person-to-person transmission emerges (Luby 2013).

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock and other domestic animals, which can serve as a source of exposure to humans (Islam 2023). NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen—Hendra virus (HeV)—which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia (Murray 1995, Selvey 1995). To date, at least 66 spillover events—all in Australia—involving more than 100 horses and seven humans have been identified (Wang 2023). Changes in bat behavior related to habitat loss and climate change appear to have increased the spillover risk of HeV from bats to horses (Eby 2023). An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014 (Ching 2015). This outbreak likely involved spillover of the virus into horses and subsequent disease in humans following consumption of contaminated horsemeat; disease also occurred in healthcare workers who cared for infected patients. Detailed genomic information for this virus is limited.

Genomic sequencing has demonstrated that there are two main clades of NiV: the M genotype, which comprises the Malaysian NiV isolates (NiV-M), and the B genotype, which includes Bangladesh (NiV-B) and India NiV isolates (NiV-I) (Liew 2022, Yadav 2019). These three strains share a high percentage of homology (NiV-M and NiV-B strains share 91.8% homology, and NiV-I strains share 85.14to 96.15% homology with both NiV-M and NiV-B). Some strain-related differences, however, have been noted in the clinical features of infection in humans and experimentally infected non-human primates, with strains of the B clade appearing to be more pathogenic than those of the M clade (Mire 2016). Recent data indicate that strains from Bangladesh are segregated into two additional distinct sublineages that have intermingled geographically and temporally in that area over time (Rahman 2021, Whitmer 2020). At this time, however, the molecular epidemiology of NiV remains somewhat unclear and issues around strain diversity and strain evolution require further elucidation (Rahman 2021).

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of MCMs to enable effective and timely emergency response to infectious disease epidemics. NiVD is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist) (WHO 2023). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this roadmap ranges from basic research to late-stage development of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply broadly to more than one MCM category), diagnostics, therapeutics, and vaccines.

(*Note*: These topics are not presented in order of public health priority.) Each section includes barriers (inherent obstacles or technical challenges that may influence the likelihood of success for development of NiV MCMs) and gaps (key needs or unresolved limitations in knowledge that are critical to the development of new NiV MCMs). These are followed by strategic goals and milestones, which build on the gaps and barriers and are focused on achievements for the next 6 years (beginning in 2024) that are necessary for moving NiV MCMs forward. The roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed. Each section also includes additional ongoing priorities that should be considered for NiV MCM R&D.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include minimizing zoonotic NiV transmission, improving use of personal protective equipment (PPE), ensuring adequate hand hygiene and environmental hygiene, promoting effective community engagement, implementing adequate infection prevention and control practices, developing adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and promoting workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of this R&D roadmap, but need to be addressed as part of a broader public health control strategy. Further research of NiV and related henipaviruses in animal species, including development of appropriate MCMs targeted to animal populations (such as vaccines [McLean 2019]), also is needed, since disease in animals may amplify occurrence of NiV (or a related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

VISION

Robust MCMs to detect, prevent, treat, and control human outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care or near patient diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Barriers and Gaps

Barriers

- Securing funding for NiV research represents a substantial challenge, since economic incentives to invest in NiV research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with small and sporadic outbreaks (Gómez Román 2020). The development of a sustainable value proposition for industry and international philanthropic public-private partnerships is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate.

- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand (Gouglas 2018). In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- National regulatory authorities in countries where NiV outbreaks are likely to occur have different regulatory requirements for authorization, licensure, or emergency use of NiV medical countermeasures, which complicates the approval process, particularly for candidate vaccines and therapeutics (Gómez Román 2022). An international, NiV-focused regulatory group has been created and will be an important mechanism for coordinating regulatory issues as NiV MCMs are moved forward (Gómez Román 2020).
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (biosafety level 4 [BSL-4]) conditions (Geisbert 2020, Gómez Román 2022), which can increase the cost and complexity of MCM development.
- To date, NiV spillovers to human communities have been identified most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the *Pteropus* genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar (Bruno 2022). Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV or related viruses; such bats can be found across Africa and parts of the Middle East (Bruno 2022). This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to the lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- The development and accessibility of animal models that recapitulate human NiV disease are critical for NiV MCM development, given the limitations on clinical samples and the infeasibility of traditional clinical efficacy trials. While ferrets and Syrian golden hamsters are well-established animal models for NiV research, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans (Foster 2022, Geisbert 2010, Geisbert 2020, Geisbert 2021, Johnston 2015, Price 2021, van Doremalen 2022, Woolsey 2023). Studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways; however, costs, space requirements (particularly in BSL-4 containment facilities) and ethical concerns constrain their use (Arnason 2020, Bossart 2012, Geisbert 2010, Johnston 2015).
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection occurs in relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate

MCM efficacy with adequate statistical power (Gómez Román 2020). It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks; however, it is likely that at least vaccines will need to be licensed via nontraditional regulatory pathways.

- Patients usually present late in the clinical course of disease and disease progression is often rapid, making it difficult to collect clinical samples before patients succumb to the disease. Additionally, autopsies are often not the standard of care in affected areas, which further reduces opportunities to collect clinical specimens. These barriers hinder the ability to understand disease pathogenesis and immunologic responses to infection, which are important for MCM R&D (Amaya 2020, Arunkumar 2019, Gurley 2020, Liew 2022, Mazzola 2019).
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV vaccines and therapeutics.

Gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of NiV MCMs, as well as other preventive measures, depend on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of human disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas (Bruno 2022, Singhai 2021).
- Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of *Pteropus* bats and potentially other bat species around the globe (Gómez Román 2020, Plowright 2019).
- Additional research is needed to optimize and further characterize relevant animal challenge models (particularly ferret, Syrian golden hamster, and AGM models) for promoting development and evaluation of NiV MCMs (Geisbert 2020, Gómez Román 2020, Price 2021, Rockx 2014). Examples of additional issues involving animal models include the following (Dhondt 2013, Gómez Román 2020, Johnston 2015, Mathieu 2015, Mire 2019, Price 2021):
    - Determine the appropriate animal model(s) for screening assay development.
    - Standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development.
    - Determine when after-challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans.
    - Identify the best models for studying chronic (relapsing) infection.
    - Determine if different animal models are needed for different clinical endpoints such as infection, disease, and transmission.
    - Determine if models perform differently based on the route of virus administration.
    - Improve overall standardization of the models.
- Other research needs include the following:
    - Improved understanding of the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs (Gurley 2020, Liew 2022). This includes evaluating the pathophysiologic differences between different NiV strains,

  determining the mechanisms that allow NiV to escape immunologic clearance and cause delayed onset or recurrent encephalitis, and identifying factors influencing the development of permanent neurologic sequelae.
  - Ongoing phylogenetic and evolutionary analyses of NiV strains to monitor viral heterogeneity and antigenic changes that may affect the epidemiologic and clinical features of disease over time and thereby influence MCM development (Gurley 2020, McKee 2022, Rahman 2021).
  - Additional research to determine if: (1) NiV strain variations influence the ability to detect NiV infections; (2) different strains have different phenotypic characteristics, such as different clinical manifestations or transmission dynamics, and (3) if strain variability impacts efficacy of vaccines or therapeutics (CEPI 2023a, Gurley 2020, Whitmer 2020).
  - Whole-genome sequencing of NiV isolates to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses (CEPI 2023a).
  - Sociological and anthropological research to understand how to best engage populations at high risk of exposure (such as persons who consume date palm sap, healthcare workers, and workers at the human-animal interface) and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease (Singhai 2021, Lancet 2018).
  - Prospective serosurveillance of henipavirus exposure from susceptible animal species and proximate human populations in areas of predicted risk to assess the potential of human spillover and to build preparedness for detection of human cases and for limiting exposure (Daszak 2020, Deka 2018, Gómez Román 2020, Plowright 2019).
  - Expanded use of machine-learning approaches can facilitate an improved understanding of the risk of NiV spillover events (Plowright 2019).Ecological studies are also needed to enhance understanding of the dynamics governing prevalence and shedding of NiV and other henipaviruses in bats (Plowright 2019).
- Other important needs include the following:
  - Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop and manufacture NiV MCMs (Gómez Román 2020).
  - Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control (Bruno 2022).
  - Advocacy to policy makers in affected countries and to global stakeholders to ensure they understand the potential health, societal, and economic benefits of devoting resources to improving NiV surveillance, detection, prevention, and control measures (Gómez Román 2020).

  - Scenario planning to clarify regulatory pathways for product approval in countries where NiV outbreaks are likely to occur, including determining whether or not efficacy data from animal models is sufficient for regulatory approval. Such planning should take into consideration the local epidemiology of NiV infection and the different requirements of local national regulatory authorities for product approval and emergency use authorization (Gómez Román 2022). Regulatory pathways and NRA capabilities vary between countries; therefore, early engagement, potentially with support from WHO and other key international stakeholders, is essential to identify country-specific considerations. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.
  - Standardized and well-characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection (Rampling 2019, Satterfield 2016). Assays that can be used at lower biosafety levels are an important priority. WHO international standards should be used (when available) as calibrators and reported in units/ml to harmonize assay results.
  - Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection (Singhai 2021).
  - Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
  - Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
  - Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.
  - Efforts to reduce the cost of medical countermeasure development and ongoing production.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify additional and ongoing sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2025, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2)

outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.

2. By 2026, create a funding plan based on the value proposition for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.
3. By 2026, develop a coordinated strategy for promoting and incentivizing greater industry engagement in R&D for NiV MCMs.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to better define the disease burden, risk factors for infection, and risk of spillover events in affected countries.

Milestones:

1. By 2025, develop a plan for enhancing human NiV surveillance in India and Bangladesh outside of existing surveillance areas (i.e., where cases previously have been identified). This should include securing funding, identifying additional surveillance catchment areas, engaging key partners in those areas, generating standardized surveillance protocols, and conducting training for implementation.
2. By 2026, initiate enhanced human NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), promote early case detection, and better define the disease burden in India and Bangladesh, particularly outside of areas where cases previously have been identified.
3. By 2026, generate and implement standardized protocols for case investigation that are aimed at identifying risk factors for primary NiV infection and at conducting case-contact studies to better understand chains of transmission (Hedge 2023).
4. By 2026, develop plans for conducting additional research in India and Bangladesh to identify the potential for and drivers of spillover events, particularly in areas where NiV cases have not yet been identified.

Strategic Goal 3: Determine the requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure or approval of NiV MCMs by engaging an international regulatory working group representing NRAs in affected areas and other key international stakeholders.

Milestones:

1. By 2024, conduct scenario planning to clarify the regulatory procedures and determine the acceptable pathways for approval and emergency use authorization of NiV MCMs (including vaccines, novel candidate therapeutics, repurposed therapeutics, and diagnostics) in countries at highest risk for NiV outbreaks (i.e., Bangladesh and India) through an international NiV-focused regulatory group.

2. By 2025, determine if any key gaps exist for regulatory approval and emergency use authorization of candidate NiV MCMs in affected countries and develop strategies to address those gaps, with specified timelines for completion.
3. By 2026, ensure that any issues affecting approval and emergency use of candidate MCMs in affected countries have been resolved and that consensus has been achieved on the necessary regulatory steps and procedures.

Strategic Goal 4: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2025, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
2. By 2025, conduct additional research to further optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs.
3. By 2026, conduct sequencing of NiV strains from existing clinical samples obtained from past NiV cases to assess variability of NiV strains in India and Bangladesh (CEPI 2023a).
4. By 2026, establish capacity at selected surveillance sites in India and Bangladesh to investigate NiV-related deaths by using post-mortem minimally invasive tissue sampling to enhance understanding of NiV disease pathogenesis (Bassat 2021).
5. By 2028, conduct research in animal models to determine if strain variability impacts efficacy of promising NiV MCMs or the accuracy of diagnostic tests.

Additional Priority Areas/Activities

Research

- **Continue to expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach. Such research should include serosurveys in different animal species and ecologic studies in bats, and should utilize computational approaches, such as machine-learning approaches.
- **Continue to perform** phylogenetic and evolutionary analyses, using whole-genome sequencing, of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Incorporate**, on an ongoing basis, additional NiV strains into preclinical research as newer strains become available.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability of henipaviruses to improve understanding of their global distribution.

- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection, including potential regulatory pathways for MCM licensure and approval.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** an open-access shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas. These should include efforts to reduce production costs and ensure equitable global access as needed.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an “affordable and fair” price is one that can reasonably be paid by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

DIAGNOSTICS

Barriers and Gaps

Barriers

- Initial signs and symptoms of NiV infection are nonspecific, and infection is often not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent infection can last for months to years after initial exposure, which can complicate epidemiologic investigation (CDC 2020).
- The accuracy of laboratory results can be affected by a variety of factors, such as clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory (WHO 2019, Mazzola 2019).
- The time required to perform diagnostic testing using conventional laboratory methods is problematic, given the potential for rapid disease progression of NiV infection (Sayed 2019).
- Diagnostic needs vary across the latent, acute, and convalescent phases of NiV infection (Bruno 2023).
- Limited laboratory infrastructure and diagnostic capabilities in peripheral settings can lead to delays in diagnosis and in outbreak investigation and response (Berge 2019, Bruno 2022, Chua 2013, Wang 2012, WHO 2019).
- Owing to the high biosafety precautions necessary when working with NiV, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis (Watanabe 2020, Widerspick 2022).
- Limited NiV-positive clinical samples are available, which are important for validation of diagnostic tests (Mazzola 2019).
- *Pteropus* species (and likely other bat species) may carry other henipaviruses in addition to NiV and HeV, some of which could be pathogenic in humans and livestock. Antibodies to different henipaviruses appear to be highly cross-reactive, making it difficult to discriminate the particular henipaviruses that are in circulation using serologic assays, which is critical to ensuring diagnostic preparedness to respond to future outbreaks (Mazzola 2019, Wang 2012, WHO 2019, Yang 2022).

Gaps

- Further research is needed to:
    - Improve understanding of the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease (Arunkumar 2019, Berge 2019, Mazzola 2019, Thakur 2019).
    - Determine criteria for test performance and further evaluate performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as rapid diagnostic tests) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
    - Continue to develop commercialized or standardized rapid nucleic acid tests that can quickly confirm active NiV infection at the point of care or at the level of near-patient

    care (Berge 2019, Pollack 2023, WHO 2019). This effort may require clarification of the regulatory pathways for commercialization of NiV diagnostic tests. To date, one rapid test has been approved for emergency use in India during an outbreak that occurred in 2018 (Yadav 2021).
  - Generate international reference standards to calibrate diagnostic assays to ensure proficiency testing of new diagnostics (Berge 2019, WHO 2019).
  - Clinically validate the performance and operational suitability of new promising diagnostics, particularly rapid diagnostic tests, in endemic geographic regions (Berge 2019, Pollack 2023).
  - Continue to assess and operationally validate safe and simple methods of sample inactivation that do not interfere with diagnosis and that can be used at peripheral sites (Pollack 2023, WHO 2019, Watanabe 2020, Widerspick 2022, Yadav 2021).
  - Establish operational suitability at the point of care (i.e., at peripheral community settings) for NiV diagnostic tests by developing an integrated approach to facilitate rapid, accurate, and safe testing procedures (Pollack 2023).This could include a minimal protocol or best practices approach for sample inactivation before testing (WHO 2019).
- Other needs include the following:
  - Development of a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples to assess and validate diagnostic tests (Berge 2019, WHO 2019). As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6) identify who would have access to the samples; (7) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
  - Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection (Berge 2019, WHO 2019).
  - In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
  - Systems for external quality assessment (EQA) monitoring of tests using up-to-date clinical specimen panels and reference standards (Mazzola 2019).
  - A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for NiV diagnostics, particularly given the costs of regulatory approval.
  - Improvement of diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses as they arise (Wang 2012).

  - Ongoing efforts to develop affordable and easy-to-use multiplex panels for detection of a range of pathogens using a syndromic approach, if such panels can be deemed cost-saving (Mazzola 2019).
- Use cases and target product profiles (TPPs) have been developed for NiV diagnostics (WHO 2019); however, the criteria may need to be modified over time as new lineages are identified (Mazzola 2019).
- Since NiV strains are continuing to evolve, it's possible that current diagnostic tests could fail to detect an emergent variant of NiV. Recently, a novel strain of HeV was identified in Australia that was not detected through conventional PCR testing owing to gene sequence mismatches (Annand 2022). Diagnostic tools that can detect a broader range of NiV, HeV, or related viruses capable of spillover may be needed to advance the ability to forecast spillover risks and to detect emergent viruses (Peel 2022).

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2026, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).
2. By 2027, identify sustainable, long-term funding, determine sites for sample storage, and initiate creation of the virtual reference repository in Bangladesh and India, with samples to be collected during future outbreaks.

Strategic Goal 2: Continue to develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are suitable for use in peripheral settings and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2025, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays. This effort should include clarifying regulatory pathways for approval (including approval for emergency use) of NiV diagnostics.
2. By 2026, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP and can be used in peripheral sites.
3. By 2026, create international reference standards for calibrating and harmonizing NiV diagnostic assays.
4. By 2026, develop a minimum protocol or set of best practices (that biosafety committees will accept) for inactivation of clinical samples from humans and animals that are being tested for NiV.

5. By 2027, complete clinical validation of performance and operational suitability for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP and can be used in peripheral sites.
6. By 2028, obtain regulatory approval for at least one rapid, point-of-care or near-patient care NiV diagnostic test that can be commercialized and standardized.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known spillover risk to promote early detection of NiV.

Milestones:

1. By 2026, expand national laboratory networks for NiV detection in the primary affected countries (Bangladesh and India) that include plans for enhancing laboratory preparedness to enable earlier and timely detection of NiV infection during future outbreaks.
2. By 2027, generate well-characterized and up-to-date proficiency panels for NiV diagnostic testing to be used in selected laboratories in Bangladesh and India.
3. By 2029, implement routine EQA monitoring of NiV diagnostic testing at selected laboratories in Bangladesh and India.

Additional Priority Areas/Activities

Research

- **Continue to explore** new diagnostic approaches that may allow earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** criteria for test performance and **continue to evaluate** performance characteristics for promising new assays for diagnosis of NiV infection.
- **Continue to conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection as they become available.
- **Continue to research** methods of diagnostic testing that are able to differentiate between various pathogenic henipaviruses.
- **Continue to develop** affordable and easy-to-use multiplex panels for detection of a range of pathogens using a syndromic approach.
- **Consider** development of diagnostic tools that can detect a broader range of NiV, HeV, or related viruses capable of spillover.

Product development

- **Refine** over time, as needed, criteria in the existing TPPs to include identification of different NiV lineages/strains.
- **Continue to develop and evaluate** point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their

intended use), can capture antigenically diverse strains of the virus, and can be performed accurately and safely in peripheral settings under a variety of circumstances.

Key capacities

- **Establish** operational suitability in peripheral laboratories of rapid diagnostic tests over time, as new tests become available.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed.

Policy and commercialization

- **Develop** guidance on optimal strategies for the deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

THERAPEUTICS

Barriers and Gaps

Barriers

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- A limiting constraint to assessing the effectiveness of promising therapeutics is the number of patients with NiV infection who can be enrolled in clinical trials over time, given the small number of cases that are identified annually.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment of patients and PEP for exposed persons, which can significantly impact clinical evaluation of therapeutic candidates.
- NiV can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in affected countries often do not have adequate infection control– programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.
- One promising therapy is the monoclonal antibody (mAb) m102.4; however, the projected cost per patient for this agent is expected to be more than $1,000 (Gómez Román 2022); this high cost poses an important barrier to its use.

Gaps

- Patients may benefit from optimal supportive care, independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials to inform best-practice guidelines and evidence-based policy decisions. Standard of care guidelines will be important for conducting clinical efficacy trials of therapeutic agents.

- During the 1998-99 NiV outbreak in Malaysia, clinicians used ribavirin to treat 140 patients. Outcomes data revealed a lower mortality rate among treated patients (Chong 2001), but the findings may have been biased by the use of historical controls. No additional clinical studies of ribavirin have been conducted, and limited studies in animals have not demonstrated efficacy of ribavirin following NiV or Hendra virus challenge (Georges-Courbot 2006, Rockx 2010). Ribavirin, however, may prove useful for PEP (Banerjee 2019); therefore, further challenge studies in animal models should considered to explore this possibility.
- Studies in animals have evaluated the usefulness of several agents (including remdesivir, favipiravir, and fusion inhibitory peptides) when delivered prior to disease onset or early during the disease course (Dawes 2018, Lo 2019, Mathieu 2018). One recent study showed that AGMs were only partially protected when remdesivir was administered 3 days post-inoculation; therefore, early administration seems critical for effective treatment (de Wit 2023). Patients with NiV infection are often detected late in the clinical course, which creates challenges for predicting how well a therapeutic agent will work in the field. Additional challenge studies in animals, therefore, are needed to: (1) assess the clinical benefit of these therapeutics as treatment options when administered after symptom onset or at least more than 24 hours after initial exposure, (2) determine whether these agents may be appropriate for PEP, and (3) clarify the most feasible and cost-effective route of administration (e.g., oral, intranasal, intravenous) appropriate for real-world conditions, particularly if being considered for mass prophylaxis in an outbreak setting (Gómez Román 2022).
- m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV (Broder 2012, Guillaume 2004, Playford 2020). Several other mAbs have also been assessed in animal models and appear promising (Gómez Román 2022). A phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia during 2015 and 2016 (Playford 2020). In that trial, m102.4 was well tolerated and safe, with no evidence of an immunogenic response.
- Additional research needs for mAbs as treatment or PEP for NiV infection include:
    - Additional clinical trials in endemic areas to further assess the safety, tolerability, efficacy, and pharmacokinetic parameters of m102.4 (and possibly other mAbs with adequate preclinical data) for PEP and potentially early treatment of clinical disease (CEPI 2023b).
    - Additional research to determine the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with m102.4, it may be necessary to consider mAb cocktails (Borisevich 2016, Playford 2020).
    - Animal studies to determine if mAb cocktails that combine several different mAbs into one formulation are more efficacious than administering one mAb alone (Dang 2021).
    - Future studies to ascertain the efficacy of m102.4 for treatment and prophylaxis against different viral strains of NiV and Hendra viruses, particularly among populations living in settings where there is the potential for an outbreak.
    - Adequate stockpiles of m102.4 (or potentially other mAbs) to ensure urgent access at the onset of an NiV outbreak.

- Given the limited number of NiV cases identified each year, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how best to allocate scarce resources for conducting such trials.
- A prepositioned, agreed-upon protocol for conducting clinical trials of promising therapeutics during NiV outbreaks would be of value in advancing clinical evaluation of such agents (Spiropoulou 2019).
- Diagnostic criteria and standardized testing are needed for including patients in clinical trials of therapeutics.
- Additional research needs include the following:
    - Further research to broaden the number of novel antiviral candidates (including repurposed drugs) for treatment of NiV infection and strengthen the therapeutic pipeline. Computational aided drug design is one tool that can be useful for this discovery (Yang 2023).
    - Additional data to establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship of promising therapeutic candidates.
    - Additional data to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP stockpiling and distribution in both affected and at-risk areas, particularly Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
    - Additional information to determine whether or not strain differences will affect the response to therapeutic candidates and results from clinical trials.
    - Additional data to determine the therapeutic windows for promising therapeutics for the different NiV strains, as highlighted by a recent study in AGMs that showed that the therapeutic window for m102.4 against a strain from Bangladesh/India was shorter than for a strain from Malaysia (Mire 2016).

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2024, convene a consortium of key stakeholders—in affected areas and internationally—to address the key challenges with conducting clinical trials of therapeutic agents during future NiV outbreaks. This consortium could potentially be modeled after the West Africa Lassa Fever Consortium (WALC) (ISARIC 2023).

2. By 2025, develop NiV standard of care guidelines to be used in affected countries, disseminate the guidelines, and conduct outreach to clinicians as appropriate.

3. By 2025, complete an agreed-upon generic prepositioned protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates (mAbs and small molecules, including

repurposed drugs) to be implemented in NiV-affected areas during outbreaks and develop plans for operationalizing the protocol.

4. By 2025, complete an agreed-upon generic prepositioned protocol for conducting PEP trials of promising therapeutic candidates (mAbs and small molecules, including repurposed drugs) to be implemented in NiV-affected areas during outbreaks and develop plans for operationalizing the protocol.
5. By 2026, complete a broader, harmonized regional protocol (to be used across Bangladesh and India) for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during outbreaks or potentially during periods of endemic disease (if comparable data can be generated over time and across different clinical sites) and develop plans for operationalizing the protocol.
6. By 2026, generate a reliable source or stockpile of an mAB (m102.4 or other mAb) to be used in outbreak-related clinical trials for both PEP and early clinical treatment.

Strategic Goal 2: Develop and evaluate therapeutic agents for the treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2025, create and implement a prioritization process for determining which promising NiV therapeutic candidates should be further evaluated in clinical trials, once adequate animal data demonstrating safety and efficacy are available.
2. By 2026, complete preclinical evaluation in animal models—with administration of the therapeutic agent more than 24 hours after challenge and potentially after symptom onset—of the preliminary safety, tolerability, and efficacy of at least two promising small-molecule therapeutic candidates or combination therapies for the treatment of NiV infection.
3. By 2027, further explore in animal models whether two or three of the most promising small-molecule therapeutic candidates are likely suitable for PEP and, therefore, should be assessed as PEP in clinical trials.
4. By 2027, determine the most cost-effective and feasible routes of administration for use in real-world settings for at least two promising small-molecule therapeutic candidates.
5. By 2027, complete at least one additional clinical trial (phase 2 or 2/3) in an NiV-affected area of m102.4 or other suitable mAb to further assess safety, tolerability, and efficacy (if NiV incidence allows efficacy assessment).
6. By 2028, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising small-molecule therapeutic candidates or combination therapies for the treatment of NiV infection.

Additional Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as m102.4, remdesivir, and favipiravir) for treating and preventing NiV infection, including conducting additional studies in animal models and clinical trials as appropriate and feasible. This should include determining the therapeutic windows for use of therapeutic agents as treatment or PEP.
- **Clarify,** in animal models, the potential for development of escape mutants from use of mAbs.
- **Continue to conduct** preclinical research on mAbs other than m102.4 and on mAb cocktails to assess safety, tolerability, and efficacy for treating NiV infection
- **Continue to expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation, potentially using pseudotyped viruses for initial screening of compounds (Li 2023).
- **Consider conducting** additional challenge studies in animal models to assess whether or not ribavirin may be suitable for PEP following NiV exposure.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Continue to develop, evaluate, and license** safe and effective therapeutic agents for the treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in affected areas to improve clinical management and supportive care of patients with NiV infection and to improve infection control practices to limit person-to-person spread.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- **Explore** strategies for decreasing the costs associated with m102.4, such as exploring the possibility of administering mAbs subcutaneously rather than intravenously.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

VACCINES

Barriers and Gaps

Barriers

- Large clinical efficacy trials, which typically are required for vaccine licensure, will likely not be feasible for NiV vaccines, owing to the sporadic and unpredictable nature of NiV outbreaks and the low case numbers usually involved (Gómez Román 2022, Nikolay 2021, Satterfield 2016).
- In the absence of large clinical efficacy trials, authorization and licensure will likely involve nontraditional regulatory pathways to guide the evaluation of safety and efficacy (Gómez Román 2022). However, experience is limited with these routes (such as the US FDA's Animal Rule or Accelerated Approval Program, the EMA's conditional market authorization, and authorization under exceptional circumstances) and there are few successful models for vaccine authorization and approval.
- The limited commercial value of NiV vaccines may impede industry's involvement in developing and producing NiV vaccines without significant financial support (e.g., through partnerships with organizations such as CEPI, PATH, and high-income country government agencies) (Gómez Román 2022).
- The affordability of creating and maintaining an NiV vaccine stockpile and deploying vaccines during outbreaks is a key issue for low- and middle-income countries; supplementary funding will likely be required to ensure vaccine preparedness for NiV outbreaks (Gómez Román 2022).
- The absence of improved diagnostic assays for the timely diagnosis of infection creates an important challenge by delaying implementation of a rapid reactive vaccination strategy for NiV outbreak control.

Gaps

- NiV vaccines are needed that: (1) are readily accessible with adequate supply chains, particularly in low-resourced areas, (2) can protect against different NiV strains, and (3) provide rapid onset of immunity to adequately prevent and control outbreaks in a timely manner.
- Use cases for NiV vaccines need to be better defined, as how vaccines are to be used will affect vaccine deployment and manufacturing plans.
- Reference standards for NiV antibodies are needed to evaluate candidate NiV vaccines.
- Expanded partnerships among researchers, funders, and regulators are needed to advance the development of promising NiV vaccine candidates (Amaya 2020, Gómez Román 2022). NiV vaccine candidates in preclinical development target the F/G glycoproteins and a variety of platform technologies are being considered (e.g., virus vectors, protein subunits, mRNA, and virus-like particles) (Amaya 2020, Geisbert 2021, Gómez Román 2022, Loomis 2021, Monath 2022). NiV vaccine candidates based on three different platforms (protein subunits, mRNA, and viral vectors) are currently in phase 1 clinical trials (Auro Vaccines 2022, NIAID 2023, Public Health Vaccines 2023).
- Demonstrating vaccine-induced protection against NiV infection or disease in an animal model will require an immune correlate of protection (CoP) or immune surrogate that can predict the likelihood of protective efficacy and that reflects the protective immune responses generated in humans (Amaya 2020, Escudero-Pérez 2023, Price 2021).
    - Accurate and reliable CoPs for determining the protective efficacy of NiV vaccines have not yet been identified (Loomis 2021). Neutralizing IgG is generally used as a CoP, although the protective threshold still needs to be defined to allow additional vaccine

  testing in animal challenge models and eventual immunobridging of antibody responses to humans, through phase 1/2 clinical studies (Escudero-Pérez 2023, Price 2021).
  - Once the protective threshold for neutralizing IgG antibodies is determined, the most appropriate and feasible assays (e.g., assays that are developed using live NiV vs. assays developed using pseudoviruses expressing the NiV F and G glycoproteins [Luo 2023]) need to be identified and standardized for use in animal models (Price 2021).
  - Different types or titers of CoPs may ultimately be needed for different vaccine platforms, antigens, clinical outcomes (e.g., protection against infection, severe disease, chronic disease, or death) and potentially host or population characteristics to support the assessment of candidate NiV vaccines using immunogenicity and efficacy data from preclinical studies.
  - Other humoral immune responses that may be relevant as COPs include specific titers of IgM, antibodies and numbers of plasmablasts and activated B cells (Escudero-Pérez 2023). With regard to cellular immune responses, CD8+ T cell measurements may be useful as a CoP (Escudero-Pérez 2023). Additional research is needed to better define these potential CoPs, which may be particularly important for next-generation vaccines.
- Most current NiV vaccine candidates target the immunodominant fusion (F) and attachment (G) glycoproteins, which elicit potent neutralizing antibody responses (Byrne 2023, Geisbert 2021, Ithinji 2022, Loomis 2020, Loomis 2021, van Doremalen 2022, Wang 2022, Woolsey 2023). Data are needed on the potential role of additional immunogens, such as nucleoproteins and other non-enveloped proteins, in stimulating B and T cell responses that contribute to viral clearance, cross-protection, or immune memory. Robust vaccine-induced humoral and cell-mediated immunity to NiV might include protective antibodies with durable immunologic memory and rapid and efficient effector functions (Escudero-Pérez 2023).
- Additional immunologic research is needed to assess the following key elements of protective immunity against NiV infection and disease:
  - The relative contributions of innate, cell-mediated, and humoral immune responses that lead to protective immunity against NiV.
  - Specific cell types and interactions between different immune compartments in achieving viral clearance, surviving acute disease, and modulating chronic infection (Escudero-Pérez 2023, Liew 2022).
  - The roles of neutralizing and non-neutralizing or binding antibodies in protection against NiV (Liew 2022).
  - Mechanisms and cell subsets of cellular immune responses (e.g., CD8 T cell activation) that play a role in cross-neutralizing (heterologous) protection against co-circulating NiV strains (e.g., NiV-M, NiV-B, and NiV-I) (Amaya 2020, Arunkumar 2019, Escudero-Pérez 2023, Liew 2022).
  - If researchers and regulators agree that a nontraditional regulatory pathway is appropriate for licensure of NiV vaccines, then a number of issues need to be addressed, such as the following (Price 2021):
    - Generate a fully characterized and controlled virus challenge stock (or potentially one each for NiV-M and NiV-B) for assessing candidate vaccines in animal models.

  - Further characterize, as needed, at least one animal model suitable for vaccine efficacy evaluation.
  - Standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development. Challenge strains used in experimental research also need to be compared against any circulating strains in humans (CEPI 2023a).
  - Determine the most appropriate CoP or surrogate marker (e.g., IgG neutralizing antibodies) for measuring protection and generate standardized assays for measurement.
  - Bridge NiV vaccine efficacy data from animal models to humans, including identifying thresholds of vaccine protection, to determine appropriate human vaccine doses.
  - If the accelerated approval process is deemed an acceptable regulatory approval pathway for NiV vaccines, then plans will be needed to conduct well-controlled clinical trials to establish that vaccines have an effect on an appropriate surrogate endpoint that is likely to predict clinical benefit against NiVD.
  - Post-licensure clinical trials will also be needed to confirm the clinical benefit of any NiV vaccines that are approved via nontraditional pathways.
- Additional research is also needed to address the following areas:
  - Clarification of vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
  - Alternative vaccine delivery approaches, such as oral tablets or transdermal patches, to facilitate rapid NiV vaccine deployment in response to NiV outbreaks in low-resource settings.
  - Further evaluation of optimal NiV antigen combinations (e.g., including stabilized prefusion F protein trimers, multimeric G constructs, and chimeric proteins containing both pre-F and G glycoproteins), and antigen/vaccine platform combinations for generating rapid and durable protective responses to NiV infection (Byrne 2023, Loomis 2020, Loomis 2021, Srivastava 2023).
  - Research in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- Mathematical modelling and forecasting may be useful in (Nikolay 2021): (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- Researchers should consider efforts toward developing pan-henipavirus vaccines to maximize potential benefit, similar to projects aimed at developing pan-coronavirus

vaccines or universal influenza vaccines (Tan 2023). One strategy for developing broadly protective henipavirus vaccines involves identifying conserved epitopes or cross-reactive antibodies targeting Henipavirus F proteins (Byrne 2023, Ithinji 2022). Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.

- Once vaccines are available, the following will be needed:
  - Guidance on the use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
  - Enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.
  - Strategic planning for stockpiling and deploying NiV vaccines.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Develop the tools and policies necessary for evaluating and potentially approving one or more NiV candidate vaccines through a nontraditional regulatory pathway.

1. By 2024, generate a fully characterized and controlled virus challenge stock (or potentially one each for NiV-M and NiV-B) for assessing candidate vaccines in animal models.
2. By 2024, establish benchmark parameters (e.g., route of challenge, timing of challenge, and challenge dose) for testing of NiV candidate vaccines in animal models, with particular focus toward meeting criteria necessary for approval via a nontraditional pathway.
3. By 2024, further characterize as needed at least one animal model suitable for determining surrogate markers that correlate with vaccine efficacy.
4. By 2025, define the protective threshold against NiV infection for serum neutralizing IgG antibodies (as a CoP or surrogate marker), which can be used in animal studies and for immunobridging to humans.
5. By 2025, convene a group of key stakeholders to agree on, through a consensus approach, the most suitable assays (e.g., assays that are developed using live NiV vs. assays developed using pseudoviruses) for determining serum neutralizing IgG antibody titers for NiV vaccine R&D and develop a strategy for standardizing those assays.
6. By 2026, generate standardized assays to measure neutralizing IgG antibodies for NiV vaccine R&D, with a particular focus on assays to be used for regulatory approval via nontraditional pathways.

Strategic Goal 2: Continue to move the current NiV vaccine pipeline forward toward licensure.

Milestones:

1. By 2024, complete current phase 1 clinical trials for at least three promising NiV candidate vaccines.
2. By 2024, define use cases for NiV vaccines to inform vaccine deployment and manufacturing plans.
3. By 2026, further assess vaccine safety and immunogenicity through additional phase 1 and initial phase 2 clinical trials (preferably in affect areas) for at least two of the most promising NiV candidate vaccines.
4. By 2027, complete immunogenicity and efficacy studies in a well-characterized animal model for at least one cross-protective NiV vaccine candidate and define a surrogate marker that demonstrates likely clinical benefit of candidate vaccines.
5. By 2029, conduct well-controlled clinical trials (preferably in affected areas) to assess a surrogate endpoint in human subjects for at least one NiV candidate vaccine.
6. By 2030, complete a regulatory dossier for at least one NiV vaccine candidate based on a suitable animal model with subsequent immunobridging to humans for review via a nontraditional approval pathway.

Additional Priority Areas/Activities

Research

- **Improve understanding** of humoral (e.g., NiV-specific IgG and IgM antibodies) and cellular (e.g., CD8 T cell) immune responses to NiV infection in animal models to inform the design of vaccines and the identification of correlates of protection (Arunkumar 2019, Escudero-Pérez 2023).
- **Improve understanding** of innate immune responses (e.g., involving interferon impairment and pro-inflammatory cytokine release) in relation to humoral and cellular immune responses to NiV infection (Escudero-Pérez 2023).
- **Continue research** on identifying the key NiV antigens (including surface glycoproteins and internal proteins) that modulate the host immune response to NiV infection to inform future vaccine design (Escudero-Pérez 2023).
- **Continue to research** different types of CoPs (both humoral and cell-mediated) for NiV vaccines that are currently in the R&D pipeline and next-generation vaccines, taking into consideration different vaccine platforms, antigens, and clinical outcomes.
- **Generate** international reference standards to calibrate serologic assays for vaccine potency analyses.
- **Continue to conduct** preclinical evaluation of promising candidate NiV vaccines (current and future vaccines) for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

- **Explore** the possibility of creating pan-henipavirus vaccines that will protect against NiV, HeV, and other henipaviruses.

Product development

- **Continue to develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans.
- **Expand** partnerships among researchers, government agencies, and industry to provide the resources necessary for ongoing R&D of NiV vaccines.
- **Define** vaccine attributes (such as time from administration to immune protection, durability of protection, and the need for booster doses) for the most promising candidate vaccines.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Support** plans for adequate manufacturing and stockpiling of NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptance and promotion within the community.
- **Consider** developing a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for faster spread.

REFERENCES

Agrawal R, Murmu J, Pattnaik S, Kanungo S, Pati S. Bangladesh sees spike in Nipah virus cases: A matter of public health concern? New Microbes New Infect 2023 Mar 30;53:101119 [Full text]

Amaya M, Broder CC. Vaccines to emerging viruses: Nipah and Hendra. Annu Rev Virol 2020 Sep 29;7(1):447-473 [Full text]

Anderson DE, Islam A, Crameri G, et al. Isolation and full-genome characterization of Nipah viruses from bats, Bangladesh. Emerg Infect Dis 2019;25:166-70 [Full text]

Annand EJ, Horsburgh BA, Xu K, et al. Novel Hendra virus variant detected by sentinel surveillance of horses in Australia. Emerg Infect Dis 2022 Mar;28(3):693-704 [Full text]

Arnason G. The emergence and development of animal research ethics: A review with a focus on nonhuman primates. Sci Eng Ethics 2020 Aug;26(4):2277-2293 [Full text]

Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2019 May 24;219(12):1867-1878 [Full text]

Arunkumar G, Devadiga S, McElroy AK, et al. Adaptive immune responses in humans during Nipah virus acute and convalescent phases of infection. Clin Infect Dis 2019 Oct 30;69(10):1752-1756 [Full text]

Auro Vaccines LLC. Safety and immunogenicity of a Nipah virus vaccine. ClinicalTrials.gov Identifier: NCT04199169. First posted 2019 Dec 12; most recent update 2022 Nov 16 [Trial record]

Banerjee S, Niyas VKM, Soneja M, et al. First experience of ribavirin postexposure prophylaxis for Nipah virus, tried during the 2018 outbreak in Kerala, India. J Infect 2019;78:491-503 [PubMed]

Bassat Q, Varo R, Hurtado JC, et al. Minimally invasive tissue sampling as an alternative to complete diagnostic autopsies in the context of epidemic outbreaks and pandemics: The example of coronavirus disease 2019 (COVID-19). Clin Infect Dis 2021 Dec 15;73(Suppl_5):S472-S479 [Full text]

Berge C and Torheim E. Nipah@20: Nipah virus international conference. Conference proceedings. 2019 December 9 and 10 [Full text]

Borisevich V, Lee B, Hickey A, et al. Escape from monoclonal antibody neutralization affects henipavirus fitness in vitro and in vivo. J Infect Dis 2016 Feb 1;213(3):448-55 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua New Guinea. Emerg Infect Dis 2010;16:1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012;2:176-87 [Full text]

Bruno L, Nappo MA, Ferrari L, et al. Nipah virus disease: Epidemiological, clinical, diagnostic and legislative aspects of this unpredictable emerging zoonosis. Animals (Basel) 2022 Dec 31;13(1):159 [Full text]

Byrne PO, Fisher BE, Ambrozak DR, et al. Structural basis for antibody recognition of vulnerable epitopes on Nipah virus F protein. Nat Commun 2023 Mar 17;14(1):1494 [Full text]

CDC (US Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 6 Oct 2020 [Web page]

CEPI (Coalition for Epidemic Preparedness Innovations). CEPI-funded project to enhance scientific understanding of deadly Nipah virus strains. 19 June 2023 [News Release] CEPI 2023a

CEPI. Call for proposals: Clinical development of a Nipah monoclonal antibody (mAb) to protect against Nipah virus (NiV) disease. 6 June 2023 [Full text] CEPI 2023b

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2):235–240 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-283 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Full text]

Ching PKG, de los Reyes V, Sucaldito M, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015 Feb;21(2):328-31 [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. Malaysian J Pathol 2010; 32(2):69-73 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microb Infect 2013 Jun;2(6):e37 [Full text]

Dang HV, Cross RW, Borisevich V, et al. Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. Nat Struct Mol Biol 2021 May;28(5):426-434 [Full text]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8:7604 [Full text]

Daszak P, Olival KJ, Li H. A strategy to prevent future epidemics similar to the 2019-nCoV outbreak. Biosaf Health 2020 Mar;2(1):6-8 [Full text]

Deka MA, Morshed N. Mapping disease transmission risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2).pii:E57 [Full text]

de Wit E, Williamson BN, Feldmann F, et al. Late remdesivir treatment initiation partially protects African green monkeys from lethal Nipah virus infection. Antiviral Res 2023 Jun 23;216:105658 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Eby P, Peel AJ, Hoegh A, Madden W, Giles JR, Hudson PJ, Plowright RK. Pathogen spillover driven by rapid changes in bat ecology. Nature. 2023 Jan;613(7943):340-344 [Full text]

Epstein JH, Anthony SJ, Islam A, et al. Nipah virus dynamics in bats and implications for spillover to humans. Proc Natl Acad Sci USA 2020 Nov 17;117(46):29190-29201 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug;14(8):1309–1311 [Full text]

Escudero-Pérez B, Lawrence P, Castillo-Olivares J. Immune correlates of protection for SARS-CoV-2, Ebola and Nipah virus infection. Front Immunol 2023 Apr 17;14:1156758 [Full text]

Field H, Young P, Yob JM, et al. The natural history of Hendra and Nipah viruses. Microbes Infect 2001 Apr;3(4):307-14 [Abstract]

Foster SL, Woolsey C, Borisevich V, et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. Proc Natl Acad Sci USA 2022 Mar 22;119(12):e2200065119 [Full text]

Geisbert JB, Borisevich V, Prasad AN, et al. An intranasal exposure model of lethal Nipah virus infection in African green monkeys. J Infect Dis 2020 May 11;221(Suppl 4):S414-S418 [Full text]

Geisbert TW, Bobb K, Borisevich V, et al. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. NPJ Vaccines 2021 Feb 8;6(1):23 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010 May 18;5(5):e0010690 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-7 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med 2000;342:1229-35 [Full text]

Gómez Román R, Tornieporth N, Cherian NG, et al. Medical countermeasures against henipaviruses: a review and public health perspective. Lancet Infect Dis 2022 Jan;22(1):e13-e27 [Full text]

Gómez Román R, Wang LF, Lee B, et al. Nipah@20: Lessons learned from another virus with pandemic potential. mSphere 2020 Jul 8;5(4):e00602-20 [Full text]

Gouglas D, Thanh Le T, Henderson K, et al. Estimating the cost of vaccine development against epidemic infectious diseases: A cost minimisation study. Lancet Glob Health 2018 Dec;6(12):e1386-e1396 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Gurley ES, Spiropoulou CF, de Wit E. Twenty years of Nipah virus research: Where do we go from here? J Infect Dis 2020 May 11;221(Suppl 4):S359-S362 [Full text]

Hasebe F, Thuy NTT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012;18:536-7 [Full text]

Hedge S, Lee KH, Styczynski A, et al. Potential for person-to-person transmission of henipaviruses: A systematic review of the literature. Preprint on Medrxiv. doi: https://doi.org/10.1101/2023.02.26.23286473 [Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of Nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium). West Africa Lassa Fever Consortium. Accessed June 2023 [Webpage]

Islam A, Cannon DL, Rahman MZ,et al. Nipah virus exposure in domestic and peridomestic animals living in human outbreak sites, Bangladesh, 2013-2015. Emerg Infect Dis 2023 Feb;29(2):393-396 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Ithinji DG, Buchholz DW, Ezzatpour S, et al. Multivalent viral particles elicit safe and efficient immunoprotection against Nipah Hendra and Ebola viruses. NPJ Vaccines 2022 Dec 17;7(1):166 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2):e0117817 [Full text]

Lancet. Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137):2295 [Full text]

Li T, Liang Z, Huang W, Wang Y. Pseudotyped virus for Henipavirus. Adv Exp Med Biol 2023;1407:175-190 [Abstract]

Liew YJM, Ibrahim PAS, Ong HM, et al. The immunobiology of Nipah virus. Microorganisms 2022 Jun 6;10(6):1162 [Full text]

Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med 2019 May 29;11(494):eaau9242 [Full text]

Loomis RJ, DiPiazza AT, Falcone S, et al. Chimeric fusion (F) and attachment (G) glycoprotein antigen delivery by mRNA as a candidate Nipah vaccine. Front Immunol 2021 Dec 8;12:772864 [Full text]

Loomis RJ, Stewart-Jones GBE, Tsybovsky Y, et al. Structure-based design of Nipah virus vaccines: a generalizable approach to paramyxovirus immunogen development. Front Immunol 2020 Jun 11;11:842 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013 Oct;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug; 15(8): 1229–1235 [Full text]

Luo X, Wang C, Huang Y, Cong S, Tan J, Hou W, Ma F, Zheng L. Establishment of a neutralization assay for Nipah virus using a high-titer pseudovirus system. Biotechnol Lett 2023 Apr;45(4):489-498 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015 Mar;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-227 [Full text]

Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. BMJ Global Health 2019;4:e001118 [Full text]

McKee CD, Islam A, Rahman MZ, et al. Nipah virus detection at bat roosts after spillover events, Bangladesh, 2012-2019. Emerg Infect Dis 2022 Jul;28(7):1384-1392 [Full text]

McLean RK, Graham SP. Vaccine Development for Nipah Virus Infection in Pigs. Front Vet Sci 2019 Feb 4;6:16 [Full text]

Mire CE, Geisbert JB, Agans KN, et al. Use of single-injection recombinant vesicular stomatitis virus vaccine to protect nonhuman primates against lethal Nipah virus disease. Emerg Infect Dis 2019;25:1144-52 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;6:30916 [Full text]

Monath TP, Nichols R, Tussey L, et al. Recombinant vesicular stomatitis vaccine against Nipah virus has a favorable safety profile: Model for assessment of live vaccines with neurotropic potential. PLoS Pathog 2022 Jun 27;18(6):e1010658 [Full text]

Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. Science 1995 Apr 7;268(5207):94-7 [Abstract]

NIAID. Dose Escalation, Open-label clinical trial to evaluate safety, tolerability and immunogenicity of a Nipah virus (NiV) mRNA vaccine, mRNA-1215, in healthy adults. ClinicalTrials.gov Identifier: NCT05398796. First Posted 2022 May 31; most recent update 2023 Apr 15 [Trial record]

Nikolay B, Ribeiro Dos Santos G, et al. Assessing the feasibility of Nipah vaccine efficacy trials based on previous outbreaks in Bangladesh. Vaccine 2021 Sep 15;39(39):5600-5606 [Abstract]

Nikolay B, Salje H, Hossain MJ, et al. Transmission of Nipah virus — 14 years of investigations in Bangladesh. N Engl J Med 2019;380:1804-14 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Full text]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Peel AJ, Yinda CK, Annand EJ, et al. Novel Hendra virus variant circulating in black flying foxes and grey-headed flying foxes, Australia. Emerg Infect Dis 2022 May;28(5):1043-1047 [Full text]

Playford EG, Munro T, Mahler SM, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: A first-in-human, randomised, controlled, phase 1 study. Lancet Infect Dis 2020 Apr;20(4):445-454 [Abstract]

Plowright RK, Becker DJ, Crowley DE, et al. Prioritizing surveillance of Nipah virus in India. PloS Negl Trop Dis 2019 Jun 27;13(6):e0007393 [Full text]

Pollak NM, Olsson M, et al. Evaluation of three rapid low-resource molecular tests for Nipah virus. Front Microbiol 2023;13:1101914 [Full text]

Price A, Sizemore D, Hassell T, Gómez Román R, Holst J, Kristiansen P. Nipah virus assays and animal models for vaccine development. Landscape Analysis, January 2021. Coalition for Epidemic Preparedness Innovations [Full text]

Public Health Vaccines LLC. A phase 1 study to evaluate safety & immunogenicity of rVSV-Nipah virus vaccine candidate PHV02 in healthy adult subjects. ClinicalTrials.gov Identifier: NCT05178901. First Posted 2022 Jan 4; most recent update 2023 Mar 10 [Trial record]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Rahman MZ, Islam MM, Hossain ME, et al. Genetic diversity of Nipah virus in Bangladesh. Int J Infect Dis 2021 Jan;102:144-151 [Full text]

Rampling T, Page M, Horby P. International biological reference preparations for epidemic infectious diseases. Emerg Infect Dis 2019;25:205-11 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Full text]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-4 [Abstract]

Sayed A, Bottu A, Qaisar M, Mane MP, Acharya Y. Nipah virus: a narrative review of viral characteristics and epidemiological determinants. Public Health 2019;173:97-104 [Abstract]

Selvey LA, Wells RM, McCormack JG, et al. Infection of humans and horses by a newly described morbillivirus. Med J Aust 1995 Jun 19;162(12):642-5 [Abstract]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat Pteropus vampyrus in Sumatera, Indonesia PLoS One 2013; 8(7): e69544. Published online 2013 Jul 22 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Full text]

Singhai M, Jain R, Jain S, et al. Nipah virus: Recent perspective and One Health approach. Ann Glob Health 2021 Oct 12;87(1):102 [Full text]

Soman Pillai V, Krishna G, Valiya Veettil M. Nipah virus: Past outbreaks and future containment. Viruses 2020 Apr 20;12(4):465 [Full text]

Spiropoulou CF. Nipah virus outbreaks: Still small but extremely lethal. J Infect Dis 2019;219:1855-7 [Full text]

Srivastava S, Verma S, Kamthania M, et al. Exploring the structural basis to develop efficient multi-epitope vaccines displaying interaction with HLA and TAP and TLR3 molecules to prevent NIPAH infection, a global threat to human health. PLoS One 2023 Mar 15;18(3):e0282580 [Full text]

Sudeep AB, Yadav PD, Gokhale MD, et al. Detection of Nipah virus in Pteropus medius in 2019 outbreak from Ernakulam district, Kerala, India. BMC Infect Dis 2021 Feb 9;21(1):162 [Full text]

Tan CW, Valkenburg SA, Poon LLM, Wang LF. Broad-spectrum pan-genus and pan-family virus vaccines. Cell Host Microbe 2023 Jun 14;31(6):902-916 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Thakur N, Bailey D. Advances in diagnostics, vaccines and therapeutics for Nipah virus. Microbes Infect 2019;21(7):278-286 [Abstract]

van Doremalen N, Avanzato VA, Goldin K, et al. ChAdOx1 NiV vaccination protects against lethal Nipah Bangladesh virus infection in African green monkeys. NPJ Vaccines 2022 Dec 21;7(1):171 [Full text]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec; 11(12):1949–1951 [Full text]

Wang Z, Amaya M, Addetia A, et al. Architecture and antigenicity of the Nipah virus attachment glycoprotein. Science 2022 Mar 25;375(6587):1373-1378 [Abstract]

Wang LF, Daniels P. Diagnosis of henipavirus infection: Current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Wang X, Wise JC, Stewart AJ. Hendra Virus: An update on diagnosis, vaccination, and biosecurity protocols for horses. Vet Clin North Am Equine Pract 2023 Apr;39(1):89-98 [Abstract]

Watanabe S, Fukushi S, et al. Effective inactivation of Nipah virus in serum samples for safe processing in low-containment laboratories. Virol J 2020 Oct 9;17(1):151 [Full text]

Whitmer SLM, Lo MK, Sazzad HMS, et al. Inference of Nipah virus evolution, 1999-2015. Virus Evol 2020 Aug 19;7(1):veaa062 [Full text]

WHO (World Health Organization). Nipah Virus fact sheet. 2018 May 30 [Web page]

WHO. WHO R&D Blueprint. Accessed June 2023 [Webpage]

WHO. WHO R&D Blueprint: Priority diagnostics for Nipah use cases and target product profiles. Draft v0.3. 2019 [Full text]

Widerspick L, Vázquez CA, et al. Inactivation methods for experimental Nipah virus infection. Viruses 2022;14(5):1052 [Full text]

Woolsey C, Borisevich V, Fears AC, et al. Recombinant vesicular stomatitis virus-vectored vaccine induces long-lasting immunity against Nipah virus disease. J Clin Invest 2023 Feb 1;133(3):e164946 [Full text]

Yadav PD, Majumdar T, Gupta N, et al. Standardization & validation of Truenat™ point-of-care test for rapid diagnosis of Nipah. Indian J Med Res 2021 Apr;154(4):645-649 [Full text]

Yadav PD, Shete AM, Kumar GA, et al. Nipah virus sequences from humans and bats during Nipah Outbreak, Kerala, India, 2018. Emerg Infect Dis 2019 May;25(5):1003-1006 [Full text]

Yang S, Kar S. Are we ready to fight the Nipah virus pandemic? An overview of drug targets, current medications, and potential leads. Struct Chem 2023 Mar 8:1-19 [Full text]

Yang M, Zhu W, Truong T, et al. Detection of Nipah and Hendra viruses using recombinant human ephrin B2 capture virus in immunoassays. Viruses 2022 Jul 28;14(8):1657 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Nipah R&D Roadmap Taskforce Meeting
July 31 & August 1, 2023

Background

The Nipah Research and Development (R&D) Roadmap provides a 6-year framework beginning in 2024 for identifying the vision, underpinning strategic goals, and prioritizing areas and activities for accelerating the collaborative development of medical countermeasures – diagnostics, therapeutics, and vaccines – against Nipah virus infection. The roadmap is a key component of the World Health Organization's (WHO's) R&D Blueprint Initiative, and was first drafted in 2017/2018 with input from subject matter experts, including a core group of taskforce members. WHO now aims to finalize and formally launch the Nipah R&D Roadmap, and the draft has been updated to incorporate recent scientific advances and research. The roadmap will be delivered to WHO in late 2023 for publication on the WHO website.

Meeting Objectives

The meeting objective include the following:

1. Briefly recap each of the four topic areas of the Nipah R&D Roadmap (cross-cutting, diagnostics, therapeutics, and vaccines).
2. Review the goals and milestones in each section and develop consensus on the wording and timelines for each milestone, as time allows.
3. Identify the milestones or issues that are highest priority.
4. Discuss challenges and opportunities for implementing the roadmap goals and milestones.

Meeting Format

The meeting will be held in a hybrid format; participants will join either in person at Wellcome in London, UK, or virtually. Presentations and facilitated discussions will be used throughout the 1.5-day meeting.

Meeting Agenda

Agenda times listed in British Standard Time (BST)

Day 1: Monday, July 31

8:45 am *Registration, tea, coffee, and light breakfast*

Session 1: Welcome, Introductions, and Overview

9:15 am Welcome (Josie Golding, Wellcome)

9:20 am Introductions (Michael Osterholm, Center for Infectious Disease Research and Policy [CIDRAP])

9:30 am Update on the WHO Blueprint Initiative (Marie-Pierre Preziosi, WHO)

9:40 am Overview and meeting objectives (Kristine Moore, CIDRAP)

Session 2: Nipah Cross-Cutting Issues

Session Facilitator: Emily Gurley, Johns Hopkins Bloomberg School of Public Health

9:50 am General comments on Nipah cross-cutting issues in the current roadmap draft

- Key progress in the past 5 years
- Critical areas where additional efforts are needed

10:10 am Review the Nipah cross-cutting goals and milestones

10:30 am *Break*

10:45 am Review the Nipah cross-cutting goals and milestones (continued)

11:45 am Concluding comments for Nipah cross-cutting issues

- High-priority milestones
- Barriers to implementing the goals and milestones
- Ideas Roadmap implementation for this section

Session 3: Nipah Diagnostics

Session Facilitator: Joel Montgomery, US Centers for Disease Control and Prevention (US CDC)

12:05 pm General comments on Nipah diagnostics in the current roadmap draft

- Key progress in the past 5 years
- Critical areas where additional efforts are needed

12:30 pm *Lunch*

1:30 pm Review the Nipah diagnostics goals and milestones

2:50 pm *Break*

3:05 pm Concluding comments for Nipah diagnostics

- High-priority milestones
- Barriers to implementing the goals and milestones
- Ideas Roadmap implementation for this section

Session 4: Nipah Therapeutics

Session Facilitator: Emmie de Wit, US National Institute of Allergy and Infectious Diseases (US NIAID)

3:25 pm General comments on Nipah therapeutics in the current roadmap draft

- Key progress in the past 5 years
- Critical areas where additional efforts are needed

3:45 pm Review the Nipah therapeutics goals and milestones

5:10 pm Concluding comments for Nipah therapeutics

- High-priority milestones

  - Barriers to implementing the goals and milestones
  - Ideas Roadmap implementation for this section

5:30 pm *Adjourn Day 1*
Drinks and reception

6:00 pm Dinner

Day 2: Tuesday, August 1

8:45 am *Registration, tea, coffee, and light breakfast*

9:15 am Welcome for Day 2 (Michael Osterholm, CIDRAP)

Session 5: Nipah Vaccines

Session Facilitator: Pending

9:20 am General comments on Nipah vaccines in the current roadmap draft
  - Key progress in the past 5 years
  - Critical areas where additional efforts are needed

9:40 am Review the Nipah vaccines goals and milestones

10:15 am *Break*

10:30 am Review the Nipah vaccines goals and milestones (continued)

11:10 am Concluding comments for Nipah vaccines
  - High-priority milestones
  - Barriers to implementing the goals and milestones
  - Ideas Roadmap implementation for this section

Session 6: Nipah R&D Roadmap Implementation and Next Steps

11:30 am Discussion: Roadmap implementation (Marie-Pierre Preziosi, WHO)

12:15 pm Discussion: Publication process (Kristine Moore, CIDRAP)

12:25 pm Wrap up and next steps (Michael Osterholm, CIDRAP)

12:30 pm *Meeting close*

12:30 pm *Lunch*

From: Kristine Moore on behalf of Kristine Moore <kamoore@umn.edu>
To: christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; anfreibe@utmb.edu; egurley1@jhu.edu; kim.halpin@csiro.au; sluby@stanford.edu; ztq9@cdc.gov; dtmourya@gmail.com; shahana@icddrb.org; mahmudur57@gmail.com; ccs8@cdc.gov; linfa.wang@duke-nus.edu.sg; Laura.Mazzola@finddx.org; cathy.roth@fcdo.gov.uk; jo.mulligan@fcdo.gov.uk; zakiul.hassan@reuben.ox.ac.uk
Cc: benassiv@who.int; preziosim@who.int; formentyp@who.int; anaeliast@gmail.com; A.Cehovin@wellcome.org; P.Fay@wellcome.org; J.Golding@wellcome.org; s.mathias@wellcome.org; s.barman@wellcome.org; lackritz@umn.edu; mehr0035@umn.edu; kamoore@umn.edu; mto@umn.edu; oneil005@umn.edu; jto@umn.edu; ulric063@umn.edu; tabitha.leighton@gmail.com; amack5@me.com; amack5@icloud.com; "Nicolina Moua"
Subject: Revised version of the Nipah R&D roadmap and near-final agenda for the July 31 and August 1 meeting at Wellcome in London
Date: Thursday, July 13, 2023 2:55:19 PM
Attachments: Nipah RD Roadmap.Revision.July2023.ForTFReview.docx
Nipah_Agenda_NearFinal.Draft.docx

Dear Colleagues: Attached please find the revised version of the Nipah R&D Roadmap for your review. This version contains extensive updates from progress made over the past 5 years and includes many additional references from the recent literature.

Please review this version **in advance** of the Nipah taskforce meeting. In the interest of time, during the meeting **we will focus primarily on discussing the goals and milestones**. We would also like to spend some time discussing roadmap implementation.

Following the meeting, we will revise the roadmap and will send it out to taskforce members for your written feedback and comments.

We have also included a near-final agenda of the meeting for your information.

Thank you, again, for your involvement in this important effort. I look forward to seeing many of you either in-person or virtually at the end of the month.

Kind regards,

Kris

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A. BACKGROUND AND SIGNIFICANCE. Paramyxoviruses, nairoviruses, and arenaviruses are widely distributed RNA viruses that cause severe human syndromes. Because of their geographical distribution and expansion, changes in human demographics and increased exposures to wild animals including rodents and bats, and their capacity for human-to-human spread, these viruses have the potential to cause pandemics. Together with the other investigators in **PABVAX**, we have selected several important prototype paramyxoviruses, nairoviruses, and arenaviruses to study to learn the rules governing immunity to these pathogens. The work on the pathogens selected is justified based on their prevalence and severity alone, but also because of their ability to serve as prototypes for genetically related viruses. We chose the viruses for focus in this RP4 on human antibody development because of their clinical relevance and because several will be studied as prototypes for vaccine development in **RP1** (arenaviruses, nairoviruses) and **RP2** (paramyxoviruses). Among the arenaviruses, we will target Lassa virus (LASV) and Machupo virus (MACV), and among the nairoviruses, we will study Crimean–Congo hemorrhagic fever virus (CCHFV). In tandem with the vaccine project in **RP2** focused on paramyxoviruses, we will study Nipah virus (NiV) and Hendra virus (HeV) as the prototypes. Information about the principles we discover about critical neutralizing epitopes identified here in **RP4** with prototype viruses can feed back into all other **Projects** that address the subsequent plug and play test cases in later years (Lujo, Chapare, Kasokero, Langya).

LASV is an Old World arenavirus (OWAV) with a single-stranded RNA virus belonging to the Arenaviridae family. Infection in humans is often the result of exposure to infected excreta from the rodent reservoir, *Mastomys natalensis* where up to 500,000 cases and an estimated 5,000 deaths occur annually in West Africa[2]. While rodent infection is largely benign, human infection can result in severe hemorrhagic disease with neurological complications and long-term hearing and vision sequelae[3,4]. There are no approved vaccines or treatments for LASV infection. **MACV** is a New World Arenavirus (NWAV) and the causative agent of Bolivian Hemorrhagic fever. Humans are infected after exposure to excreta from the reservoir, *Calomys callosus,* the large vesper mouse. Disease in humans has a slow onset, but can progress to severe hemorrhagic fever coupled to insidious neurological complications including tremors, seizures, and paralysis[5]. There are no approved vaccines or therapies approved for MACV. **CCHFV** is a highly pathogenic tick-borne virus classified under the Nairovirus genus within the Bunyaviridae family[6]. CCHF outbreaks have a case fatality rate of up to 40%, and there are no vaccines or treatments approved for human use. **NiV/HeV.** Bat-borne, NiV and HeV are enveloped, negative-sense, single-stranded RNA viruses belonging to the Henipavirus genus within the Paramyxoviridae family[7]. These emerging zoonotic viruses pose a significant threat to human and animal health due to their high fatality rates and broad host range. As with the other prototype virus in the **PABAX** Center, there are no vaccines or treatments against henipaviruses licensed for human use.

Antibodies (Abs) are important correlates of protection for paramyxoviruses, nairoviruses, and arenaviruses. Immune sera against members of each of these groups of viruses has been shown to exhibit protective efficacy *in vivo*, which supports the use for Abs as treatment options. However, the specificity, functional properties, and other attributes of protective antibodies are poorly defined for most of these viruses. MAbs to the attachment and fusion surface proteins of paramyxoviruses (such as the G and F proteins of Hendra/Nipah[8,9] and the GP proteins of the arenaviruses like LASV[10,11]) can neutralize and protect against infection in rodent models. CCHFV is a bit of a conundrum in that ultrapotent human neutralizing antibodies to the related Rift Valley fever virus surface proteins are highly protective *in vivo*[12,13], but CCHFV neutralizing antibodies are not protective. In contrast, some non-neutralizing antibodies do protect partially for CCHFV[14]. Clearly, there is a lot to learn about the rules of immunity to these viruses, and the genetic, molecular, and structural basis of antibody-mediated protection for viruses like CCHFV. Based on these data, we propose the use of mAbs as treatment options to learn the rule of how to protect against emerging paramyxoviruses, nairoviruses, and arenaviruses and to develop candidate antibody countermeasures for translational development with **Core D**. To do so, we will further develop our technical methods for rapidly discovering mAbs, in the case of a future unexpected epidemic caused by viruses of these families. Most importantly, we will define the principles governing combination antibody therapy to prevent virus escape from treatment or prevention and to optimize the efficacy of the beneficial effects conferred by passive immunity.

B. SCIENTIFIC PREMISE. The work proposed here in **RP4** is based on the extensive basic, translational, and clinical trial data on the role of neutralizing Abs in protection against infection and disease caused by paramyxoviruses, nairoviruses, and arenaviruses. Although a significant amount of data supports the use of mAbs to prevent or control paramyxovirus, nairovirus, and arenavirus infection, many questions remain, such as the ideal epitopes to target with mAb combination therapy to improve upon neutralization potency, *in vivo* efficacy, and resistance to viral escape. Identifying these epitopes can help inform vaccine design to elicit optimal protective polyclonal responses (in **RP1** and **RP2**, especially with designs for test cases in years 4 and 5). Here we aim to refine our understanding of the correlates of mAb protection against paramyxoviruses, nairoviruses, and arenaviruses to enable the design of a modular "plug-and-play" workflow to rapidly respond to potential

pandemics by generating optimized combinations of vaccine-like mAbs with an extended half-life that could be used for long-term prophylaxis (> 6 months) and therapy.

C. INNOVATION. RP4 has many innovative conceptual and technical features including: Conceptual. **(1)** determining the principles that govern optimal combinations of human mAbs against paramyxoviruses, nairoviruses, and arenaviruses for protection and resistance to viral escape; **(2)** the development of a clear workflow that generates therapeutic or preventative antibodies (long half-life mAbs that serve as vaccine surrogates) against emerging or new paramyxoviruses, nairoviruses, and arenaviruses with pandemic potential. Our goal is to define the principles and features, including epitope, combination ratios, and mechanisms of action, that result in optimal efficacy of mAbs against paramyxoviruses, nairoviruses, and arenaviruses in animal models and create a higher barrier for resistance. These studies will serve as paradigms for rapid antibody discovery in response to future paramyxovirus, nairovirus, and arenavirus pandemic threats. Technical. **(3)** large-scale B cell repertoire sequencing with customized methods and software; (**4**) use of humanized (Alloy) mice and novel immunogens from **RP1** and **RP2** and **Core D**; **(5)** single B cell functional (neutralization) assays performed on a Beacon instrument; **(6)** development of high-throughput real-time label-free virus neutralization assays (xCELLigence platform); and **(7)** use of *simultaneous* in parallel screening with several different high-throughput virus-specific B cell isolation approaches (Beacon, 10X Genomics, Rhapsody) to generate and validate best-in-class human mAbs as candidate medical countermeasures.

D. LINKAGE TO OTHER PABVAX PROJECTS AND CORES. The group will focus on developing a modular test case platform for rapid selection of highly neutralizing and/or protective mAbs against paramyxoviruses, nairoviruses, and arenaviruses. Members of our group have active collaborations with most members of the **PABVAX Center** as reflected in previous collaborations and publications (see **Overall** document). We will interact extensively with the Animal Model and Preclinical Evaluation Core (**Core E)** to study neutralization against authentic viruses and to test for protection in small and large animals as well as with **RP3** for testing paramyxovirus mAbs. The knowledge gained from our studies on the structure-function relationships of neutralizing mAb combinations can be applied to rational vaccine design for **RP 1** and **RP2** in later years of the effort when we pivot to apply the lessons learned to the same test case targets as **RP1** and **RP2.** Numerous antigens will be made available to our RP. Finally, long-half-life Fc mutations are now available based on engineering of human IgG1 for enhanced binding to the neonatal Fc receptor (FcRn) such as LS (M428L /N434S)[15], or YTE (M252Y/S254T/T256E)[16] mutations. With ≥ 90 days half-life in humans, our approach will generate mAbs with vaccine-like properties that remain effective for months during a virus epidemic and thus provides a platform for response to future paramyxovirus, nairovirus, and arenavirus pandemic threats, a key goal of our **PABVAX Center.** *Our laboratory previously validated this vaccine surrogate concept when we isolated the human mAbs that formed the basis for Evusheld with YTE long-half-life Fc mutations (used in 70 countries and millions of people) that exhibited > 6 months protective levels in humans after single IM injections*.

E. APPROACH

E.1. Specific Aim 1. Discover potently neutralizing arenavirus mAb combination therapies. For the prototypes, we will focus on LASV, an Old-World virus and MACV, a New-World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[17,18]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV or MACV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.

Preliminary Data. ***(a) Potently neutralizing human anti-arenavirus mAbs.*** The Crowe laboratory has previously characterized panels of neutralizing human anti-viral human mAbs from virus-immune or vaccinated individuals for about 50 viruses, mostly RNA viruses. Isolated peripheral blood mononuclear cells (PBMCs) are used to generate hybridomas or alternatively antigen-sorted at single B cell level and antibody variable genes sequenced to generate mAbs recombinantly. Panels of human anti-viral mAbs are produced rapidly (we recently reported isolation of 15,000 mAb gene pairs from single Ebola-virus-specific human B cells from a single blood sample of an immune individual[19]. Previous studies by others clearly show that representative neutralizing and protective human antibodies can be isolated from LASV-immune subjects[20-24]. In this Aim, we will refine the approaches we have developed previously for other RNA viruses in a proof-of-concept study by identifying potently neutralizing combinations of anti-LASV and anti-MACV human mAbs. We will rapidly discover human anti-LASV mAbs from LASV-immune individuals and human anti-MACV mAbs by immunizing Alloy transgenic mice. Synergy studies will be performed to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify optimal anti-LASV and anti-MACV mAb combinations and further advance the discovery platform for rapid response against emerging arenaviruses.

(b) Advanced mAb technologies. The Crowe laboratory has developed some of the highest yield systems to isolate naturally occurring human mAbs using human hybridoma or advanced single-cell RNA-seq methods (**Fig 1**). We have used sorting of antigen-specific cells with recombinant viral surface protein antigens (like the GP1 and GP2 proteins we will use here), cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. One variation we will use is to capture Ab genes directly from viral glycoprotein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), as we have done for Ebola virus[19,25] and SARS-CoV-2[1,26-31]. These single-cell techniques enhance the capture of rare antigen-specific B cells.

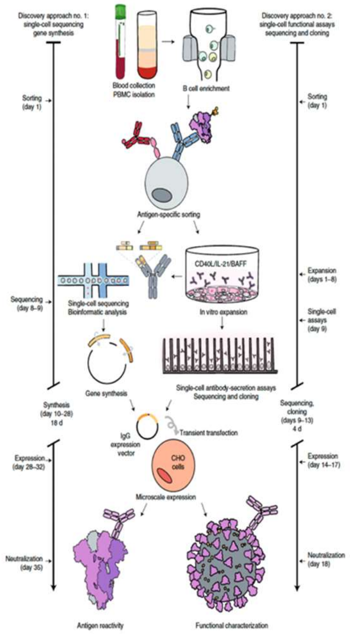

Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion allows us to further assess the functionality of the B cells or for antibody sequencing. Recombinant mAb expression validates the mAb sequence and functional activity. Figure is adapted from [1].

E.1.1. Rapid isolation of human anti-LASV and anti-MACV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from one previously LASV-infected individual who was medically managed previously by Emory University in their infectious diseases containment unit. This person has a documented robust neutralizing serum antibody response, suggesting isolation of neutralizing human mAbs from these PBMC aliquots will be straightforward for us. Our colleagues in **RP1** have ongoing field operations for over a decade in Sierra Leone, Liberia, and Nigeria. This work is currently in association with the NIH-Centers for Research on Emerging Infectious Diseases (CREID) with a heavy focus on the study of LASV ecology, epidemiology, and host responses. **RP4** is currently working with **RP1** on obtaining PBMCs from ~10 individuals with prior laboratory-confirmed cases of LASV. The acquisition of these samples is being managed by the Vanderbilt Vaccine Center Biorepository with separate institutional funding to be de-identified prior to use in the studies proposed here. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies have been approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples will be deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded. For anti-MACV mAbs, as a human PBMC source is not readily available, we will immunize Alloy humanized mice. These animals yield PBMCs that secrete fully human mAbs. Alloy mice are used routinely in the Crowe lab workflow.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-LASV and anti-MACV mAbs in a rapid manner to mimic a pandemic response to an outbreak. We will single-cell sort antigen-specific B cells using recombinant LASV or MACV proteins (*e.g.*, **Table 1)** or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including LASV and MACV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe GP protein or virus-particle binding to single B cells. These cells are exported for sequencing of the variable gene regions and analysis using the PyIR software developed in the Crowe laboratory. We will select a panel of up to 500 neutralizing anti-LASV mAbs and a similar number of anti-MACV mAbs to express recombinantly and advance for further characterization. We can easily conduct multiple approaches simultaneously in parallel[1,32].

Table 1. Antigens from Core D
Lassa Josiah NP
Lassa Josiah GPe
Machupo GPe
Machupo NP

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using a custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors.

(d) Micro-scale mAb expression. The Crowe laboratory has the capacity for large-throughput micro-scale mAb expression and purification. The lower end of this range allows expression of thousands of mAbs, which will feed into automated mAb purification platforms. This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based LASV and MACV neutralization assay using RTCA. We have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of cytopathic effect (CPE). Here, we will apply this method to assess neutralization of LASV. Recently, we validated the inclusion of simultaneous live-cell fluorescence microscopy into this real-time CPE-based screening platform (xCelligence RTCA eSight). These capabilities expand the platform to screen viruses that may or may not induce CPE, allowing us to also use a replication-competent vesicular stomatitis virus (VSV) LASV surrogate that expresses LASV surface proteins and causes CPE[33]. Currently, we have a VSV-LASV-Josiah (lineage IV)[33] and a Sauerwald version (lineage II) of these recombinants. Making additional LASV VSV-LASVs is not difficult, and **RP1** will make the other missing lineages or needed arenaviruses based on the same approaches as previously published. **RP1** also can easily make versions of these VSVs that express the green fluorescent protein (GFP) reporter gene. Existing VSV-CCHFV, VSV-NiV, and VSV-HeV vectors[34-36] will be provided by Dr. Geisbert overall PI and Lead of **Core E**. We already have Vanderbilt IBC approval for use of these constructs. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression if used will identify mAbs with neutralizing activity against LASV. These mAbs will be moved forward for additional studies to identify an optimal anti-LASV mAb combination. Similar processes will be followed to determine neutralization capacity of MACV mAb candidates.

E.1.2. Identifying LASV and MACV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind LASV or MACV and validate their epitopes. We will perform competition-binding studies with GP or subunit proteins (as above) or virus-like particles (VLPs)[37] available from **Core E** at UTMB. **Core E** will also inactivate LASV and MACV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from previously reported antibodies for the identification of mAbs that can concurrently bind LASV or MACV with a goal of finding more potent clones at those sites or clones that complement these inhibitory mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-LASV or anti-MACV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations for each virus, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key residues binding residues of human anti-LASV and anti-MACV mAbs.*** To determine the key residues for binding of down-selected mAbs, we will perform deep mutational scanning[38]. Briefly, all amino acids of the GP proteins will be mutated to the other 19 amino acids with a unique barcode using PCR-based mutagenesis. The LASV or MACV GP protein library will be incubated with the appropriate mAbs to identify residues with loss-of-binding phenotypes. A sequence 'logo' showing the relative contributions of each amino acid mutation will identify residues critical for binding. Multiple amino acid substitutions can then be assessed by reverse genetics at UTMB to confirm the epitopes recognized by the down-selected mAbs. This method also serves as a surrogate for *in vitro* neutralization escape studies. ***(d) Neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-LASV or VSV-MACV constructs at BSL2 in the presence of saturating concentrations of mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Confirmatory reverse genetics studies will be performed by introducing mutations into an infectious cDNA clone of LASV or MACV by **RP1** to test neutralization. We hypothesize that escape will be less likely in the presence of mAb combinations than single mAbs. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of escape variants in the presence of mAbs.

E.1.3. Structural basis of neutralization by mAb combinations. The studies above should identify optimal anti-LASV and anti-MACV mAb combinations that simultaneously bind, neutralize, and increase the barrier for viral escape under mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb

combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for arenavirus by identifying key epitope targets (**RP1**).

E.1.4. Protective efficacy of human anti-arenavirus mAbs. ***(a) Studies in guinea pigs***. MAbs that satisfy the criteria stated above and that display stage-appropriate manufacturability characteristics will advance to *in vivo* testing. We will use established models of LASV[11,39] and MACV[40,41] infection in guinea pigs. PABVAX **Core E** has access to many strains from different LASV and MACV lineages, which will be tested. For prioritization, we will focus on LASV lineage II, III, and IV strains and MACV lineage I, II, and VIII strains for which uniformly lethal models are available. Groups of 6 outbred Hartley guinea pigs will be treated with low, medium, and high doses of single or combinations of anti-LASV or anti-MACVs mAb, or isotype controls at day +1 after inoculation with the LASVs or MACVs This approach allows us to determine whether combinations provide advantage over the best monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-LASV and anti-MACV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements (blood) at day 5 or 6 after LASV or MACV challenge and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal LASV and MACV mAb pairs that are comparable to or outperform previously reported mAbs for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-LASV and anti-MACV mAb combination against LASV or MACV in NHPs. As a final test of an optimal anti-LASV and anti-MACV mAb combinations, we will assess protective efficacy of the lead anti-LASV mAb pair in NHPs as well as the anti-MACV mAb pair in NHPs. These anti-LASV and anti-MACV mAb pairs will be selected based on the *in vitro* and guinea pig data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys will only be considered pending success in the guinea pig studies and consultation with the external Scientific Advisory Board (SAB) and NIAID program staff.

E.1.5. Applying lessons learned in LASV and MACV research with the selected test case pathogens for mAb generation: Lujo and Chapare mAbs. Once we have completed the LASV and MACV discovery campaigns, will turn to the test case pathogens to see if we can use the prototype pathogen approach to related viruses, Lujo (LUJV) and Chapare (CHAPV). We do not have access to human PBMCs from LUJV - or CHAPV-immune donors. As an alternative approach, we will use humanized mouse models as a source of B cells encoding fully human monoclonal antibodies. The Vanderbilt core investigators have an established fully executed agreement to use Alloy human antibody mice for this purpose (See Letter of Support). The Alloy ATX-Gx™ mouse is one of the most effective *in vivo* human antibody discovery platforms, with over 140 partners and counting. This mouse system was originally invented and validated inside a major pharma company and then further developed by Alloy. This foundational suite of highly immunocompetent transgenic mice is engineered to drive the greatest potential diversity of unique human antibodies binding to the viral target of interest, with broad epitopic coverage. The comprehensive functional human antibody repertoire in these mice is optimized for human Ab sequence developability and diversity. Alloy and Vanderbilt have already executed a simple licensing process for use of the ATX-Gx platform, and as an established partner the Vanderbilt core team will access the expanding portfolio of transgenic Alloy strains designed to address a range of discovery challenges (including diverse Ab isotypes). Mice will be immunized with conformationally correct recombinant protein antigens (**Table 2**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for the target of interest. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for LASV and MACV.

Table 2. Antigens available from RP1
LUJV NP NTD
LUJV GPe
LUJV NP NTD +GPe
CHAPV NP NTD
CHAPV GPe
CHAPV NP NTD +GPe

<u>**Anticipated results, potential problems, and alternative approaches**</u>**.** In this Aim, we will identify human anti-LASV and anti-MACV mAb combinations with optimal neutralizing and protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging LASV, MACV, or related arenaviruses. Given our previous ability to generate human antiviral mAbs, we do not anticipate problems in isolating anti-LASV or anti-MACV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently neutralizing anti-LASV or anti-MACV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody™) on enriched B cells (*i.e.*, memory B cells) isolated from human LASV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in LASV-immune PBMC samples and identify clonal lineages, including

siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-LASV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-LASV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-LASV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-LASV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the high-throughput Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E2. Specific Aim 2. Discover a protective CCHF virus mAb combination therapy. Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[14,42,43]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E).** As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.

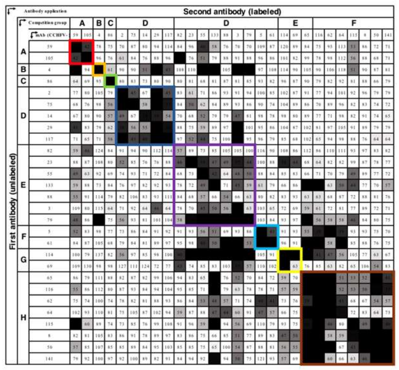

Figure 2. Competition assessment of human Abs to CCHFV using M-segment expressing cells. We tested 28 mAbs in competition assays. MAbs are displayed in 8 groups (A-H) based on their ability to compete for binding. Values shown are the % of binding that occurred during competition compared to non-competed binding of the mAb. This value was normalized to 100%. The values are also indicated by the box fill color; darker colors toward black indicate higher competition and lighter colors toward white indicate less competition, on a gradient scale.

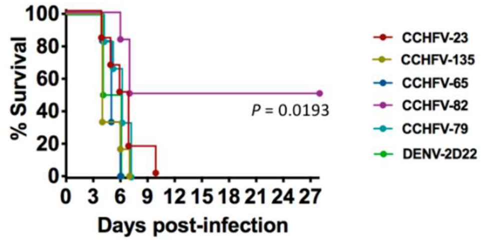

Figure 3. Human mAb CCHF-82 provides partial protection as a post-exposure prophylaxis against the Turkish strain of CCHFV in the STAT1$^{-/-}$ mouse model. A single dose of mAb was administered by the IP route to mice (n=6 per group (3 male, 3 female)) at 30 min post infections. A lethal dose of Turkish strain of CCHFV was administered. CCHF mAbs or DENV-2D22 (an isotype-matched negative control mAb) were tested in a single dose of 250 µg per mouse. Kaplan-Meier survival curves were statistically analyzed using a log-rank (Mantel-Cox) test where mAb-treated animals (*P* value shown in figure) were compared to animals treated with the DENV-2D22 negative control mAb. Unpublished data.

<u>Preliminary Data</u>. ***(a) Potently neutralizing and non-neutralizing human anti-CCHFV mAbs.*** In this Aim, we will rapidly discover human anti-CCHFV mAbs from CCHFV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify an optimal anti-CCHFV mAb combination and further advance the discovery platform for rapid response against emerging nairoviruses. In preliminary experiments in collaboration with investigators in **RP1** and **Core E**, the Crowe laboratory has previously isolated and characterized panels of neutralizing human anti-CCHFV human mAbs from individuals who were convalescent after naturally-acquired laboratory-confirmed infection in Spain or Turkey. We isolated a panel of antibodies against CCHFV that recognize the M-segment in transfected cells, and they exhibit a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 2**). This panel contains cross-binding and cross-neutralizing antibodies for diverse strains. We observed a human mAb designated CCHF-82 competing with competition group 1 and group 2 antibodies from the previously isolated GP38-reactive murine antibodies. CCHF-82 showed some protection in a STAT1$^{-/-}$ mouse model of infection conducted by investigators in **RP1 (Fig 3)**. Further investigation with this antibody in the IbAr10200 prophylaxis and therapeutic models is warranted. In contrast, the CCHFV-neutralizing mAbs that we isolated and tested *in vivo* did not perform well in the Turkish strain STAT1$^{-/-}$ mouse model. We now know protection in this model is possible, given the work from Fels *et al*., 2021 who showed that potent antibodies against various sites on the Gc surface can afford protection in this model[42]. However, in their study they did not test GP38-reactive antibodies. To our knowledge, our preliminary

data is the first indication that a human GP38-targeting antibody can provide some protection in this mouse model.

Non-neutralizing murine mAbs protect in mouse models of infection[14], but it is unknown if the human antibody response could provide similar levels of protection. Murine mAb 13G8 binds to GP38 and does not neutralize CCHFV. This mAb provides protection against lethal challenge as a prophylaxis and a therapeutic against CCHFV. In preliminary experiments we sought to understand if human survivors of CCHFV infection possess B cells encoding mAbs that bind a similar epitope as 13G8 and provide protection. CCHF-82 competes for binding with 13G8 to preGn-transfected cells containing GP38. This antibody offered some protection as a post-exposure prophylaxis against the Turkish strain of CCHFV (**Fig 3**). Human antibodies like this should be further studied and the frequency of these clones should be elucidated to understand if this is a feasible strategy for future vaccine efforts for humans. The epitope that 13G8 and CCHF-82 identifies could inform vaccine design if these antibodies offer protection in larger animal models. Understanding how non-neutralizing antibodies function is critical in efforts to validate the GP38-binding class of antibodies. Golden *et al.* undertook a study to understand the mechanisms 13G8 functioned and observed that complement activation contributes to the protective capacity of this antibody[14]. Here, in studies of CCHF-82, we also will assess complement activation in mouse models of infection to fully understand the function of this mAb *in vivo*. Furthermore, if this mAb protects in the CCHFV IbAr10200 strain challenge model, assessing its mechanism is warranted. Together with **Core D**, we will assess IgG molecules with complement knockout ("KA") and Fc gamma receptor knockout (LALA-PG) mutations in the Fc domain to study this matter. Human mAb CCHF-82 displayed limited cross-protection against experimental infection with the Turkish strain of CCHFV. Our antibody isolation here will aim to identify cross-protective non-neutralizing antibodies reactive with GP38. Recently, vaccinating with NP was shown to be another way to elicit non- neutralizing antibodies[44]. The NP of CCHFV is more conserved than the glycoproteins and might also be a non-canonical target for the identification of cross-protective non-neutralizing antibodies[44].

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant viral surface protein antigens, cloning of Ab variable genes, and then expression of mAbs as as described above in Specific Aim 1.

E.2.1. Rapid isolation of human anti-CCHFV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from a number of previously CCHFV-infected individuals. One was a health care survivor who was infected while caring for a patient in Spain[45]. We also collected a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Turkey, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples were only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies were approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples were deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-CCHFV mAbs rapidly, as if we had to respond to a nairovirus outbreak in real time. Our ability to respond rapidly in the setting of outbreaks is well-documented in the lay press (60 Minutes features and others) and the scientific literature, e.g., 78 days from sample to protection of NHPs for Zika virus[32], and 25 days from PBMC sample receipt to delivery of the antibody genes for the approved COVID-19 drug Evusheld to AstraZeneca[1]. Here, we will single-cell sort antigen-specific B cells using recombinant CCHFV proteins (GP38, Gc, Gn, Gc/Gn) or non-infectious particles as bait as described in Specific Aim 1. We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D**, (**Table 3**) or by **RP1 (Table 4)**.

Table 3. Core D CCHFV antigens
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF NP

Table 4. Antigens available from RP1
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF Turkey NP
CCHF Turkey GnGc+GP38+NP
CCHF Turkey GnGc+GP38
CCHF Turkey GP38+NP
CCHF Turkey GnGc+NP

(c) Recombinant mAb cloning. As described in Specific Aim 1 above.

(d) Micro-scale mAb expression. As described in Specific Aim 1 above.

(e) A CPE-based CCHFV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of CCHFV. We will use a rVSV CCHFV surrogate that expresses CCHFV surface proteins and causes CPE[35]. Investigators in **RP1** have the VSV-CCHFV-GFP versions of clades 1 through 7 based on this publication. We will obtain these VSV constructs from investigators in **PABVAX**. Existing VSV vectors will be provided by investigators in **RP1 and Core E**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against CCHFV. These mAbs will be moved forward for additional studies to identify an optimal anti-CCHFV mAb combination. GP38-reactive clones will be advanced based on avidity ranking since they are not likely to be neutralizing but may be protective *in vivo*.

E.2.2. Identifying CCHFV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind CCHFV surface proteins and validate their epitopes. We will perform competition-binding studies with CCHFV Gc/Gn or subunit proteins (as above) or CCHFV virus-like particles (VLPs) made from a replicon system in hand. **Core E** will inactivate CCHFV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site. Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from some of the mAbs shown in the unpublished preliminary data above for the identification of mAbs that can concurrently bind CCHFV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-CCHFV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. GP-38-reactive clones will be screened for synergistic binding as they are not expected to be neutralizing. ***(c) Identification of key binding residues of human anti-CCHFV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-CCHFV constructs at BSL2 in the presence of saturating concentrations of CCHFV mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of VSV-CCHFV escape variants in the presence of mAbs.

E.2.3. Structural basis of neutralization by anti-CCHFV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.2.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies in STAT1$^{-/-}$ mouse models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (Core D), will advance to *in vivo* testing. We will use an established STAT1$^{-/-}$ mouse model of CCHFV infection that the **RP1** and **Core E** investigators have deployed[35]. To benchmark our mAbs, a separate set of STAT1$^{-/-}$ mice will be treated with CCHF-82, which we showed in unpublished preliminary data above confers substantial but incomplete protection (**Fig 3**). The **Core E** laboratory has access to strains representing all 6 clades that can be tested. Groups of 6-8 STAT1$^{-/-}$ mice will be treated with low, medium, and high doses) of single or combinations of anti-CCHFV mAb, or isotype controls at day +1 after inoculation with CCHFVs. This approach allows us to determine whether combinations provide advantage over the best CCHFV monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after CCHFV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal CCHFV mAb pairs that are comparable to or outperform the mAbs we reported above. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-CCHFV mAb combination against CCHFV in NHPs. As a final test of an optimal anti-CCHFV mAb combination, we will assess efficacy of the lead anti-CCHFV mAb pair in NHPs (**Core E**), to be selected based on the *in vitro,* STAT1$^{-/-}$ mice, and manufacturability data (**Core D**). Down-selection for testing in cynomolgus monkeys would only be considered with success in the STAT1$^{-/-}$ mouse studies and in consultation with the external SAB and NIAID program staff.

E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs. Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human mAbs, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-CCHFV mAb combinations with optimal protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging CCHFV or related nairoviruses. Given our previous ability to generate human anti-CCHFV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-CCHFV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-CCHFV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (i.e., memory B cells) isolated from human CCHFV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in CCHFV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-CCHFV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-CCHFV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-CCHFV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-CCHFV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

Table 5. Antigens available from RP1
Kasokero GnGC
Kasokero GP38
Kasokero NP
Kasokero GnGc +GP38 +NP
Kasokero GnGc +GP38
Kasokero GnGc +NP
Kasokero GP38 +NP

E3. Specific Aim 3. Establish optimal mAb combination therapies against henipaviruses Hendra (HeV) and Nipah (NiV) viruses. We will use a large panel of potent anti-henipavirus mAbs that we previously made from the memory B cells of immune individuals and generate new antibodies targeting the fusion (F) protein. **RP2** will provide new antigens for mAb selection and BSL-2-compatible viruses for neutralization screens including Cedar virus constructs displaying HeV and NiV F and G proteins. We will identify optimal mAb combinations to enhance henipavirus therapy, minimize viral escape, and improve treatment efficacy through synergy. *In vitro* competition-binding, neutralization, and mechanistic studies will be conducted, including authentic virus neutralization with **PABVAX Core E**. We will refine the recognized epitopes and investigate the structural basis for optimal combination neutralization of henipaviruses using HeV and NiV as prototypes. *In vivo* assessment of mAb combinations will determine the minimal effective dose for protection and the ability to prevent *in vivo* escape. Protective efficacy of lead mAb combinations will be assessed in hamster models of NiV and HeV infection with **Core E**. Leads will be transferred to **Core D**. As a final test of optimal henipavirus mAb combinations, we will assess protective efficacy of the lead henipavirus mAb pairs in NHPs, to be selected based on the *in vitro* and hamster data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in African green monkey (AGM) henipavirus model studies would only be considered pending success in the hamster studies and consultation with the external SAB and NIAID program staff. The HeV and NiV studies will be conducted in years 1-3 and then applied to the plug-and-play test case Langya virus in years 2-5. If **RP2** and **Core E** cannot rescue recombinant Langya virus, the lessons learned in Years 1-3 will be applied to the alternate "plug and play" test viruses Angavokely or Sosuga (SOSV) virus. Preliminary data on SOSV mAbs are provided.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-NIV/HeV mAbs.*** In this Aim, we will further adapt approaches we have developed in preliminary experiments with henipavirus mAb studies by identifying a potently protective combination of anti-NiV/HeV human mAbs. Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups. This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains. In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and outstanding potency anti-G antibodies with extensive characterization, including synergistic neutralization.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant henipavirus F protein antigens, as described above in Specific Aim 1.

E.3.1. Rapid isolation of human anti-HeV/NiV mAbs. ***(a) Immune cells.*** We are obtaining human PBMCs from a number of previously NiV-infected individuals who acquired immunity following natural infection in collaboration with the **icddr,b**, an international health research institute based in Dhaka, Bangladesh. We are collecting a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Bangladesh, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies are approved by the icddr,b and Vanderbilt University Medical Center IRBs. PBMCs isolated from the blood samples are deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their use in this **RP4,** if funded. ***(b) Applying mAb technologies for new mAb isolation***. We will use sorting of antigen-specific B cells, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer antigen-specific B cells. We will sort soluble fusion (sF) or attachment (G) protein-specific cells with recombinant viral surface protein antigens from **Core D** (**Table 6**).

(c) Ab screening and sequence analysis. Our goal is to identify human anti-NiV/HeV mAbs rapidly, as if we had to respond to a paramyxovirus outbreak in real time. We will single-cell sort antigen-specific B cells using recombinant HeV and NiV proteins (sF or G) as bait as described in Specific Aim 1 above.

(d) Recombinant mAb cloning. As described in Specific Aim 1 above.

(e) Micro-scale mAb expression. As described in Specific Aim 1 above.

Table 6. Core D Henipavirus antigens
Nipah sF
Hendra sF
NiV G

(f) CPE-based HeV/NiV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of HeV and NiV. We will use rVSV HeV and NiV surrogates that expresses henipaviurs surface proteins and cause CPE. Investigators in **RP2 and RP3** have VSV-NiV F and VSV-NiV G with GFP[34], VSV-NiV G without GFP[36] and VSV-HeVG (Geisbert and Cross, unpublished). We will obtain these all these VSV constructs from investigators in **PABVAX**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against HeV and NiV. We also can use Cedar virus constructs for neutralization assays as the have reduced pathogenicity[46] and can be used in high-throughput screening assays for henipavirus antiviral discovery[47]. Cedar viruses will be provided by Dr. Broder of **RP2**. The mAbs with most potent activity will be moved forward for additional studies to identify an optimal anti-HeV/NiV mAb combination.

E.3.2. Identifying HeV/NiV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind HeV/NiV surface proteins and validate their epitopes. We will perform competition-binding studies with HeV/NiV sF or G proteins (as above). We will include positive controls made recombinantly from some of the mAbs shown in the preliminary data above for the identification of mAbs that can concurrently bind HeV/NiV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti- HeV/NiV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key binding residues of human anti-HeV/NiV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-NiV F, VSV-NiV G and VSV-HeV G constructs at BSL-2 in the presence of saturating concentrations of HeV/NiV mAbs (single or combination) using RTCA to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test the level of infection of VSV-HeV/NiV escape variants in the presence of mAbs.

E.3.3. Structural basis of neutralization by anti-HeV/NiV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.3.4. Protective efficacy of human anti-henipavirus mAbs. ***(a) Studies in hamsters***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (**Core D**), will advance to *in vivo* testing. We will use an established hamster model of NiV or HeV infection that the **Core E** investigators have deployed. To

benchmark our mAbs, a separate set of hamsters will be treated with G mAbs HENV-103+HENV-117, which we showed in previous data above confers substantial protection[8]. Groups of 6-8 hamsters will be treated with low, medium, or high IgG doses) of single or combinations of anti-HeV/NiV mAbs, or isotype controls at day +1 after inoculation with HeV or NiV. This approach allows us to determine whether combinations provide advantage over the best HeV or NiV mAb monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-HeV and anti-NiV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after HeV or NiV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal HeV/NiV mAb pairs that are comparable to or outperform the mAbs we reported previously. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-HeV/NiV mAb combination against HeV and NiV in NHPs. As a final test of an optimal anti-HeV/NiV mAb combination, we may assess efficacy of the lead anti-HeV/NiV mAb pair in NHPs (**Core E**), selected based on the *in vitro,* hamster data, and manufacturability data (**Core D**). Down-selection for testing in AGMs would only be considered with success in the hamster studies and in consultation with the external SAB and NIAID program staff.

E.3.5. Applying lessons learned in HeV/NiV research to Langya, Angavokely and Sosuga viruses. Once we have completed the HeV/NiV discovery campaigns, will turn to the test case pathogen Langya (or alternate Angavokely and Sosuga (SOSV) viruses) to see if we can use the prototype pathogen approach for this virus. We have plenty of human PBMCs from the sole identified SOSV-immune individual and have already shown that it is possible to obtain HN- and F-specific neutralizing antibodies from this individual's B cells[48] and **Fig 4**. We also have already prepared recombinant SOSV prefusion F and HN proteins[48]. We do not have access to human PBMCs from Langya- or Angavokely-immune donors. For those mAb discovery campaigns, we will use Alloy humanized mouse models as a source of B cells encoding human mAbs, as above. Mice will be immunized with recombinant protein antigens (**Table 7**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Langya or Angavokely. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above using single-cell RNAseq or human hybridoma methods. The development will follow as for Hev/NiV described above.

Table 7. Paramyxovirus antigens from Core D
Mojang sF
Angavokely sF
Langya sF

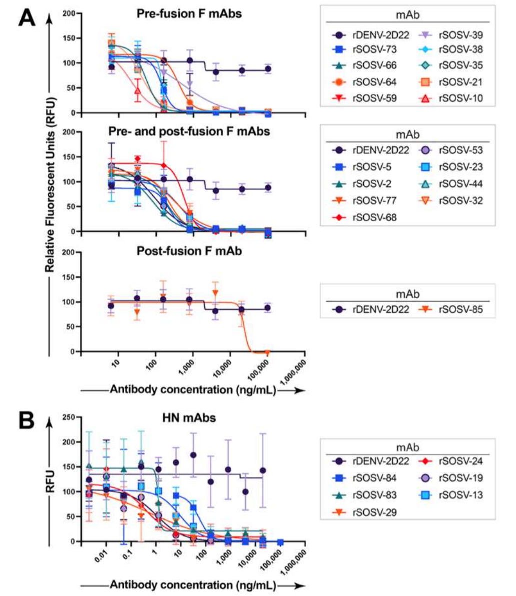

Figure 4. Neutralization assay of SOSV mAbs against live virus. SOSV mAbs were tested for inhibition of authentic rSOSV-ZsG in quadruplicate on Vero-E6 cell culture monolayers. (A) Neutralization data for anti-F mAbs. Data are grouped according to the pattern of antigen-reactivity: pre-fusion F, pre- and post-fusion F, or post-fusion F protein. (B) Neutralization data for the HN-specific mAbs.

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-HeV/NiV mAb combinations with optimal protective activity. These mAb combinations will be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging HeV/NiV or related paramyxoviruses. Given our previous ability to generate human anti- HeV/NiV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-HeV/NiV mAbs. We may observe that new mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-HeV/NiV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells *(i.e., memory B cells) isolated from human NiV-immune PBMC samples. In addition, we can deep sequence the B* cell repertoires present in NiV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-HeV/NiV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-HeV/NiV mAb combinations that can inform vaccine design (**RP2**) for related viruses used as test cases in years 2 through 5.

F. Scientific rigor. ***Experimental design.*** Experiments will include biological and technical replicates, and results will be analyzed by parametric or non-parametric analyses (depending on data distribution) and corrections for multiple comparisons. EC_{50} (binding) and IC_{50} (neutralization) values will be reported only following at least 3 identically conducted experiments. All mAb screening will incorporate appropriate + and - controls. Positive signals in binding assays will be defined as >3 SD above background. ***Protein quality.*** We have a high capacity to purify proteins using a Protein Maker workstation that allows purification chromatography on 24 parallel columns. We perform QC on protein reagents using a Maurice instrument with capillary electrophoresis technology to automate our protein profiling either by size or charge, providing cIEF and CE-SDS data. Animal ***studies*** will be performed blinded with independent replicates and include negative (*e.g.*, placebo), and positive (when available) controls. We will use power calculations (**Data Management Core B**) to determine sample sizes. For virological studies, power analysis was performed using accepted values for type I error (0.05) and power (80%). The values for population variance (15×) are based on existing data sets and our experience with rodent challenge studies. We will perform experiments at least 2-3 independent times (n = 4-5 each) to attain n = 12-15 per arm. Smaller group sizes can be used if differences are larger than expected.

G. Relevant biological variables. The human mAbs are derived from immune human donors or Alloy mice. Humans. Human Abs will be isolated for biological, structural, and functional studies at a clonal level. The sample size is not powered for large scale epidemiologic or clinical study conclusions. There is no evidence that sex, age, body mass index, or underlying health conditions other than immunodeficiency or immunosuppressive treatments alters the activity of cloned Abs in structure/function studies. The samples are de-identified without patient specific identifiers to protect the health information of the donors; thus, research with these samples is considered *Not Human Subjects Research* category activity. Mice, hamsters, guinea pigs, NHP, and viruses are also biological systems used in this application. Conventional animal housing conditions will be used because there is no evidence that altered room temperatures and light/dark cycles influence paramyxovirus, nairovirus, or arenavirus pathogenesis. Mice, hamsters, guinea pigs, and NHPs of both sexes will be used, and phenotypes will be monitored for sex-based differences. Virus stocks will be either low passage seed stocks from natural isolates or generated from existing cDNA clones and sequence-confirmed prior to use for infection studies.

H. Milestones and quantitative Go/No-Go criteria. Through **Aims 1-3**, we have proposed experiments that will advance our knowledge of the mAb-mediated mechanisms of action against paramyxoviruses, nairoviruses, and arenaviruses. In each Aim, we will use down-selection criteria (as below) to advance mAb combinations or variants for additional studies. By year 3, we will have defined the principles that govern optimal mAb combinations for paramyxoviruses, nairoviruses, and arenaviruses and the rational approaches needed to select mAbs for potency and breadth. We will use the following metrics to advance mAbs: ***(a) Neutralizing activity.*** All mAbs will have neutralizing potency < 10 ng/mL, EC_{50} value; ***(b) Binding affinity***. MAbs will be of high affinity to surface proteins or virions (K_D < 5 nM). ***(c) Protein stability and production quality.*** MAbs must produce at high levels without optimization (> 100 mg/L) and show biochemical/functional stability at 4°C or -80°C (>90% retention of binding and neutralization activity at 30 d). ***(d) Synergy***. Combinations of 2 mAbs against a given virus ideally will show synergy of neutralization *in vitro* or at a minimum, additive inhibition (no antagonism); ***(e) Epitopes***. To avoid resistance, mAbs in combination will bind 2 epitopes (determined by competition binding (<20% reduction in binding), mapping, and structural studies. ***(f) Resistance*** against mAbs will be assessed for virus escape. Deep sequencing of viruses that emerge from cells or animals treated with mAbs will inform prioritization. Combinations of mAbs with high genetic barriers to resistance (<10× loss in EC_{50}) will be advanced. ***(g) Protection in mice, hamsters, guinea pigs, or NHPs.*** The following metrics can be used: (i) Protection against lethal challenge by homologous virus (>90% survival; (ii) Protection against clinical disease by homologous virus (>80%); (iii) Protection against infection by homologous virus (>100-fold reduction in viral titers in multiple organs). ***(h) pK measurements***. For lead protective mAbs with mutations that should confer extended half-life, we will perform pK studies in NHPs (with **Core D** and **E**) to assess levels over time with a goal of a human predicted serum half-life of >6 months.

Timeline for RP4	Year 1	Year 2	Year 3		Year 4	Year 5
Prototype mAb generation and testing: **LASV and MACV mAbs**	X	X	X		X	
Test case mAb generation and testing: **Lujo and Chapare mAbs**			X		X	X
Prototype mAb generation and testing: **CCHFV mAbs**	X	X	X		X	
Test case mAb generation and testing: **Kasokero mAbs**			X		X	X
Prototype mAb generation and testing: **NiV/HeV mAbs**	X	X	X			
Test case mAb generation: **Langya, Angavokely/Sosuga mAbs**		X	X		X	X
In vivo efficacy studies with **Core E**	X	X	X		X	X
Translational activities with **Core D**	X	X	X		X	X

LITERATURE CITED

1. Zost, S.J., *et al.* Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* **26**, 1422-1427 (2020).
2. Fischer, W.A., II & Wohl, D.A. Moving Lassa Fever Research and Care Into the 21st Century. *The Journal of Infectious Diseases* **215**, 1779-1781 (2017).
3. Ezeomah, C., *et al.* Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. *Open Forum Infect Dis* **6**, ofz512 (2019).
4. Li, A.L., *et al.* Ophthalmic manifestations and vision impairment in Lassa fever survivors. *PloS one* **15**, e0243766 (2020).
5. Webb, P.A., Johnson, K.M., Mackenzie, R.B. & Kuns, M.L. Some characteristics of Machupo virus, causative agent of Bolivian hemorrhagic fever. *Am J Trop Med Hyg* **16**, 531-538 (1967).
6. Hawman, D.W. & Feldmann, H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology* (2023).
7. Mackenzie, J., Wang, L. & Broder, C.C. Henipaviruses. in *Fields Virology*, Vol. 2 1070-1085 (2013).
8. Doyle, M.P., *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021).
9. Dang, H.V., *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021).
10. Cross, R.W., *et al.* Antibody therapy for Lassa fever. *Curr Opin Virol* **37**, 97-104 (2019).
11. Cross, R.W., *et al.* Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral research* **133**, 218-222 (2016).
12. Cartwright, H.N., Barbeau, D.J. & McElroy, A.K. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere* **6**, e0055621 (2021).
13. Gutjahr, B., *et al.* Two monoclonal antibodies against glycoprotein Gn protect mice from Rift Valley Fever challenge by cooperative effects. *PLoS Negl Trop Dis* **14**, e0008143 (2020).
14. Golden, J.W., *et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv* **5**, eaaw9535 (2019).
15. Zalevsky, J., *et al.* Enhanced antibody half-life improves in vivo activity. *Nat Biotechnol* **28**, 157-159 (2010).
16. Dall'Acqua, W.F., *et al.* Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* **169**, 5171-5180 (2002).
17. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Levis, S.C. & Maiztegui, J.I. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* **2**, 255-256 (1984).
18. Jahrling, P.B. & Peters, C.J. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infection and immunity* **44**, 528-533 (1984).
19. Chen, E.C., *et al.* Systematic analysis of human antibody response to ebolavirus glycoprotein shows high prevalence of neutralizing public clonotypes. *Cell Rep* **42**, 112370 (2023).
20. Buck, T.K., *et al.* Neutralizing Antibodies against Lassa Virus Lineage I. *mBio* **13**, e0127822 (2022).
21. Enriquez, A.S., *et al.* Delineating the mechanism of anti-Lassa virus GPC-A neutralizing antibodies. *Cell Rep* **39**, 110841 (2022).
22. Hastie, K.M., *et al.* Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell* **178**, 1004-1015 e1014 (2019).
23. Hastie, K.M., *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).
24. Li, H., *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991 (2022).
25. Gilchuk, P., *et al.* Pan-ebolavirus protective therapy by two multifunctional human antibodies. *Cell* **184**, 5593-5607 e5518 (2021).
26. Chen, E.C., *et al.* Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* **36**, 109604 (2021).
27. Dong, J., *et al.* Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-1244 (2021).
28. Greaney, A.J., *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).

29. Kramer, K.J., *et al.* Potent neutralization of SARS-CoV-2 variants of concern by an antibody with an uncommon genetic signature and structural mode of spike recognition. *Cell Rep* **37**, 109784 (2021).
30. Suryadevara, N., *et al.* Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell* **184**, 2316-2331 e2315 (2021).
31. Zost, S.J., *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449 (2020).
32. Gilchuk, P., *et al.* Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics. *Nat Biomed Eng* **4**, 1030-1043 (2020).
33. Cross, R.W., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep* **40**, 111094 (2022).
34. Mire, C.E., *et al.* Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J* **10**, 353 (2013).
35. Rodriguez, S.E., *et al.* Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep* **9**, 7755 (2019).
36. Foster, S.L., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. *Proc Natl Acad Sci U S A* **119**, e2200065119 (2022).
37. Branco, L.M., *et al.* Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* **7**, 279 (2010).
38. Starr, T.N., *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
39. Deschambault, Y., *et al.* An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. *Viruses* **15**(2023).
40. Bell, T.M., *et al.* Pathogenesis of Bolivian Hemorrhagic Fever in Guinea Pigs. *Vet Pathol* **53**, 190-199 (2016).
41. Golden, J.W., *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* **7**, 4679 (2017).
42. Fels, J.M., *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell* **184**, 3486-3501 e3421 (2021).
43. Mishra, A.K., *et al.* Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies. *Science* **375**, 104-109 (2022).
44. Leventhal, S.S., *et al.* Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *EBioMedicine* **82**, 104188 (2022).
45. Negredo, A., *et al.* Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med* **377**, 154-161 (2017).
46. Laing, E.D., *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018).
47. Amaya, M., *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084 (2021).
48. Parrington, H.M., *et al.* Potently neutralizing human mAbs against the zoonotic pararubulavirus Sosuga virus. *JCI Insight* **8**(2023).

A. BACKGROUND AND SIGNIFICANCE. Paramyxoviruses, nairoviruses, and arenaviruses are widely distributed RNA viruses that cause severe human syndromes. Because of their geographical distribution and expansion, changes in human demographics and increased exposures to wild animals including rodents and bats, and their capacity for human-to-human spread, these viruses have the potential to cause pandemics. Together with the other investigators in **PABVAX**, we have selected several important prototype paramyxoviruses, nairoviruses, and arenaviruses to study to learn the rules governing immunity to these pathogens. The work on the pathogens selected is justified based on their prevalence and severity alone, but also because of their ability to serve as prototypes for genetically related viruses. We chose the viruses for focus in this RP4 on human antibody development because of their clinical relevance and because several will be studied as prototypes for vaccine development in **RP1** (arenaviruses, nairoviruses) and **RP2** (paramyxoviruses). Among the arenaviruses, we will target Lassa virus (LASV) and Machupo virus (MACV), and among the nairoviruses, we will study Crimean–Congo hemorrhagic fever virus (CCHFV). In tandem with the vaccine projects in **RP2** focused on paramyxoviruses, we will study Nipah virus (NiV) and Hendra virus (HeV) as the prototypes. Information about the principles we discover about critical neutralizing epitopes identified here in **RP4** with prototype viruses can feed back into all other **Projects** that address the subsequent plug and play test cases in later years (Lujo, Chapare, Kasokero, Langya).

LASV is an Old World arenavirus (OWAV) with a single-stranded RNA virus belonging to the Arenaviridae family. Infection in humans is often the result of exposure to infected excreta from the rodent reservoir, *Mastomys natalensis* where up to 500,000 cases and an estimated 5,000 deaths occur annually in West Africa[2]. While rodent infection is largely benign, human infection can result in severe hemorrhagic disease with neurological complications and long-term hearing and vision sequelae[3,4]. There are no approved vaccines or treatments for LASV infection. **MACV** is a New World Arenavirus (NWAV) and the causative agent of Bolivian Hemorrhagic fever. Humans are infected after exposure to excreta from the reservoir, *Calomys callosus,* the large vesper mouse. Disease in humans has a slow onset, but can progress to severe hemorrhagic fever coupled to insidious neurological complications including tremors, seizures, and paralysis[5]. There are no approved vaccines or therapies approved for MACV. **CCHFV** is a highly pathogenic tick-borne virus classified under the Nairovirus genus within the Bunyaviridae family[6]. CCHF outbreaks have a case fatality rate of up to 40%, and there are no vaccines or treatments approved for human use. **NiV/HeV.** Bat-borne, NiV and HeV are enveloped, negative-sense, single-stranded RNA viruses belonging to the Henipavirus genus within the Paramyxoviridae family[7]. These emerging zoonotic viruses pose a significant threat to human and animal health due to their high fatality rates and broad host range. As with the other prototype virus in the **PABAX** Center, there are no vaccines or treatments against henipaviruses licensed for human use.

Antibodies (Abs) are important correlates of protection for paramyxoviruses, nairoviruses, and arenaviruses. Immune sera against members of each of these groups of viruses has been shown to exhibit protective efficacy *in vivo*, which supports the use for Abs as treatment options. However, the specificity, functional properties, and other attributes of protective antibodies are poorly defined for most of these viruses. MAbs to the attachment and fusion surface proteins of paramyxoviruses (such as the G and F proteins of Hendra/Nipah[8,9] and the GP proteins of the arenaviruses like LASV[10,11]) can neutralize and protect against infection in rodent models. CCHFV is a bit of a conundrum in that ultrapotent human neutralizing antibodies to the related Rift Valley fever virus surface proteins are highly protective *in vivo*[12,13], but CCHFV neutralizing antibodies are not protective. In contrast, some non-neutralizing antibodies do protect partially for CCHFV[14]. Clearly, there is a lot to learn about the rules of immunity to these viruses, and the genetic, molecular, and structural basis of antibody-mediated protection for viruses like CCHFV. Based on these data, we propose the use of mAbs as treatment options to learn the rule of how to protect against emerging paramyxoviruses, nairoviruses, and arenaviruses and to develop candidate antibody countermeasures for translational development with **Core D**. To do so, we will further develop our technical methods for rapidly discovering mAbs, in the case of a future unexpected epidemic caused by viruses of these families. Most importantly, we will define the principles governing combination antibody therapy to prevent virus escape from treatment or prevention and to optimize the efficacy of the beneficial effects conferred by passive immunity.

B. SCIENTIFIC PREMISE. The work proposed here in **RP4** is based on the extensive basic, translational, and clinical trial data on the role of neutralizing Abs in protection against infection and disease caused by paramyxoviruses, nairoviruses, and arenaviruses. Although a significant amount of data supports the use of mAbs to prevent or control paramyxovirus, nairovirus, and arenavirus infection, many questions remain, such as the ideal epitopes to target with mAb combination therapy to improve upon neutralization potency, *in vivo* efficacy, and resistance to viral escape. Identifying these epitopes can help inform vaccine design to elicit optimal protective polyclonal responses (in **RP1** and **RP2**, especially with designs for test cases in years 4 and 5). Here we aim to refine our understanding of the correlates of mAb protection against paramyxoviruses, nairoviruses, and arenaviruses to enable the design of a modular “plug-and-play” workflow to rapidly respond to potential

pandemics by generating optimized combinations of vaccine-like mAbs with an extended half-life that could be used for long-term prophylaxis (> 6 months) and therapy.

C. INNOVATION. RP4 has many innovative conceptual and technical features including: Conceptual. **(1)** determining the principles that govern optimal combinations of human mAbs against paramyxoviruses, nairoviruses, and arenaviruses for protection and resistance to viral escape; **(2)** the development of a clear workflow that generates therapeutic or preventative antibodies (long half-life mAbs that serve as vaccine surrogates) against emerging or new paramyxoviruses, nairoviruses, and arenaviruses with pandemic potential. Our goal is to define the principles and features, including epitope, combination ratios, and mechanisms of action, that result in optimal efficacy of mAbs against paramyxoviruses, nairoviruses, and arenaviruses in animal models and create a higher barrier for resistance. These studies will serve as paradigms for rapid antibody discovery in response to future paramyxovirus, nairovirus, and arenavirus pandemic threats. Technical. **(3)** large-scale B cell repertoire sequencing with customized methods and software; (**4**) use of humanized (Alloy) mice and novel immunogens from **RP1** and **RP2** and **Core D**; **(5)** single B cell functional (neutralization) assays performed on a Beacon instrument; **(6)** development of high-throughput real-time label-free virus neutralization assays (xCELLigence platform); and **(7)** use of *simultaneous* in parallel screening with several different high-throughput virus-specific B cell isolation approaches (Beacon, 10X Genomics, Rhapsody) to generate and validate best-in-class human mAbs as candidate medical countermeasures.

D. LINKAGE TO OTHER PABVAX PROJECTS AND CORES. The group will focus on developing a modular test case platform for rapid selection of highly neutralizing and/or protective mAbs against paramyxoviruses, nairoviruses, and arenaviruses. Members of our group have active collaborations with most members of the **PABVAX Center** as reflected in previous collaborations and publications (see **Overall** document). We will interact extensively with the Animal Model and Preclinical Evaluation Core (**Core E)** to study neutralization against authentic viruses and to test for protection in small and large animals as well as with **RP3** for testing paramyxovirus mAbs. The knowledge gained from our studies on the structure-function relationships of neutralizing mAb combinations can be applied to rational vaccine design for **RP 1** and **RP2** in later years of the effort when we pivot to apply the lessons learned to the same test case targets as **RP1** and **RP2.** Numerous antigens will be made available to our RP. Finally, long-half-life Fc mutations are now available based on engineering of human IgG1 for enhanced binding to the neonatal Fc receptor (FcRn) such as LS (M428L /N434S)[15], or YTE (M252Y/S254T/T256E)[16] mutations. With ≥ 90 days half-life in humans, our approach will generate mAbs with vaccine-like properties that remain effective for months during a virus epidemic and thus provides a platform for response to future paramyxovirus, nairovirus, and arenavirus pandemic threats, a key goal of our **PABVAX Center.** *Our laboratory previously validated this vaccine surrogate concept when we isolated the human mAbs that formed the basis for Evusheld with YTE long-half-life Fc mutations (used in 70 countries and millions of people) that exhibited > 6 months protective levels in humans after single IM injections*.

E. APPROACH

E.1. Specific Aim 1. Discover potently neutralizing arenavirus combination therapies. For the prototypes, we will focus on LASV, an Old-World virus and MACV, a New-World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[17,18]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV or MACV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.

Preliminary Data. ***(a) Potently neutralizing human anti-LASV and anti-MACV mAbs.*** The Crowe laboratory has previously characterized panels of neutralizing human anti-viral human mAbs from virus-immune or vaccinated individuals for about 50 viruses, mostly RNA viruses. Isolated peripheral blood mononuclear cells (PBMCs) are used to generate hybridomas or alternatively antigen-sorted at single B cell level and antibody variable genes sequenced to generate mAbs recombinantly. Panels of human anti-viral mAbs are produced rapidly (we recently reported isolation of 15,000 mAb gene pairs from single Ebola-virus-specific human B cells from a single blood sample of an immune individual[19]. Previous studies by others clearly show that representative neutralizing and protective human antibodies can be isolated from LASV-immune subjects[20-24]. In this Aim, we will refine the approaches we have developed previously for other RNA viruses in a proof-of-concept study by identifying potently neutralizing combinations of anti-LASV and anti-MACV human mAbs. We will rapidly discover human anti-LASV mAbs from LASV-immune individuals and human anti-MACV mAbs by immunizing Alloy transgenic mice. Synergy studies will be performed to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify optimal anti-LASV and anti-MACV mAb combinations and further advance the discovery platform for rapid response against emerging arenaviruses.

(b) Advanced mAb technologies. The Crowe laboratory has developed some of the highest yield systems to isolate naturally occurring human mAbs using human hybridoma or advanced single-cell RNA-seq methods (**Fig 1**). We have used sorting of antigen-specific cells with recombinant viral surface protein antigens (like the GP1 and GP2 proteins we will use here), cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. One variation we will use is to capture Ab genes directly from viral glycoprotein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), as we have done for Ebola virus[19,25] and SARS-CoV-2[1,26-31]. These single-cell techniques enhance the capture of rare antigen-specific B cells.

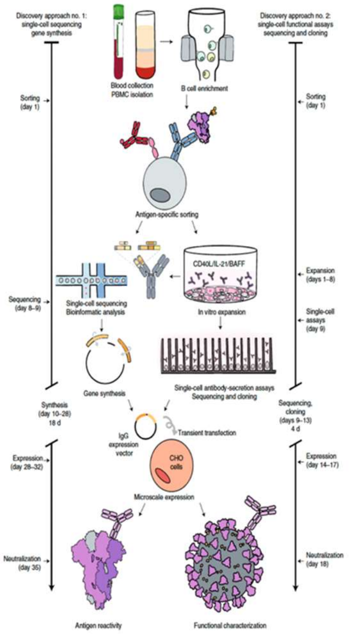

Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion allows us to further assess the functionality of the B cells or for antibody sequencing. Recombinant mAb expression validates the mAb sequence and functional activity. Figure is adapted from [1].

E.1.1. Rapid isolation of human anti-LASV and anti-MACV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from one previously LASV-infected individual who was medically managed previously by Emory University in their infectious diseases containment unit. This person has a documented robust neutralizing serum antibody response, suggesting isolation of neutralizing human mAbs from these PBMC aliquots will be straightforward for us. Our colleagues in **RP1** have ongoing field operations for over a decade in Sierra Leone, Liberia, and Nigeria. This work is currently in association with the NIH-Centers for Research on Emerging Infectious Diseases (CREID) with a heavy focus on the study of LASV ecology, epidemiology, and host responses. **RP4** is currently working with **RP1** on obtaining PBMCs from ~10 individuals with prior laboratory-confirmed cases of LASV. The acquisition of these samples is being managed by the Vanderbilt Vaccine Center Biorepository with separate institutional funding to be de-identified prior to use in the studies proposed here. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies have been approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples will be deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded. For anti-MACV mAbs, as a human PBMC source is not readily available, we will immunize Alloy humanized mice. These animals yield PBMCs that secrete fully human mAbs. Alloy mice are used routinely in the Crowe lab workflow.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-LASV and anti-MACV mAbs in a rapid manner to mimic a pandemic response to an outbreak. We will single-cell sort antigen-specific B cells using recombinant LASV or MACV proteins (*e.g.*, **Table 1)** or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including LASV and MACV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe GP protein or virus-particle binding to single B cells. These cells are exported for sequencing of the variable gene regions and analysis using the PyIR software developed in the Crowe laboratory. We will select a panel of up to 500 neutralizing anti-LASV mAbs and a similar number of anti-MACV mAbs to express recombinantly and advance for further characterization. We can easily conduct multiple approaches simultaneously in parallel[1,32].

Table 1. Antigens from Core D
Lassa Josiah NP
Lassa Josiah GPe
Machupo GPe
Machupo NP

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using a custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors.

(d) Micro-scale mAb expression. The Crowe laboratory has the capacity for large-throughput micro-scale mAb expression and purification. The lower end of this range allows expression of thousands of mAbs, which will feed into automated mAb purification platforms. This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based LASV and MACV neutralization assay using RTCA. We have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of cytopathic effect (CPE). Here, we will apply this method to assess neutralization of LASV. Recently, we validated the inclusion of simultaneous live-cell fluorescence microscopy into this real-time CPE-based screening platform (xCelligence RTCA eSight). These capabilities expand the platform to screen viruses that may or may not induce CPE, allowing us to also use a replication-competent vesicular stomatitis virus (VSV) LASV surrogate that expresses LASV surface proteins and causes CPE[33]. Currently, we have a VSV-LASV-Josiah (lineage IV)[33] and a Sauerwald version (lineage II) of these recombinants. Making additional LASV VSV-LASVs is not difficult, and **RP1** will make the other missing lineages or needed arenaviruses based on the same approaches as previously published. **RP1** also can easily make versions of these VSVs that express the green fluorescent protein (GFP) reporter gene. Existing VSV-CCHFV, VSV-NiV, and VSV-HeV vectors[34-36] will be provided by Dr. Geisbert overall PI and Lead of **Core E**. We already have Vanderbilt IBC approval for use of these constructs. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression if used will identify mAbs with neutralizing activity against LASV. These mAbs will be moved forward for additional studies to identify an optimal anti-LASV mAb combination. Similar processes will be followed to determine neutralization capacity of MACV mAb candidates.

E.1.2. Identifying LASV and MACV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind LASV or MACV and validate their epitopes. We will perform competition-binding studies with GP or subunit proteins (as above) or virus-like particles (VLPs)[37] available from **Core E** at UTMB. **Core E** will also inactivate LASV and MACV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from previously reported antibodies for the identification of mAbs that can concurrently bind LASV or MACV with a goal of finding more potent clones at those sites or clones that complement these inhibitory mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-LASV or anti-MACV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations for each virus, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key residues binding residues of human anti-LASV and anti-MACV mAbs.*** To determine the key residues for binding of down-selected mAbs, we will perform deep mutational scanning[38]. Briefly, all amino acids of the GP proteins will be mutated to the other 19 amino acids with a unique barcode using PCR-based mutagenesis. The LASV or MACV GP protein library will be incubated with the appropriate mAbs to identify residues with loss-of-binding phenotypes. A sequence ‘logo’ showing the relative contributions of each amino acid mutation will identify residues critical for binding. Multiple amino acid substitutions can then be assessed by reverse genetics at UTMB to confirm the epitopes recognized by the down-selected mAbs. This method also serves as a surrogate for *in vitro* neutralization escape studies. ***(d) Neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-LASV or VSV-MACV constructs at BSL2 in the presence of saturating concentrations of mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Confirmatory reverse genetics studies will be performed by introducing mutations into an infectious cDNA clone of LASV or MACV by **RP1** to test neutralization. We hypothesize that escape will be less likely in the presence of mAb combinations than single mAbs. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of escape variants in the presence of mAbs.

E.1.3. Structural basis of neutralization by mAb combinations. The studies above should identify optimal anti-LASV and anti-MACV mAb combinations that simultaneously bind, neutralize, and increase the barrier for viral escape under mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb

combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for arenavirus by identifying key epitope targets (**RP1**).

E.1.4. Protective efficacy of human anti-arenavirus mAbs. ***(a) Studies in guinea pigs***. MAbs that satisfy the criteria stated above and that display stage-appropriate manufacturability characteristics will advance to *in vivo* testing. We will use established models of LASV[11,39] and MACV[40,41] infection in guinea pigs. PABVAX **Core E** has access to many strains from different LASV and MACV lineages, which will be tested. For prioritization, we will focus on LASV lineage II, III, and IV strains and MACV lineage I, II, and VIII strains for which uniformly lethal models are available. Groups of 6 outbred Hartley guinea pigs will be treated with low, medium, and high doses of single or combinations of anti-LASV or anti-MACVs mAb, or isotype controls at day +1 after inoculation with the LASVs or MACVs This approach allows us to determine whether combinations provide advantage over the best monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-LASV and anti-MACV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements (blood) at day 5 or 6 after LASV or MACV challenge and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal LASV and MACV mAb pairs that are comparable to or outperform previously reported mAbs for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-LASV and anti-MACV mAb combination against LASV or MACV in NHPs. As a final test of an optimal anti-LASV and anti-MACV mAb combinations, we will assess protective efficacy of the lead anti-LASV mAb pair in NHPs as well as the anti-MACV mAb pair in NHPs. These anti-LASV and anti-MACV mAb pairs will be selected based on the *in vitro* and guinea pig data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys will only be considered pending success in the guinea pig studies and consultation with the external Scientific Advisory Board (SAB) and NIAID program staff.

E.1.5. Applying lessons learned in LASV and MACV research with the selected test case pathogens for mAb generation: Lujo and Chapare mAbs. Once we have completed the LASV and MACV discovery campaigns, will turn to the test case pathogens to see if we can use the prototype pathogen approach to related viruses, Lujo (LUJV) and Chapare (CHAPV). We do not have access to human PBMCs from LUJV - or CHAPV-immune donors. As an alternative approach, we will use humanized mouse models as a source of B cells encoding fully human monoclonal antibodies. The Vanderbilt core investigators have an established fully executed agreement to use Alloy human antibody mice for this purpose (See Letter of Support). The Alloy ATX-Gx™ mouse is one of the most effective *in vivo* human antibody discovery platforms, with over 140 partners and counting. This mouse system was originally invented and validated inside a major pharma company and then further developed by Alloy. This foundational suite of highly immunocompetent transgenic mice is engineered to drive the greatest potential diversity of unique human antibodies binding to the viral target of interest, with broad epitopic coverage. The comprehensive functional human antibody repertoire in these mice is optimized for human Ab sequence developability and diversity. Alloy and Vanderbilt have already executed a simple licensing process for use of the ATX-Gx platform, and as an established partner the Vanderbilt core team will access the expanding portfolio of transgenic Alloy strains designed to address a range of discovery challenges (including diverse Ab isotypes). Mice will be immunized with conformationally correct recombinant protein antigens (**Table 2**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for the target of interest. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for LASV and MACV.

Table 2. Antigens available from RP1
LUJV NP NTD
LUJV GPe
LUJV NP NTD +GPe
CHAPV NP NTD
CHAPV GPe
CHAPV NP NTD +GPe

<u>**Anticipated results, potential problems, and alternative approaches**</u>**.** In this Aim, we will identify human anti-LASV and anti-MACV mAb combinations with optimal neutralizing and protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging LASV, MACV, or related arenaviruses. Given our previous ability to generate human antiviral mAbs, we do not anticipate problems in isolating anti-LASV or anti-MACV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently neutralizing anti-LASV or anti-MACV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody™) on enriched B cells (*i.e.*, memory B cells) isolated from human LASV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in LASV-immune PBMC samples and identify clonal lineages, including

siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-LASV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-LASV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-LASV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-LASV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the high-throughput Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E2. Specific Aim 2. Discover a protective CCHF virus mAb combination therapy. Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[14,42,43]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E).** As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.

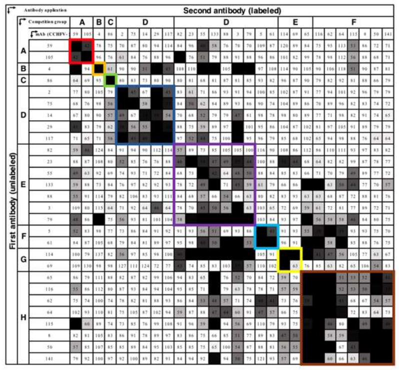

Figure 2. Competition assessment of human Abs to CCHFV using M-segment expressing cells. We tested 28 mAbs in competition assays. MAbs are displayed in 8 groups (A-H) based on their ability to compete for binding. Values shown are the % of binding that occurred during competition compared to non-competed binding of the mAb. This value was normalized to 100%. The values are also indicated by the box fill color; darker colors toward black indicate higher competition and lighter colors toward white indicate less competition, on a gradient scale.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-CCHFV mAbs.*** In this Aim, we will rapidly discover human anti-CCHFV mAbs from CCHFV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify an optimal anti-CCHFV mAb combination and further advance the discovery platform for rapid response against emerging nairoviruses. In preliminary experiments in collaboration with investigators in **RP1** and **Core E**, the Crowe laboratory has previously isolated and characterized panels of neutralizing human anti-CCHFV human mAbs from individuals who were convalescent after naturally-acquired laboratory-confirmed infection in Spain or Turkey. We isolated a panel of antibodies against CCHFV that recognize the M-segment in transfected cells, and they exhibit a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 2**). This panel contains cross-binding and cross-neutralizing antibodies for diverse strains. We observed a human mAb designated CCHF-82 competing with competition group 1 and group 2 antibodies from the previously isolated GP38-reactive murine antibodies. CCHF-82 showed some protection in a STAT1$^{-/-}$ mouse model of infection conducted by investigators in **RP1 (Fig 3)**. Further investigation with this antibody in the IbAr10200 prophylaxis and therapeutic models is warranted. In contrast, the CCHFV-neutralizing mAbs that we isolated and tested *in vivo* did not perform well in the Turkish strain STAT1$^{-/-}$ mouse model. We now know protection in this model is possible, given the work from Fels *et al*., 2021 who showed that potent antibodies against various sites on the Gc surface can afford protection in this model[42]. However, in their study they did not test GP38-reactive antibodies. To our knowledge, our preliminary

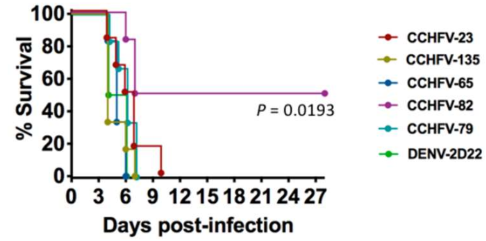

Figure 3. Human mAb CCHF-82 provides partial protection as a post-exposure prophylaxis against the Turkish strain of CCHFV in the STAT1$^{-/-}$ mouse model. A single dose of mAb was administered by the IP route to mice (n=6 per group (3 male, 3 female)) at 30 min post infections. A lethal dose of Turkish strain of CCHFV was administered. CCHF mAbs or DENV-2D22 (an isotype-matched negative control mAb) were tested in a single dose of 250 µg per mouse. Kaplan-Meier survival curves were statistically analyzed using a log-rank (Mantel-Cox) test where mAb-treated animals (*P* value shown in figure) were compared to animals treated with the DENV-2D22 negative control mAb. Unpublished data.

data is the first indication that a human GP38-targeting antibody can provide some protection in this mouse model.

Non-neutralizing murine mAbs protect in mouse models of infection[14], but it is unknown if the human antibody response could provide similar levels of protection. Murine mAb 13G8 binds to GP38 and does not neutralize CCHFV. This mAb provides protection against lethal challenge as a prophylaxis and a therapeutic against CCHFV. In preliminary experiments we sought to understand if human survivors of CCHFV infection possess B cells encoding mAbs that bind a similar epitope as 13G8 and provide protection. CCHF-82 competes for binding with 13G8 to preGn-transfected cells containing GP38. This antibody offered some protection as a post-exposure prophylaxis against the Turkish strain of CCHFV (**Fig 3**). Human antibodies like this should be further studied and the frequency of these clones should be elucidated to understand if this is a feasible strategy for future vaccine efforts for humans. The epitope that 13G8 and CCHF-82 identifies could inform vaccine design if these antibodies offer protection in larger animal models. Understanding how non-neutralizing antibodies function is critical in efforts to validate the GP38-binding class of antibodies. Golden *et al.* undertook a study to understand the mechanisms 13G8 functioned and observed that complement activation contributes to the protective capacity of this antibody[14]. Here, in studies of CCHF-82, we also will assess complement activation in mouse models of infection to fully understand the function of this mAb *in vivo*. Furthermore, if this mAb protects in the CCHFV IbAr10200 strain challenge model, assessing its mechanism is warranted. Together with **Core D**, we will assess IgG molecules with complement knockout ("KA") and Fc gamma receptor knockout (LALA-PG) mutations in the Fc domain to study this matter. Human mAb CCHF-82 displayed limited cross-protection against experimental infection with the Turkish strain of CCHFV. Our antibody isolation here will aim to identify cross-protective non-neutralizing antibodies reactive with GP38. Recently, vaccinating with NP was shown to be another way to elicit non- neutralizing antibodies[44]. The NP of CCHFV is more conserved than the glycoproteins and might also be a non-canonical target for the identification of cross-protective non-neutralizing antibodies[44].

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant viral surface protein antigens, cloning of Ab variable genes, and then expression of mAbs as as described above in Specific Aim 1.

E.2.1. Rapid isolation of human anti-CCHFV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from a number of previously CCHFV-infected individuals. One was a health care survivor who was infected while caring for a patient in Spain[45]. We also collected a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Turkey, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples were only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies were approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples were deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-CCHFV mAbs rapidly, as if we had to respond to a nairovirus outbreak in real time. Our ability to respond rapidly in the setting of outbreaks is well-documented in the lay press (60 Minutes features and others) and the scientific literature, e.g., 78 days from sample to protection of NHPs for Zika virus[32], and 25 days from PBMC sample receipt to delivery of the antibody genes for the approved COVID-19 drug Evusheld to AstraZeneca[1]. Here, we will single-cell sort antigen-specific B cells using recombinant CCHFV proteins (GP38, Gc, Gn, Gc/Gn) or non-infectious particles as bait as described in Specific Aim 1. We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D**, (**Table 3**) or by **RP1 (Table 4)**.

Table 3. Core D CCHFV antigens
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF NP

(c) Recombinant mAb cloning. As described in Specific Aim 1 above.

(d) Micro-scale mAb expression. As described in Specific Aim 1 above.

(e) A CPE-based CCHFV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of CCHFV. We will use a rVSV CCHFV surrogate that expresses CCHFV surface proteins and causes CPE[35]. Investigators in **RP1** have the VSV-CCHFV-GFP versions of clades 1 through 7 based on this publication. We will obtain these VSV constructs from investigators in **PABVAX**. Existing VSV vectors will be provided by investigators in **RP1 and Core E**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against CCHFV. These mAbs will be moved forward for additional studies to identify an optimal anti-CCHFV mAb combination. GP38-reactive clones will be advanced based on avidity ranking since they are not likely to be neutralizing but may be protective *in vivo*.

Table 4. Antigens available from RP1
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF Turkey NP
CCHF Turkey GnGc+GP38+NP
CCHF Turkey GnGc+GP38
CCHF Turkey GP38+NP
CCHF Turkey GnGc+NP

E.2.2. Identifying CCHFV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind CCHFV surface proteins and validate their epitopes. We will perform competition-binding studies with CCHFV Gc/Gn or subunit proteins (as above) or CCHFV virus-like particles (VLPs) made from a replicon system in hand. **Core E** will inactivate CCHFV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site. Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from some of the mAbs shown in the unpublished preliminary data above for the identification of mAbs that can concurrently bind CCHFV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-CCHFV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. GP-38-reactive clones will be screened for synergistic binding as they are not expected to be neutralizing. ***(c) Identification of key binding residues of human anti-CCHFV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-CCHFV constructs at BSL2 in the presence of saturating concentrations of CCHFV mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of VSV-CCHFV escape variants in the presence of mAbs.

E.2.3. Structural basis of neutralization by anti-CCHFV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.2.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies in STAT1$^{-/-}$ mouse models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (Core D), will advance to *in vivo* testing. We will use an established STAT1$^{-/-}$ mouse model of CCHFV infection that the **RP1** and **Core E** investigators have deployed[35]. To benchmark our mAbs, a separate set of STAT1$^{-/-}$ mice will be treated with CCHF-82, which we showed in unpublished preliminary data above confers substantial but incomplete protection (**Fig 3**). The **Core E** laboratory has access to strains representing all 6 clades that can be tested. Groups of 6-8 STAT1$^{-/-}$ mice will be treated with low, medium, and high doses) of single or combinations of anti-CCHFV mAb, or isotype controls at day +1 after inoculation with CCHFVs. This approach allows us to determine whether combinations provide advantage over the best CCHFV monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after CCHFV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal CCHFV mAb pairs that are comparable to or outperform the mAbs we reported above. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-CCHFV mAb combination against CCHFV in NHPs. As a final test of an optimal anti-CCHFV mAb combination, we will assess efficacy of the lead anti-CCHFV mAb pair in NHPs (**Core E**), to be selected based on the *in vitro,* STAT1$^{-/-}$ mice, and manufacturability data (**Core D**). Down-selection for testing in cynomolgus monkeys would only be considered with success in the STAT1$^{-/-}$ mouse studies and in consultation with the external SAB and NIAID program staff.

E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs. Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human mAbs, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-CCHFV mAb combinations with optimal protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging CCHFV or related nairoviruses. Given our previous ability to generate human anti-CCHFV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-CCHFV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-CCHFV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (i.e., memory B cells) isolated from human CCHFV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in CCHFV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-CCHFV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-CCHFV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-CCHFV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-CCHFV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

Table 5. Antigens available from RP1
Kasokero GnGC
Kasokero GP38
Kasokero NP
Kasokero GnGc +GP38 +NP
Kasokero GnGc +GP38
Kasokero GnGc +NP
Kasokero GP38 +NP

E3. Specific Aim 3. Establish optimal mAb combination therapies against henipaviruses Hendra (HeV) and Nipah (NiV) viruses. We will use a large panel of potent anti-henipavirus mAbs that we previously made from the memory B cells of immune individuals and generate new antibodies targeting the fusion (F) protein. **RP2** will provide new antigens for mAb selection and BSL-2-compatible viruses for neutralization screens including Cedar virus constructs displaying HeV and NiV F and G proteins. We will identify optimal mAb combinations to enhance henipavirus therapy, minimize viral escape, and improve treatment efficacy through synergy. *In vitro* competition-binding, neutralization, and mechanistic studies will be conducted, including authentic virus neutralization with **PABVAX Core E**. We will refine the recognized epitopes and investigate the structural basis for optimal combination neutralization of henipaviruses using HeV and NiV as prototypes. *In vivo* assessment of mAb combinations will determine the minimal effective dose for protection and the ability to prevent *in vivo* escape. Protective efficacy of lead mAb combinations will be assessed in hamster models of NiV and HeV infection with **Core E**. Leads will be transferred to **Core D**. As a final test of optimal henipavirus mAb combinations, we will assess protective efficacy of the lead henipavirus mAb pairs in NHPs, to be selected based on the *in vitro* and hamster data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in African green monkey (AGM) henipavirus model studies would only be considered pending success in the hamster studies and consultation with the external SAB and NIAID program staff. The HeV and NiV studies will be conducted in years 1-3 and then applied to the plug-and-play test case Langya virus in years 2-5. If **RP2** and **Core E** cannot rescue recombinant Langya virus, the lessons learned in Years 1-3 will be applied to the alternate “plug and play” test viruses Angavokely or Sosuga (SOSV) virus. Preliminary data on SOSV mAbs are provided.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-NIV/HeV mAbs.*** In this Aim, we will further adapt approaches we have developed in preliminary experiments with henipavirus mAb studies by identifying a potently protective combination of anti-NiV/HeV human mAbs. Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups. This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains. In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and outstanding potency anti-G antibodies with extensive characterization, including synergistic neutralization.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant henipavirus F protein antigens, as described above in Specific Aim 1.

E.3.1. Rapid isolation of human anti-HeV/NiV mAbs. ***(a) Immune cells.*** We are obtaining human PBMCs from a number of previously NiV-infected individuals who acquired immunity following natural infection in collaboration with the **icddr,b**, an international health research institute based in Dhaka, Bangladesh. We are collecting a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Bangladesh, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies are approved by the icddr,b and Vanderbilt University Medical Center IRBs. PBMCs isolated from the blood samples are deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their use in this **RP4,** if funded. ***(b) Applying mAb technologies for new mAb isolation***. We will use sorting of antigen-specific B cells, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer antigen-specific B cells. We will sort soluble fusion (sF) or attachment (G) protein-specific cells with recombinant viral surface protein antigens from **Core D** (**Table 6**).

(c) Ab screening and sequence analysis. Our goal is to identify human anti-NiV/HeV mAbs rapidly, as if we had to respond to a paramyxovirus outbreak in real time. We will single-cell sort antigen-specific B cells using recombinant HeV and NiV proteins (sF or G) as bait as described in Specific Aim 1 above.

(d) Recombinant mAb cloning. As described in Specific Aim 1 above.

(e) Micro-scale mAb expression. As described in Specific Aim 1 above.

(f) CPE-based HeV/NiV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of HeV and NiV. We will use rVSV HeV and NiV surrogates that expresses henipaviurs surface proteins and cause CPE. Investigators in **RP2 and RP3** have VSV-NiV F and VSV-NiV G with GFP[34], VSV-NiV G without GFP[36] and VSV-HeVG (Geisbert and Cross, unpublished). We will obtain these all these VSV constructs from investigators in **PABVAX**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against HeV and NiV. We also can use Cedar virus constructs for neutralization assays as the have reduced pathogenicity[46] and can be used in high-throughput screening assays for henipavirus antiviral discovery[47]. Cedar viruses will be provided by Dr. Broder of **RP2**. The mAbs with most potent activity will be moved forward for additional studies to identify an optimal anti-HeV/NiV mAb combination.

Table 6. Core D Henipavirus antigens
Nipah sF
Hendra sF
NiV G

E.3.2. Identifying HeV/NiV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind HeV/NiV surface proteins and validate their epitopes. We will perform competition-binding studies with HeV/NiV sF or G proteins (as above). We will include positive controls made recombinantly from some of the mAbs shown in the preliminary data above for the identification of mAbs that can concurrently bind HeV/NiV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti- HeV/NiV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key binding residues of human anti-HeV/NiV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-NiV F, VSV-NiV G and VSV-HeV G constructs at BSL-2 in the presence of saturating concentrations of HeV/NiV mAbs (single or combination) using RTCA to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test the level of infection of VSV-HeV/NiV escape variants in the presence of mAbs.

E.3.3. Structural basis of neutralization by anti-HeV/NiV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.3.4. Protective efficacy of human anti-henipavirus mAbs. ***(a) Studies in hamsters***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (**Core D**), will advance to *in vivo* testing. We will use an established hamster model of NiV or HeV infection that the **Core E** investigators have deployed. To

benchmark our mAbs, a separate set of hamsters will be treated with G mAbs HENV-103+HENV-117, which we showed in previous data above confers substantial protection[8]. Groups of 6-8 hamsters will be treated with low, medium, or high IgG doses) of single or combinations of anti-HeV/NiV mAbs, or isotype controls at day +1 after inoculation with HeV or NiV. This approach allows us to determine whether combinations provide advantage over the best HeV or NiV mAb monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-HeV and anti-NiV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after HeV or NiV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal HeV/NiV mAb pairs that are comparable to or outperform the mAbs we reported previously. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-HeV/NiV mAb combination against HeV and NiV in NHPs. As a final test of an optimal anti-HeV/NiV mAb combination, we may assess efficacy of the lead anti-HeV/NiV mAb pair in NHPs (**Core E**), selected based on the *in vitro,* hamster data, and manufacturability data (**Core D**). Down-selection for testing in AGMs would only be considered with success in the hamster studies and in consultation with the external SAB and NIAID program staff.

E.3.5. Applying lessons learned in HeV/NiV research to Langya, Angavokely and Sosuga viruses. Once we have completed the HeV/NiV discovery campaigns, will turn to the test case pathogen Langya (or alternate Angavokely and Sosuga (SOSV) viruses) to see if we can use the prototype pathogen approach for this virus. We have plenty of human PBMCs from the sole identified SOSV-immune individual and have already shown that it is possible to obtain HN- and F-specific neutralizing antibodies from this individual's B cells[48] and **Fig 4**. We also have already prepared recombinant SOSV prefusion F and HN proteins[48]. We do not have access to human PBMCs from Langya- or Angavokely-immune donors. For those mAb discovery campaigns, we will use Alloy humanized mouse models as a source of B cells encoding human mAbs, as above. Mice will be immunized with recombinant protein antigens (**Table 7**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Langya or Angavokely. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above using single-cell RNAseq or human hybridoma methods. The development will follow as for Hev/NiV described above.

Table 7. Paramyxovirus antigens from Core D
Mojang sF
Angavokely sF
Langya sF

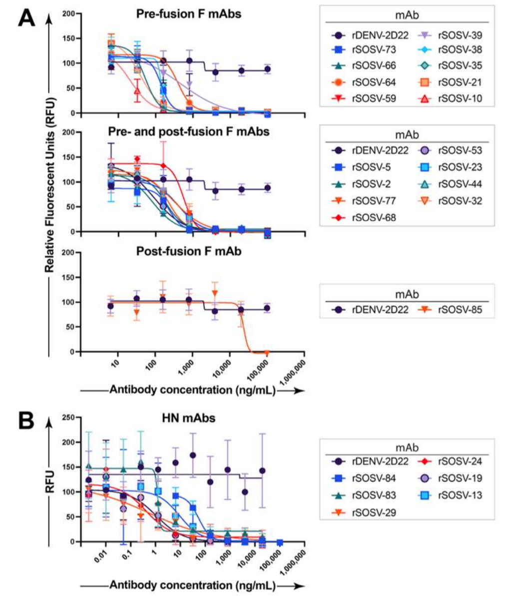

Figure 4. Neutralization assay of SOSV mAbs against live virus. SOSV mAbs were tested for inhibition of authentic rSOSV-ZsG in quadruplicate on Vero-E6 cell culture monolayers. (A) Neutralization data for anti-F mAbs. Data are grouped according to the pattern of antigen-reactivity: pre-fusion F, pre- and post-fusion F, or post-fusion F protein. (B) Neutralization data for the HN-specific mAbs.

<u>Anticipated results, potential problems, and alternative approaches</u>. In this Aim, we will identify human anti-HeV/NiV mAb combinations with optimal protective activity. These mAb combinations will be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging HeV/NiV or related paramyxoviruses. Given our previous ability to generate human anti- HeV/NiV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-HeV/NiV mAbs. We may observe that new mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-HeV/NiV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells *(i.e., memory B cells) isolated from human NiV-immune PBMC samples. In addition, we can deep sequence the B* cell repertoires present in NiV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-HeV/NiV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-HeV/NiV mAb combinations that can inform vaccine design (**RP2**) for related viruses used as test cases in years 2 through 5.

F. Scientific rigor. ***Experimental design.*** Experiments will include biological and technical replicates, and results will be analyzed by parametric or non-parametric analyses (depending on data distribution) and corrections for multiple comparisons. EC$_{50}$ (binding) and IC$_{50}$ (neutralization) values will be reported only following at least 3 identically conducted experiments. All mAb screening will incorporate appropriate + and - controls. Positive signals in binding assays will be defined as >3 SD above background. ***Protein quality.*** We have a high capacity to purify proteins using a Protein Maker workstation that allows purification chromatography on 24 parallel columns. We perform QC on protein reagents using a Maurice instrument with capillary electrophoresis technology to automate our protein profiling either by size or charge, providing cIEF and CE-SDS data. Animal ***studies*** will be performed blinded with independent replicates and include negative (*e.g.*, placebo), and positive (when available) controls. We will use power calculations (**Data Management Core B**) to determine sample sizes. For virological studies, power analysis was performed using accepted values for type I error (0.05) and power (80%). The values for population variance (15×) are based on existing data sets and our experience with rodent challenge studies. We will perform experiments at least 2-3 independent times (n = 4-5 each) to attain n = 12-15 per arm. Smaller group sizes can be used if differences are larger than expected.

G. Relevant biological variables. The human mAbs are derived from immune human donors or Alloy mice. Humans. Human Abs will be isolated for biological, structural, and functional studies at a clonal level. The sample size is not powered for large scale epidemiologic or clinical study conclusions. There is no evidence that sex, age, body mass index, or underlying health conditions other than immunodeficiency or immunosuppressive treatments alters the activity of cloned Abs in structure/function studies. The samples are de-identified without patient specific identifiers to protect the health information of the donors; thus, research with these samples is considered *Not Human Subjects Research* category activity. Mice, hamsters, guinea pigs, NHP, and viruses are also biological systems used in this application. Conventional animal housing conditions will be used because there is no evidence that altered room temperatures and light/dark cycles influence paramyxovirus, nairovirus, or arenavirus pathogenesis. Mice, hamsters, guinea pigs, and NHPs of both sexes will be used, and phenotypes will be monitored for sex-based differences. Virus stocks will be either low passage seed stocks from natural isolates or generated from existing cDNA clones and sequence-confirmed prior to use for infection studies.

H. Milestones and quantitative Go/No-Go criteria. Through **Aims 1-3**, we have proposed experiments that will advance our knowledge of the mAb-mediated mechanisms of action against paramyxoviruses, nairoviruses, and arenaviruses. In each Aim, we will use down-selection criteria (as below) to advance mAb combinations or variants for additional studies. By year 3, we will have defined the principles that govern optimal mAb combinations for paramyxoviruses, nairoviruses, and arenaviruses and the rational approaches needed to select mAbs for potency and breadth. We will use the following metrics to advance mAbs: ***(a) Neutralizing activity.*** All mAbs will have neutralizing potency < 10 ng/mL, EC$_{50}$ value; ***(b) Binding affinity***. MAbs will be of high affinity to surface proteins or virions (K$_D$ < 5 nM). ***(c) Protein stability and production quality.*** MAbs must produce at high levels without optimization (> 100 mg/L) and show biochemical/functional stability at 4°C or -80°C (>90% retention of binding and neutralization activity at 30 d). ***(d) Synergy***. Combinations of 2 mAbs against a given virus ideally will show synergy of neutralization *in vitro* or at a minimum, additive inhibition (no antagonism); ***(e) Epitopes***. To avoid resistance, mAbs in combination will bind 2 epitopes (determined by competition binding (<20% reduction in binding), mapping, and structural studies. ***(f) Resistance*** against mAbs will be assessed for virus escape. Deep sequencing of viruses that emerge from cells or animals treated with mAbs will inform prioritization. Combinations of mAbs with high genetic barriers to resistance (<10× loss in EC$_{50}$) will be advanced. ***(g) Protection in mice, hamsters, guinea pigs, or NHPs.*** The following metrics can be used: (i) Protection against lethal challenge by homologous virus (>90% survival; (ii) Protection against clinical disease by homologous virus (>80%); (iii) Protection against infection by homologous virus (>100-fold reduction in viral titers in multiple organs). ***(h) pK measurements***. For lead protective mAbs with mutations that should confer extended half-life, we will perform pK studies in NHPs (with **Core D** and **E**) to assess levels over time with a goal of a human predicted serum half-life of >6 months.

Timeline for RP4	**Year 1**	**Year 2**	**Year 3**		**Year 4**	**Year 5**
Prototype mAb generation and testing: **LASV and MACV mAbs**	X	X	X		X	
Test case mAb generation and testing: **Lujo and Chapare mAbs**			X		X	X
Prototype mAb generation and testing: **CCHFV mAbs**	X	X	X		X	
Test case mAb generation and testing: **Kasokero mAbs**			X		X	X
Prototype mAb generation and testing: **NiV/HeV mAbs**	X	X	X			
Test case mAb generation: **Langya, Angavokely/Sosuga mAbs**		X	X		X	X
In vivo efficacy studies with **Core E**	X	X	X		X	X
Translational activities with **Core D**	X	X	X		X	X

LITERATURE CITED – RP4

1. Zost, S.J.*, et al.* Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* **26**, 1422-1427 (2020).
2. Fischer, W.A., II & Wohl, D.A. Moving Lassa Fever Research and Care Into the 21st Century. *The Journal of Infectious Diseases* **215**, 1779-1781 (2017).
3. Ezeomah, C.*, et al.* Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. *Open Forum Infect Dis* **6**, ofz512 (2019).
4. Li, A.L.*, et al.* Ophthalmic manifestations and vision impairment in Lassa fever survivors. *PloS one* **15**, e0243766 (2020).
5. Webb, P.A., Johnson, K.M., Mackenzie, R.B. & Kuns, M.L. Some characteristics of Machupo virus, causative agent of Bolivian hemorrhagic fever. *Am J Trop Med Hyg* **16**, 531-538 (1967).
6. Hawman, D.W. & Feldmann, H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology* (2023).
7. Mackenzie, J., Wang, L. & Broder, C.C. Henipaviruses. in *Fields Virology*, Vol. 2 1070-1085 (2013).
8. Doyle, M.P.*, et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021).
9. Dang, H.V.*, et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021).
10. Cross, R.W.*, et al.* Antibody therapy for Lassa fever. *Curr Opin Virol* **37**, 97-104 (2019).
11. Cross, R.W.*, et al.* Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral research* **133**, 218-222 (2016).
12. Cartwright, H.N., Barbeau, D.J. & McElroy, A.K. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere* **6**, e0055621 (2021).
13. Gutjahr, B.*, et al.* Two monoclonal antibodies against glycoprotein Gn protect mice from Rift Valley Fever challenge by cooperative effects. *PLoS Negl Trop Dis* **14**, e0008143 (2020).
14. Golden, J.W.*, et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv* **5**, eaaw9535 (2019).
15. Zalevsky, J.*, et al.* Enhanced antibody half-life improves in vivo activity. *Nat Biotechnol* **28**, 157-159 (2010).
16. Dall'Acqua, W.F.*, et al.* Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* **169**, 5171-5180 (2002).
17. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Levis, S.C. & Maiztegui, J.I. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* **2**, 255-256 (1984).
18. Jahrling, P.B. & Peters, C.J. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infection and immunity* **44**, 528-533 (1984).
19. Chen, E.C.*, et al.* Systematic analysis of human antibody response to ebolavirus glycoprotein shows high prevalence of neutralizing public clonotypes. *Cell Rep* **42**, 112370 (2023).
20. Buck, T.K.*, et al.* Neutralizing Antibodies against Lassa Virus Lineage I. *mBio* **13**, e0127822 (2022).
21. Enriquez, A.S.*, et al.* Delineating the mechanism of anti-Lassa virus GPC-A neutralizing antibodies. *Cell Rep* **39**, 110841 (2022).
22. Hastie, K.M.*, et al.* Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell* **178**, 1004-1015 e1014 (2019).
23. Hastie, K.M.*, et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).
24. Li, H.*, et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991 (2022).

25. Gilchuk, P., *et al.* Pan-ebolavirus protective therapy by two multifunctional human antibodies. *Cell* **184**, 5593-5607 e5518 (2021).
26. Chen, E.C., *et al.* Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* **36**, 109604 (2021).
27. Dong, J., *et al.* Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-1244 (2021).
28. Greaney, A.J., *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).
29. Kramer, K.J., *et al.* Potent neutralization of SARS-CoV-2 variants of concern by an antibody with an uncommon genetic signature and structural mode of spike recognition. *Cell Rep* **37**, 109784 (2021).
30. Suryadevara, N., *et al.* Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell* **184**, 2316-2331 e2315 (2021).
31. Zost, S.J., *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449 (2020).
32. Gilchuk, P., *et al.* Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics. *Nat Biomed Eng* **4**, 1030-1043 (2020).
33. Cross, R.W., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep* **40**, 111094 (2022).
34. Mire, C.E., *et al.* Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J* **10**, 353 (2013).
35. Rodriguez, S.E., *et al.* Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep* **9**, 7755 (2019).
36. Foster, S.L., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. *Proc Natl Acad Sci U S A* **119**, e2200065119 (2022).
37. Branco, L.M., *et al.* Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* **7**, 279 (2010).
38. Starr, T.N., *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
39. Deschambault, Y., *et al.* An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. *Viruses* **15**(2023).
40. Bell, T.M., *et al.* Pathogenesis of Bolivian Hemorrhagic Fever in Guinea Pigs. *Vet Pathol* **53**, 190-199 (2016).
41. Golden, J.W., *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* **7**, 4679 (2017).
42. Fels, J.M., *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell* **184**, 3486-3501 e3421 (2021).
43. Mishra, A.K., *et al.* Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies. *Science* **375**, 104-109 (2022).
44. Leventhal, S.S., *et al.* Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *EBioMedicine* **82**, 104188 (2022).
45. Negredo, A., *et al.* Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med* **377**, 154-161 (2017).
46. Laing, E.D., *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018).
47. Amaya, M., *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084 (2021).
48. Parrington, H.M., *et al.* Potently neutralizing human mAbs against the zoonotic pararubulavirus Sosuga virus. *JCI Insight* **8**(2023).

A. BACKGROUND AND SIGNIFICANCE. Paramyxoviruses, nairoviruses, and arenaviruses are widely distributed RNA viruses that cause severe human syndromes. Because of their geographical distribution and expansion, changes in human demographics and increased exposures to wild animals including rodents and bats, and their capacity for human-to-human spread, these viruses have the potential to cause pandemics. Together with the other investigators in **PABVAX**, we have selected several important prototype paramyxoviruses, nairoviruses, and arenaviruses to study to learn the rules governing immunity to these pathogens. The work on the pathogens selected is justified based on their prevalence and severity alone, but also because of their ability to serve as prototypes for genetically related viruses. We chose the viruses for focus in this RP4 on human antibody development because of their clinical relevance and because several will be studied as prototypes for vaccine development in **RP1** (arenaviruses, nairoviruses) and **RP2** (paramyxoviruses). Among the arenaviruses, we will target Lassa virus (LASV) and Machupo virus (MACV), and among the nairoviruses, we will study Crimean–Congo hemorrhagic fever virus (CCHFV). In tandem with the vaccine projects in **RP2** focused on paramyxoviruses, we will study Nipah virus (NiV) and Hendra virus (HeV) as the prototypes. Information about the principles we discover about critical neutralizing epitopes identified here in **RP4** with prototype viruses can feed back into all other **Projects** that address the subsequent plug and play test cases in later years (Lujo, Chapare, Kasokero, Langya).

LASV is an Old World arenavirus (OWAV) with a single-stranded RNA virus belonging to the Arenaviridae family. Infection in humans is often the result of exposure to infected excreta from the rodent reservoir, *Mastomys natalensis* where up to 500,000 cases and an estimated 5,000 deaths occur annually in West Africa[2]. While rodent infection is largely benign, human infection can result in severe hemorrhagic disease with neurological complications and long-term hearing and vision sequelae[3,4]. There are no approved vaccines or treatments for LASV infection. **MACV** is a New World Arenavirus (NWAV) and the causative agent of Bolivian Hemorrhagic fever. Humans are infected after exposure to excreta from the reservoir, *Calomys callosus,* the large vesper mouse. Disease in humans has a slow onset, but can progress to severe hemorrhagic fever coupled to insidious neurological complications including tremors, seizures, and paralysis[5]. There are no approved vaccines or therapies approved for MACV. **CCHFV** is a highly pathogenic tick-borne virus classified under the Nairovirus genus within the Bunyaviridae family[6]. CCHF outbreaks have a case fatality rate of up to 40% and there are no vaccines or treatments approved for human use. **NiV/HeV.** Bat borne, NiV and HeV are enveloped, negative-sense, single-stranded RNA viruses belonging to the Henipavirus genus within the Paramyxoviridae family[7]. These emerging zoonotic viruses pose a significant threat to human and animal health due to their high fatality rates and broad host range. As with the other prototype virus in the **PABAX** Center there are no vaccines or treatments against henipaviruses licensed for human use.

Antibodies (Abs) are important correlates of protection for paramyxoviruses, nairoviruses, and arenaviruses. Immune sera against members of each of these groups of viruses has been shown to exhibit protective efficacy *in vivo*, which supports the use for Abs as treatment options. However, the specificity, functional properties, and other attributes of protective antibodies are poorly defined for most of these viruses. MAbs to the attachment and fusion surface proteins of paramyxoviruses (such as the G and F proteins of Hendra/Nipah[8,9] and the GP proteins of the arenaviruses like LASV[10,11]) can neutralize and protect against infection in rodent models. CCHFV is a bit of a conundrum in that ultrapotent human neutralizing antibodies to the related Rift Valley fever virus surface proteins are highly protective *in vivo*[12,13], but CCHFV neutralizing antibodies are not protective. In contrast, some non-neutralizing antibodies do protect partially for CCHFV[14]. Clearly, there is a lot to learn about the rules of immunity to these viruses, and the genetic, molecular, and structural basis of antibody-mediated protection for viruses like CCHFV. Based on these data, we propose the use of mAbs as treatment options to learn the rule of how to protect against emerging paramyxoviruses, nairoviruses, and arenaviruses and to develop candidate antibody countermeasures for translational development with **Core D**. To do so, we will further develop our technical methods for rapidly discovering mAbs, in the case of a future unexpected epidemic caused by viruses of these families. Most importantly, we will define the principles governing combination antibody therapy to prevent virus escape from treatment or prevention and to optimize the efficacy of the beneficial effects conferred by passive immunity.

B. SCIENTIFIC PREMISE. The work proposed here in **RP4** is based on the extensive basic, translational, and clinical trial data on the role of neutralizing Abs in protection against infection and disease caused by paramyxoviruses, nairoviruses, and arenaviruses. Although a significant amount of data supports the use of mAbs to prevent or control paramyxovirus, nairovirus, and arenavirus infection, many questions remain, such as the ideal epitopes to target with mAb combination therapy to improve upon neutralization potency, *in vivo* efficacy, and resistance to viral escape. Identifying these epitopes can help inform vaccine design to elicit optimal protective polyclonal responses (in **RP1** and **RP2**, especially with designs for test cases in years 4 and 5). Here we aim to refine our understanding of the correlates of mAb protection against paramyxoviruses, nairoviruses, and arenaviruses to enable the design of a modular “plug-and-play” workflow to rapidly respond to potential

pandemics by generating optimized combinations of vaccine-like mAbs with an extended half-life that could be used for long-term prophylaxis (> 6 months) and therapy.

C. INNOVATION. RP4 has many innovative conceptual and technical features including: Conceptual. **(1)** determining the principles that govern optimal combinations of human mAbs against paramyxoviruses, nairoviruses, and arenaviruses for protection and resistance to viral escape; **(2)** the development of a clear workflow that generates therapeutic or preventative antibodies (long half-life mAbs that serve as vaccine surrogates) against emerging or new paramyxoviruses, nairoviruses, and arenaviruses with pandemic potential. Our goal is to define the principles and features, including epitope, combination ratios, and mechanisms of action, that result in optimal efficacy of mAbs against paramyxoviruses, nairoviruses, and arenaviruses in animal models and create a higher barrier for resistance. These studies will serve as paradigms for rapid antibody discovery in response to future paramyxovirus, nairovirus, and arenavirus pandemic threats. Technical. **(3)** large-scale B cell repertoire sequencing with customized methods and software; **(4**) use of humanized (Alloy) mice and novel immunogens from **RP1** and **RP2** and **Core D**; **(5)** single B cell functional (neutralization) assays performed on a Beacon instrument; **(6)** development of high-throughput real-time label-free virus neutralization assays (xCELLigence platform); and **(7)** use of *simultaneous* in parallel screening with several different high-throughput virus-specific B cell isolation approaches (Beacon, 10X Genomics, Rhapsody) to generate and validate best-in-class human mAbs as candidate medical countermeasures.

D. LINKAGE TO OTHER PABVAX PROJECTS AND CORES. The group will focus on developing a modular test case platform for rapid selection of highly neutralizing and/or protective mAbs against paramyxoviruses, nairoviruses, and arenaviruses. Members of our group have active collaborations with most members of the **PABVAX Center** as reflected in previous collaborations and publications (see **Overall** document). We will interact extensively with the Animal Model and Preclinical Evaluation Core (**Core E)** to study neutralization against authentic viruses and to test for protection in small and large animals as well as with **RP3** for testing paramyxovirus mAbs. The knowledge gained from our studies on the structure-function relationships of neutralizing mAb combinations can be applied to rational vaccine design for **RP 1** and **RP2** in later years of the effort when we pivot to apply the lessons learned to the same test case targets as **RP1** and **RP2.** Numerous antigens will be made available to our RP. Finally, long-half-life Fc mutations are now available based on engineering of human IgG1 for enhanced binding to the neonatal Fc receptor (FcRn) such as LS (M428L /N434S)[15], or YTE (M252Y/S254T/T256E)[16] mutations. With ≥ 90 days half-life in humans, our approach will generate mAbs with vaccine-like properties that remain effective for months during a virus epidemic and thus provides a platform for response to future paramyxovirus, nairovirus, and arenavirus pandemic threats, a key goal of our **PABVAX Center.** *Our laboratory previously validated this vaccine surrogate concept when we isolated the human mAbs that formed the basis for Evusheld with YTE long-half-life Fc mutations (used in 70 countries and millions of people) that exhibited > 6 months protective levels in humans after single IM injections*.

E. APPROACH

E.1. Specific Aim 1. Discover potently neutralizing arenavirus combination therapies. For the prototypes, we will focus on LASV, an Old World virus and MACV, a New World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[17,18]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV or MACV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.

Preliminary Data. ***(a) Potently neutralizing human anti-LASV and anti-MACV mAbs.*** The Crowe laboratory has previously characterized panels of neutralizing human anti-viral human mAbs from virus-immune or vaccinated individuals for about 50 viruses, mostly RNA viruses. Isolated peripheral blood mononuclear cells (PBMCs) are used to generate hybridomas or alternatively antigen-sorted at single B cell level and antibody variable genes sequenced to generate mAbs recombinantly. Panels of human anti-viral mAbs are produced rapidly (we recently reported isolation of 15,000 mAb gene pairs from single Ebola-virus-specific human B cells from a single blood sample of an immune individual[19]. Previous studies by others clearly show that representative neutralizing and protective human antibodies can be isolated from LASV-immune subjects[20-24]. In this Aim, we will refine the approaches we have developed previously for other RNA viruses in a proof-of-concept study by identifying potently neutralizing combinations of anti-LASV and anti-MACV human mAbs. We will rapidly discover human anti-LASV mAbs from LASV-immune individuals and human anti-MACV mAbs by immunizing Alloy transgenic mice. Synergy studies will be performed to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify optimal anti-LASV and anti-MACV mAb combinations and further advance the discovery platform for rapid response against emerging arenaviruses.

(b) Advanced mAb technologies. The Crowe laboratory has developed some of the highest yield systems to isolate naturally occurring human mAbs using human hybridoma or advanced single-cell RNA-seq methods (**Fig 1**). We have used sorting of antigen-specific cells with recombinant viral surface protein antigens (like the GP1 and GP2 proteins we will use here), cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. One variation we will use is to capture Ab genes directly from viral glycoprotein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), as we have done for Ebola virus[19,25] and SARS-CoV-2[1,26-31]. These single-cell techniques enhance the capture of rare antigen-specific B cells.

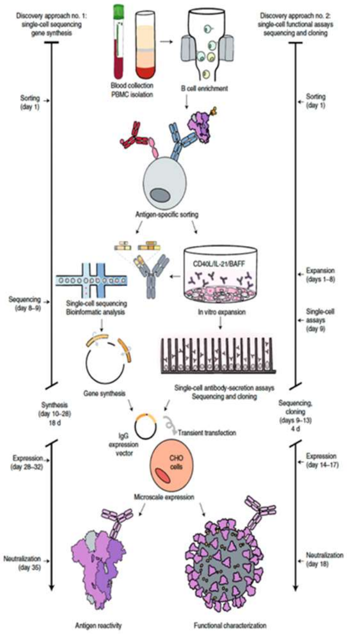

Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion allows us to further assess the functionality of the B cells or for antibody sequencing. Recombinant mAb expression validates the mAb sequence and functional activity. Figure is adapted from [1].

E.1.1. Rapid isolation of human anti-LASV and anti-MACV mAbs. *(a) Immune cells.* We have obtained human PBMCs from one previously LASV-infected individual who was medically managed previously by Emory University in their infectious diseases containment unit. This person has a documented robust neutralizing serum antibody response, suggesting isolation of neutralizing human mAbs from these PBMC aliquots will be straightforward for us. Our colleagues in **RP1** have ongoing field operations for over a decade in Sierra Leone, Liberia, and Nigeria. This work is currently in association with the NIH-Centers for Research on Emerging Infectious Diseases (CREID) with a heavy focus on the study of LASV ecology, epidemiology, and host responses. **RP4** is currently working with **RP1** on obtaining PBMCs from ~10 individuals with prior laboratory-confirmed cases of LASV. The acquisition of these samples is being managed by the Vanderbilt Vaccine Center Biorepository with separate institutional funding to be de-identified prior to use in the studies proposed here. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies have been approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples will be deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded. For anti-MACV mAbs, as a human PBMC source is not readily available, we will immunize Alloy humanized mice. These animals yield PBMCs that secrete fully human mAbs. Alloy mice are used routinely in the Crowe lab workflow.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-LASV and anti-MACV mAbs in a rapid manner to mimic a pandemic response to an outbreak. We will single-cell sort antigen-specific B cells using recombinant LASV or MACV proteins (*e.g.*, **Table 1)** or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including LASV and MACV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe GP protein or virus-particle binding to single B cells. These cells are exported for sequencing of the variable gene regions and analysis using the PyIR software developed in the Crowe laboratory. We will select a panel of up to 500 neutralizing anti-LASV mAbs and a similar number of anti-MACV mAbs to express recombinantly and advance for further characterization. We can easily conduct multiple approaches simultaneously in parallel[1,32].

Table 1. Antigens from Core D
Lassa Josiah NP
Lassa Josiah GPe
Machupo GPe
Machupo NP

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using a custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors.

(d) Micro-scale mAb expression. The Crowe laboratory has the capacity for large-throughput micro-scale mAb expression and purification. The lower end of this range allows expression of thousands of mAbs, which will feed into automated mAb purification platforms. This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based LASV and MACV neutralization assay using RTCA. We have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of cytopathic effect (CPE). Here, we will apply this method to assess neutralization of LASV. Recently, we validated the inclusion of simultaneous live-cell fluorescence microscopy into this real-time CPE-based screening platform (xCelligence RTCA eSight). These capabilities expand the platform to screen viruses that may or may not induce CPE, allowing us to also use a replication-competent vesicular stomatitis virus (VSV) LASV surrogate that expresses LASV surface proteins and causes CPE[33]. Currently, we have a VSV-LASV-Josiah (lineage IV)[33] and a Sauerwald version (lineage II) of these recombinants. Making additional LASV VSV-LASVs is not difficult, and **RP1** will make the other missing lineages or needed arenaviruses based on the same approaches as previously published. **RP1** also can easily make versions of these VSVs that express the green fluorescent protein (GFP) reporter gene. Existing VSV-CCHFV, VSV-NiV, and VSV-HeV vectors[34-36] will be provided by Dr. Geisbert overall PI and Lead of **Core E**. We already have Vanderbilt IBC approval for use of these constructs. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression if used will identify mAbs with neutralizing activity against LASV. These mAbs will be moved forward for additional studies to identify an optimal anti-LASV mAb combination. Similar processes will be followed to determine neutralization capacity of MACV mAb candidates.

E.1.2. Identifying LASV and MACV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind LASV or MACV and validate their epitopes. We will perform competition-binding studies with GP or subunit proteins (as above) or virus-like particles (VLPs)[37] available from **Core E** at UTMB. **Core E** will also inactivate LASV and MACV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from previously reported antibodies for the identification of mAbs that can concurrently bind LASV or MACV with a goal of finding more potent clones at those sites or clones that complement these inhibitory mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-LASV or anti-MACV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations for each virus, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key residues binding residues of human anti-LASV and anti-MACV mAbs.*** To determine the key residues for binding of down-selected mAbs, we will perform deep mutational scanning[38]. Briefly, all amino acids of the GP proteins will be mutated to the other 19 amino acids with a unique barcode using PCR-based mutagenesis. The LASV or MACV GP protein library will be incubated with the appropriate mAbs to identify residues with loss-of-binding phenotypes. A sequence 'logo' showing the relative contributions of each amino acid mutation will identify residues critical for binding. Multiple amino acid substitutions can then be assessed by reverse genetics at UTMB to confirm the epitopes recognized by the down-selected mAbs. This method also serves as a surrogate for *in vitro* neutralization escape studies. ***(d) Neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-LASV or VSV-MACV constructs at BSL2 in the presence of saturating concentrations of mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Confirmatory reverse genetics studies will be performed by introducing mutations into an infectious cDNA clone of LASV or MACV by **RP1** to test neutralization. We hypothesize that escape will be less likely in the presence of mAb combinations than single mAbs. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of escape variants in the presence of mAbs.

E.1.3. Structural basis of neutralization by mAb combinations. The studies above should identify optimal anti-LASV and anti-MACV mAb combinations that simultaneously bind, neutralize, and increase the barrier for viral escape under mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb

combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for arenavirus by identifying key epitope targets (**RP1**).

E.1.4. Protective efficacy of human anti-arenavirus mAbs. ***(a) Studies in guinea pigs***. MAbs that satisfy the criteria stated above and that display stage-appropriate manufacturability characteristics will advance to *in vivo* testing. We will use established models of LASV[11,39] and MACV[40,41] infection in guinea pigs. PABVAX **Core E** has access to many strains from different LASV and MACV lineages, which will be tested. For prioritization, we will focus on LASV lineage II, III, and IV strains and MACV lineage I, II, and VIII strains for which uniformly lethal models are available. Groups of 6 outbred Hartley guinea pigs will be treated with low, medium, and high doses of single or combinations of anti-LASV or anti-MACVs mAb, or isotype controls at day +1 after inoculation with the LASVs or MACVs This approach allows us to determine whether combinations provide advantage over the best monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-LASV and anti-MACV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements (blood) at day 5 or 6 after LASV or MACV challenge and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal LASV and MACV mAb pairs that are comparable to or outperform previously reported mAbs for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-LASV and anti-MACV mAb combination against LASV or MACV in NHPs. As a final test of an optimal anti-LASV and anti-MACV mAb combinations, we will assess protective efficacy of the lead anti-LASV mAb pair in NHPs as well as the anti-MACV mAb pair in NHPs. These anti-LASV and anti-MACV mAb pairs will be selected based on the *in vitro* and guinea pig data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys will only be considered pending success in the guinea pig studies and consultation with the external Scientific Advisory Board (SAB) and NIAID program staff.

E.1.5. Applying lessons learned in LASV and MACV research with the selected test case pathogens for mAb generation: Lujo and Chapare mAbs. Once we have completed the LASV and MACV discovery campaigns, will turn to the test case pathogens to see if we can use the prototype pathogen approach to related viruses, Lujo (LUJV) and Chapare (CHAPV). We do not have access to human PBMCs from LUJV - or CHAPV-immune donors. As an alternative approach, we will use humanized mouse models as a source of B cells encoding fully human monoclonal antibodies. The Vanderbilt core investigators have an established fully executed agreement to use Alloy human antibody mice for this purpose (See Letter of Support). The Alloy ATX-Gx™ mouse is one of the most effective *in vivo* human antibody discovery platforms, with over 140 partners and counting. This mouse system was originally invented and validated inside a major pharma company and then further developed by Alloy. This foundational suite of highly immunocompetent transgenic mice is engineered to drive the greatest potential diversity of unique human antibodies binding to the viral target of interest, with broad epitopic coverage. The comprehensive functional human antibody repertoire in these mice is optimized for human Ab sequence developability and diversity. Alloy and Vanderbilt have already executed a simple licensing process for use of the ATX-Gx platform, and as an established partner the Vanderbilt core team will access the expanding portfolio of transgenic Alloy strains designed to address a range of discovery challenges (including diverse Ab isotypes). Mice will be immunized with conformationally correct recombinant protein antigens (**Table 2**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for the target of interest. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for LASV and MACV.

Table 2. Antigens available from RP1
LUJV NP NTD
LUJV GPe
LUJV NP NTD +GPe
CHAPV NP NTD
CHAPV GPe
CHAPV NP NTD +GPe

<u>**Anticipated results, potential problems, and alternative approaches**</u>**.** In this Aim, we will identify human anti-LASV and anti-MACV mAb combinations with optimal neutralizing and protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging LASV, MACV, or related arenaviruses. Given our previous ability to generate human antiviral mAbs, we do not anticipate problems in isolating anti-LASV or anti-MACV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently neutralizing anti-LASV or anti-MACV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody™) on enriched B cells (*i.e.*, memory B cells) isolated from human LASV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in LASV-immune PBMC samples and identify clonal lineages, including

siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-LASV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-LASV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-LASV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-LASV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the high-throughput Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E2. Specific Aim 2. Discover a protective CCHF virus mAb combination therapy. Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[14,42,43]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E).** As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.

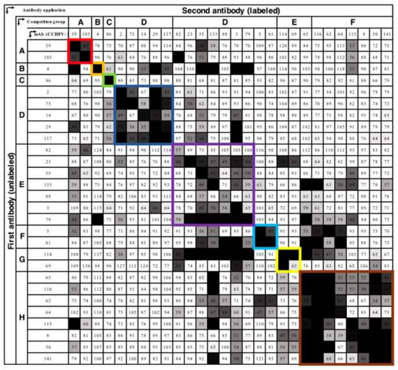

Figure 2. Competition assessment of human Abs to CCHFV using M-segment expressing cells. We tested 28 mAbs in competition assays. MAbs are displayed in 8 groups (A-H) based on their ability to compete for binding. Values shown are the % of binding that occurred during competition compared to non-competed binding of the mAb. This value was normalized to 100%. The values are also indicated by the box fill color; darker colors toward black indicate higher competition and lighter colors toward white indicate less competition, on a gradient scale.

<u>Preliminary Data</u>. ***(a) Potently neutralizing and non-neutralizing human anti-CCHFV mAbs.*** In this Aim, we will rapidly discover human anti-CCHFV mAbs from CCHFV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify an optimal anti-CCHFV mAb combination and further advance the discovery platform for rapid response against emerging nairoviruses. In preliminary experiments in collaboration with investigators in **RP1** and **Core E**, the Crowe laboratory has previously isolated and characterized panels of neutralizing human anti-CCHFV human mAbs from individuals who were convalescent after naturally-acquired laboratory-confirmed infection in Spain or Turkey. We isolated a panel of antibodies against CCHFV that recognize the M-segment in transfected cells, and they exhibit a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 2**). This panel contains cross-binding and cross-neutralizing antibodies for diverse strains. We observed a human mAb designated CCHF-82 competing with competition group 1 and group 2 antibodies from the previously isolated GP38-reactive murine antibodies. CCHF-82 showed some protection in a STAT1$^{-/-}$ mouse model of infection conducted by investigators in **RP1 (Fig 3)**. Further investigation with this antibody in the IbAr10200 prophylaxis and therapeutic models is warranted. In contrast, the CCHFV-neutralizing mAbs that we isolated and tested *in vivo* did not perform well in the Turkish strain STAT1$^{-/-}$ mouse model. We now know protection in this model is possible, given the work from Fels *et al*., 2021 who showed that potent antibodies against various sites on the Gc surface can afford protection in this model[42]. However, in their study they did not test GP38-reactive antibodies. To our knowledge, our preliminary

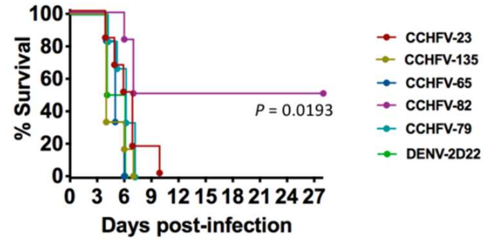

Figure 3. Human mAb CCHF-82 provides partial protection as a post-exposure prophylaxis against the Turkish strain of CCHFV in the STAT1$^{-/-}$ mouse model. A single dose of mAb was administered by the IP route to mice (n=6 per group (3 male, 3 female)) at 30 min post infections. A lethal dose of Turkish strain of CCHFV was administered. CCHF mAbs or DENV-2D22 (an isotype-matched negative control mAb) were tested in a single dose of 250 µg per mouse. Kaplan-Meier survival curves were statistically analyzed using a log-rank (Mantel-Cox) test where mAb-treated animals (*P* value shown in figure) were compared to animals treated with the DENV-2D22 negative control mAb. Unpublished data.

data is the first indication that a human GP38-targeting antibody can provide some protection in this mouse model.

Non-neutralizing murine mAbs protect in mouse models of infection[14], but it is unknown if the human antibody response could provide similar levels of protection. Murine mAb 13G8 binds to GP38 and does not neutralize CCHFV. This mAb provides protection against lethal challenge as a prophylaxis and a therapeutic against CCHFV. In preliminary experiments we sought to understand if human survivors of CCHFV infection possess B cells encoding mAbs that bind a similar epitope as 13G8 and provide protection. CCHF-82 competes for binding with 13G8 to preGn-transfected cells containing GP38. This antibody offered some protection as a post-exposure prophylaxis against the Turkish strain of CCHFV (**Fig 3**). Human antibodies like this should be further studied and the frequency of these clones should be elucidated to understand if this is a feasible strategy for future vaccine efforts for humans. The epitope that 13G8 and CCHF-82 identifies could inform vaccine design if these antibodies offer protection in larger animal models. Understanding how non-neutralizing antibodies function is critical in efforts to validate the GP38-binding class of antibodies. Golden *et al.* undertook a study to understand the mechanisms 13G8 functioned and observed that complement activation contributes to the protective capacity of this antibody[14]. Here, in studies of CCHF-82, we also will assess complement activation in mouse models of infection to fully understand the function of this mAb *in vivo*. Furthermore, if this mAb protects in the CCHFV IbAr10200 strain challenge model, assessing its mechanism is warranted. Together with **Core D**, we will assess IgG molecules with complement knockout ("KA") and Fc gamma receptor knockout (LALA-PG) mutations in the Fc domain to study this matter. Human mAb CCHF-82 displayed limited cross-protection against experimental infection with the Turkish strain of CCHFV. Our antibody isolation here will aim to identify cross-protective non-neutralizing antibodies reactive with GP38. Recently, vaccinating with NP was shown to be another way to elicit non- neutralizing antibodies[44]. The NP of CCHFV is more conserved than the glycoproteins and might also be a non-canonical target for the identification of cross-protective non-neutralizing antibodies[44].

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant viral surface protein antigens, cloning of Ab variable genes, and then expression of mAbs as as described above in Specific Aim 1.

E.2.1. Rapid isolation of human anti-CCHFV mAbs. *(a) Immune cells.* We have obtained human PBMCs from a number of previously CCHFV-infected individuals. One was a health care survivor who was infected while caring for a patient in Spain[45]. We also collected a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Turkey, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples were only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies were approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples were deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-CCHFV mAbs rapidly, as if we had to respond to a nairovirus outbreak in real time. Our ability to respond rapidly in the setting of outbreaks is well-documented in the lay press (60 Minutes features and others) and the scientific literature, e.g., 78 days from sample to protection of NHPs for Zika virus[32], and 25 days from PBMC sample receipt to delivery of the antibody genes for the approved COVID-19 drug Evusheld to AstraZeneca[1]. Here, we will single-cell sort antigen-specific B cells using recombinant CCHFV proteins (GP38, Gc, Gn, Gc/Gn) or non-infectious particles as bait as described in Specific Aim 1. We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D**, (**Table 3**) or by **RP1 (Table 4)**.

Table 3. Core D CCHFV antigens
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF NP

(c) Recombinant mAb cloning. As described in Specific Aim 1 above.

(d) Micro-scale mAb expression. As described in Specific Aim 1 above.

(e) A CPE-based CCHFV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of CCHFV. We will use a rVSV CCHFV surrogate that expresses CCHFV surface proteins and causes CPE[35]. Investigators in **RP1** have the VSV-CCHFV-GFP versions of clades 1 through 7 based on this publication. We will obtain these VSV constructs from investigators in **PABVAX**. Existing VSV vectors will be provided by investigators in **RP1 and Core E**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against CCHFV. These mAbs will be moved forward for additional studies to identify an optimal anti-CCHFV mAb combination. GP38-reactive clones will be advanced based on avidity ranking since they are not likely to be neutralizing but may be protective *in vivo*.

Table 4. Antigens available from RP1
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF Turkey NP
CCHF Turkey GnGc+GP38+NP
CCHF Turkey GnGc+GP38
CCHF Turkey GP38+NP
CCHF Turkey GnGc+NP

E.2.2. Identifying CCHFV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind CCHFV surface proteins and validate their epitopes. We will perform competition-binding studies with CCHFV Gc/Gn or subunit proteins (as above) or CCHFV virus-like particles (VLPs) made from a replicon system in hand. **Core E** will inactivate CCHFV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site. Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from some of the mAbs shown in the unpublished preliminary data above for the identification of mAbs that can concurrently bind CCHFV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-CCHFV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. GP-38-reactive clones will be screened for synergistic binding as they are not expected to be neutralizing. ***(c) Identification of key binding residues of human anti-CCHFV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-CCHFV constructs at BSL2 in the presence of saturating concentrations of CCHFV mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of VSV-CCHFV escape variants in the presence of mAbs.

E.2.3. Structural basis of neutralization by anti-CCHFV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.2.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies in STAT1$^{-/-}$ mouse models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (Core D), will advance to *in vivo* testing. We will use an established STAT1$^{-/-}$ mouse model of CCHFV infection that the **RP1** and **Core E** investigators have deployed[35]. To benchmark our mAbs, a separate set of STAT1$^{-/-}$ mice will be treated with CCHF-82, which we showed in unpublished preliminary data above confers substantial but incomplete protection (**Fig 3**). The **Core E** laboratory has access to strains representing all 6 clades that can be tested. Groups of 6-8 STAT1$^{-/-}$ mice will be treated with low, medium, and high doses) of single or combinations of anti-CCHFV mAb, or isotype controls at day +1 after inoculation with CCHFVs. This approach allows us to determine whether combinations provide advantage over the best CCHFV monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after CCHFV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal CCHFV mAb pairs that are comparable to or outperform the mAbs we reported above. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-CCHFV mAb combination against CCHFV in NHPs. As a final test of an optimal anti-CCHFV mAb combination, we will assess efficacy of the lead anti-CCHFV mAb pair in NHPs (**Core E**), to be selected based on the *in vitro,* STAT1$^{-/-}$ mice, and manufacturability data (**Core D**). Down-selection for testing in cynomolgus monkeys would only be considered with success in the STAT1$^{-/-}$ mouse studies and in consultation with the external SAB and NIAID program staff.

E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs. Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human mAbs, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-CCHFV mAb combinations with optimal protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging CCHFV or related nairoviruses. Given our previous ability to generate human anti-CCHFV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-CCHFV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-CCHFV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (i.e., memory B cells) isolated from human CCHFV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in CCHFV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-CCHFV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-CCHFV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-CCHFV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-CCHFV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

Table 5. Antigens available from RP1
Kasokero GnGC
Kasokero GP38
Kasokero NP
Kasokero GnGc +GP38 +NP
Kasokero GnGc +GP38
Kasokero GnGc +NP
Kasokero GP38 +NP

E3. Specific Aim 3. Establish optimal mAb combination therapies against henipaviruses Hendra (HeV) and Nipah (NiV) viruses. We will use a large panel of potent anti-henipavirus mAbs that we previously made from the memory B cells of immune individuals and generate new antibodies targeting the fusion (F) protein. **RP2** will provide new antigens for mAb selection and BSL-2-compatible viruses for neutralization screens including Cedar virus constructs displaying HeV and NiV F and G proteins. We will identify optimal mAb combinations to enhance henipavirus therapy, minimize viral escape, and improve treatment efficacy through synergy. *In vitro* competition-binding, neutralization, and mechanistic studies will be conducted, including authentic virus neutralization with **PABVAX Core E**. We will refine the recognized epitopes and investigate the structural basis for optimal combination neutralization of henipaviruses using HeV and NiV as prototypes. *In vivo* assessment of mAb combinations will determine the minimal effective dose for protection and the ability to prevent *in vivo* escape. Protective efficacy of lead mAb combinations will be assessed in hamster models of NiV and HeV infection with **Core E**. Leads will be transferred to **Core D**. As a final test of optimal henipavirus mAb combinations, we will assess protective efficacy of the lead henipavirus mAb pairs in NHPs, to be selected based on the *in vitro* and hamster data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in African green monkey (AGM) henipavirus model studies would only be considered pending success in the hamster studies and consultation with the external SAB and NIAID program staff. The HeV and NiV studies will be conducted in years 1-3 and then applied to the plug-and-play test case Langya virus in years 2-5. If **RP2** and **Core E** cannot rescue recombinant Langya virus, the lessons learned in Years 1-3 will be applied to the alternate "plug and play" test viruses Angavokely or Sosuga (SOSV) virus. Preliminary data on SOSV mAbs are provided.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-NIV/HeV mAbs.*** In this Aim, we will further adapt approaches we have developed in preliminary experiments with henipavirus mAb studies by identifying a potently protective combination of anti-NiV/HeV human mAbs. Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups. This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains. In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and outstanding potency anti-G antibodies with extensive characterization, including synergistic neutralization.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant henipavirus F protein antigens, as described above in Specific Aim 1.

E.3.1. Rapid isolation of human anti-HeV/NiV mAbs. ***(a) Immune cells.*** We are obtaining human PBMCs from a number of previously NiV-infected individuals who acquired immunity following natural infection in collaboration with the **icddr,b**, an international health research institute based in Dhaka, Bangladesh. We are collecting a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Bangladesh, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies are approved by the icddr,b and Vanderbilt University Medical Center IRBs. PBMCs isolated from the blood samples are deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their use in this **RP4,** if funded. ***(b) Applying mAb technologies for new mAb isolation***. We will use sorting of antigen-specific B cells, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer antigen-specific B cells. We will sort soluble fusion (sF) or attachment (G) protein-specific cells with recombinant viral surface protein antigens from **Core D** (**Table 6**).

(c) Ab screening and sequence analysis. Our goal is to identify human anti-NiV/HeV mAbs rapidly, as if we had to respond to a paramyxovirus outbreak in real time. We will single-cell sort antigen-specific B cells using recombinant HeV and NiV proteins (sF or G) as bait as described in Specific Aim 1 above.

(d) Recombinant mAb cloning. As described in Specific Aim 1 above.

(e) Micro-scale mAb expression. As described in Specific Aim 1 above.

(f) CPE-based HeV/NiV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of HeV and NiV. We will use rVSV HeV and NiV surrogates that expresses henipaviurs surface proteins and cause CPE. Investigators in **RP2 and RP3** have VSV-NiV F and VSV-NiV G with GFP[34], VSV-NiV G without GFP[36] and VSV-HeVG (Geisbert and Cross, unpublished). We will obtain these all these VSV constructs from investigators in **PABVAX**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against HeV and NiV. We also can use Cedar virus constructs for neutralization assays as the have reduced pathogenicity[46] and can be used in high-throughput screening assays for henipavirus antiviral discovery[47]. Cedar viruses will be provided by Dr. Broder of **RP2**. The mAbs with most potent activity will be moved forward for additional studies to identify an optimal anti-HeV/NiV mAb combination.

Table 6. Core D Henipavirus antigens
Nipah sF
Hendra sF
NiV G

E.3.2. Identifying HeV/NiV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind HeV/NiV surface proteins and validate their epitopes. We will perform competition-binding studies with HeV/NiV sF or G proteins (as above). We will include positive controls made recombinantly from some of the mAbs shown in the preliminary data above for the identification of mAbs that can concurrently bind HeV/NiV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti- HeV/NiV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key binding residues of human anti-HeV/NiV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-NiV F, VSV-NiV G and VSV-HeV G constructs at BSL-2 in the presence of saturating concentrations of HeV/NiV mAbs (single or combination) using RTCA to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test the level of infection of VSV-HeV/NiV escape variants in the presence of mAbs.

E.3.3. Structural basis of neutralization by anti-HeV/NiV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.3.4. Protective efficacy of human anti-henipavirus mAbs. ***(a) Studies in hamsters***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (**Core D**), will advance to *in vivo* testing. We will use an established hamster model of NiV or HeV infection that the **Core E** investigators have deployed. To

benchmark our mAbs, a separate set of hamsters will be treated with G mAbs HENV-103+HENV-117, which we showed in previous data above confers substantial protection[8]. Groups of 6-8 hamsters will be treated with low, medium, or high IgG doses) of single or combinations of anti-HeV/NiV mAbs, or isotype controls at day +1 after inoculation with HeV or NiV. This approach allows us to determine whether combinations provide advantage over the best HeV or NiV mAb monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-HeV and anti-NiV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after HeV or NiV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal HeV/NiV mAb pairs that are comparable to or outperform the mAbs we reported previously. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-HeV/NiV mAb combination against HeV and NiV in NHPs. As a final test of an optimal anti-HeV/NiV mAb combination, we may assess efficacy of the lead anti-HeV/NiV mAb pair in NHPs (**Core E**), selected based on the *in vitro,* hamster data, and manufacturability data (**Core D**). Down-selection for testing in AGMs would only be considered with success in the hamster studies and in consultation with the external SAB and NIAID program staff.

E.3.5. Applying lessons learned in HeV/NiV research to Langya, Angavokely and Sosuga viruses. Once we have completed the HeV/NiV discovery campaigns, will turn to the test case pathogen Langya (or alternate Angavokely and Sosuga (SOSV) viruses) to see if we can use the prototype pathogen approach for this virus. We have plenty of human PBMCs from the sole identified SOSV-immune individual and have already shown that it is possible to obtain HN- and F-specific neutralizing antibodies from this individual's B cells[48]. We also have already prepared recombinant SOSV prefusion F and HN proteins[48]. We do not have access to human PBMCs from Langya- or Angavokely-immune donors. For those mAb discovery campaigns, we will use Alloy humanized mouse models as a source of B cells encoding human mAbs, as above. Mice will be immunized with recombinant protein antigens (**Table 7**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Langya or Angavokely. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for Hev/NiV described above.

Table 7. Paramyxovirus antigens from Core D
Mojang sF
Angavokely sF
Langya sF

<u>Anticipated results, potential problems, and alternative approaches</u>. In this Aim, we will identify human anti-HeV/NiV mAb combinations with optimal protective activity. These mAb combinations will be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging HeV/NiV or related paramyxoviruses. Given our previous ability to generate human anti-HeV/NiV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-HeV/NiV mAbs. We may observe that new mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-HeV/NiV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody™) on enriched B cells *(i.e., memory B cells) isolated from human NiV-immune PBMC samples. In addition, we can deep sequence the B* cell repertoires present in NiV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-HeV/NiV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-HeV/NiV mAb combinations that can inform vaccine design (**RP2**) for related viruses being used as test cases in years 2 through 5.

F. Scientific rigor. *<u>Experimental design</u>.* Experiments will include biological and technical replicates, and results will be analyzed by parametric or non-parametric analyses (depending on data distribution) and corrections for multiple comparisons. EC_{50} (binding) and IC_{50} (neutralization) values will be reported only following at least 3 identically conducted experiments. All mAb screening will incorporate appropriate + and - controls. Positive signals in binding assays will be defined as >3 SD above background. ***<u>Protein quality</u>.*** We have a high capacity to purify proteins using a Protein Maker workstation that allows purification chromatography on 24 parallel columns. We perform QC on protein reagents using a Maurice instrument with capillary electrophoresis technology to automate our protein profiling either by size or charge, providing cIEF and CE-SDS data. Animal ***studies*** will be performed blinded with independent replicates and include negative (*e.g.*, placebo), and positive (when available) controls. We will use power calculations (**Data Management Core B**) to determine sample sizes. For virological studies, power analysis was performed using accepted values for type I error (0.05) and power (80%). The values for population variance (15×) are based on existing data sets and our experience with rodent challenge studies. We will perform experiments at least 2-3 independent times (n = 4-5 each) to attain n = 12-15 per arm. Smaller group sizes can be used if differences are larger than expected.

G. Relevant biological variables. The human mAbs are derived from immune human donors or Alloy mice. Humans. Human Abs will be isolated for biological, structural, and functional studies at a clonal level. The sample size is not powered for large scale epidemiologic or clinical study conclusions. There is no evidence that sex, age, body mass index, or underlying health conditions other than immunodeficiency or immunosuppressive treatments alters the activity of cloned Abs in structure/function studies. The samples are de-identified without patient specific identifiers to protect the health information of the donors; thus, research with these samples is considered *Not Human Subjects Research* category activity. Mice, hamsters, guinea pigs, NHP, and viruses are also biological systems used in this application. Conventional animal housing conditions will be used because there is no evidence that altered room temperatures and light/dark cycles influence paramyxovirus, nairovirus, or arenavirus pathogenesis. Mice, hamsters, guinea pigs, and NHPs of both sexes will be used, and phenotypes will be monitored for sex-based differences. Virus stocks will be either low passage seed stocks from natural isolates or generated from existing cDNA clones and sequence-confirmed prior to use for infection studies.

H. Milestones and quantitative Go/No-Go criteria. Through **Aims 1-3**, we have proposed experiments that will advance our knowledge of the mAb-mediated mechanisms of action against paramyxoviruses, nairoviruses, and arenaviruses. In each Aim, we will use down-selection criteria (as below) to advance mAb combinations or variants for additional studies. By year 3, we will have defined the principles that govern optimal mAb combinations for paramyxoviruses, nairoviruses, and arenaviruses and the rational approaches needed to select mAbs for potency and breadth. We will use the following metrics to advance mAbs: ***(a) Neutralizing activity.*** All mAbs will have neutralizing potency < 10 ng/mL, EC_{50} value; ***(b) Binding affinity***. MAbs will be of high affinity to surface proteins or virions ($K_D < 5$ nM). ***(c) Protein stability and production quality.*** MAbs must produce at high levels without optimization (> 100 mg/L) and show biochemical/functional stability at 4°C or -80°C (>90% retention of binding and neutralization activity at 30 d). ***(d) Synergy***. Combinations of 2 mAbs against a given virus ideally will show synergy of neutralization *in vitro* or at a minimum, additive inhibition (no antagonism); ***(e) Epitopes***. To avoid resistance, mAbs in combination will bind 2 epitopes (determined by competition binding (<20% reduction in binding), mapping, and structural studies. ***(f) Resistance*** against mAbs will be assessed for virus escape. Deep sequencing of viruses that emerge from cells or animals treated with mAbs will inform prioritization. Combinations of mAbs with high genetic barriers to resistance (<10× loss in EC_{50}) will be advanced. ***(g) Protection in mice, hamsters, guinea pigs, or NHPs.*** The following metrics can be used: (i) Protection against lethal challenge by homologous virus (>90% survival; (ii) Protection against clinical disease by homologous virus (>80%); (iii) Protection against infection by homologous virus (>100-fold reduction in viral titers in multiple organs). ***(h) pK measurements***. For lead protective mAbs with mutations that should confer extended half-life, we will perform pK studies in NHPs (with **Core D** and **E**) to assess levels over time with a goal of a human predicted serum half-life of >6 months.

Timeline for RP4	Year 1	Year 2	Year 3		Year 4	Year 5
Prototype mAb generation and testing: **LASV and MACV mAbs**	X	X	X		X	
Test case mAb generation and testing: **Lujo and Chapare mAbs**			X		X	X
Prototype mAb generation and testing: **CCHFV mAbs**	X	X	X		X	
Test case mAb generation and testing: **Kasokero mAbs**			X		X	X
Prototype mAb generation and testing: **NIV/HENV mAbs**	X	X	X			
Test case mAb generation: **Langya, Angavokely/Sosuga mAbs**		X	X		X	X
In vivo efficacy studies with **Core E**	X	X	X		X	X
Translational activities with **Core D**	X	X	X		X	X

LITERATURE CITED – RP4

1. Zost, S.J., *et al.* Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* **26**, 1422-1427 (2020).
2. Fischer, W.A., II & Wohl, D.A. Moving Lassa Fever Research and Care Into the 21st Century. *The Journal of Infectious Diseases* **215**, 1779-1781 (2017).
3. Ezeomah, C., *et al.* Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. *Open Forum Infect Dis* **6**, ofz512 (2019).
4. Li, A.L., *et al.* Ophthalmic manifestations and vision impairment in Lassa fever survivors. *PloS one* **15**, e0243766 (2020).
5. Webb, P.A., Johnson, K.M., Mackenzie, R.B. & Kuns, M.L. Some characteristics of Machupo virus, causative agent of Bolivian hemorrhagic fever. *Am J Trop Med Hyg* **16**, 531-538 (1967).
6. Hawman, D.W. & Feldmann, H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology* (2023).
7. Mackenzie, J., Wang, L. & Broder, C.C. Henipaviruses. in *Fields Virology*, Vol. 2 1070-1085 (2013).
8. Doyle, M.P., *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021).
9. Dang, H.V., *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021).
10. Cross, R.W., *et al.* Antibody therapy for Lassa fever. *Curr Opin Virol* **37**, 97-104 (2019).
11. Cross, R.W., *et al.* Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral research* **133**, 218-222 (2016).
12. Cartwright, H.N., Barbeau, D.J. & McElroy, A.K. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere* **6**, e0055621 (2021).
13. Gutjahr, B., *et al.* Two monoclonal antibodies against glycoprotein Gn protect mice from Rift Valley Fever challenge by cooperative effects. *PLoS Negl Trop Dis* **14**, e0008143 (2020).
14. Golden, J.W., *et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv* **5**, eaaw9535 (2019).
15. Zalevsky, J., *et al.* Enhanced antibody half-life improves in vivo activity. *Nat Biotechnol* **28**, 157-159 (2010).
16. Dall'Acqua, W.F., *et al.* Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* **169**, 5171-5180 (2002).
17. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Levis, S.C. & Maiztegui, J.I. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* **2**, 255-256 (1984).
18. Jahrling, P.B. & Peters, C.J. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infection and immunity* **44**, 528-533 (1984).
19. Chen, E.C., *et al.* Systematic analysis of human antibody response to ebolavirus glycoprotein shows high prevalence of neutralizing public clonotypes. *Cell Rep* **42**, 112370 (2023).
20. Buck, T.K., *et al.* Neutralizing Antibodies against Lassa Virus Lineage I. *mBio* **13**, e0127822 (2022).
21. Enriquez, A.S., *et al.* Delineating the mechanism of anti-Lassa virus GPC-A neutralizing antibodies. *Cell Rep* **39**, 110841 (2022).
22. Hastie, K.M., *et al.* Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell* **178**, 1004-1015 e1014 (2019).
23. Hastie, K.M., *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).

24. Li, H., *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991 (2022).
25. Gilchuk, P., *et al.* Pan-ebolavirus protective therapy by two multifunctional human antibodies. *Cell* **184**, 5593-5607 e5518 (2021).
26. Chen, E.C., *et al.* Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* **36**, 109604 (2021).
27. Dong, J., *et al.* Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-1244 (2021).
28. Greaney, A.J., *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).
29. Kramer, K.J., *et al.* Potent neutralization of SARS-CoV-2 variants of concern by an antibody with an uncommon genetic signature and structural mode of spike recognition. *Cell Rep* **37**, 109784 (2021).
30. Suryadevara, N., *et al.* Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell* **184**, 2316-2331 e2315 (2021).
31. Zost, S.J., *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449 (2020).
32. Gilchuk, P., *et al.* Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics. *Nat Biomed Eng* **4**, 1030-1043 (2020).
33. Cross, R.W., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep* **40**, 111094 (2022).
34. Mire, C.E., *et al.* Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J* **10**, 353 (2013).
35. Rodriguez, S.E., *et al.* Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep* **9**, 7755 (2019).
36. Foster, S.L., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. *Proc Natl Acad Sci U S A* **119**, e2200065119 (2022).
37. Branco, L.M., *et al.* Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* **7**, 279 (2010).
38. Starr, T.N., *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
39. Deschambault, Y., *et al.* An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. *Viruses* **15**(2023).
40. Bell, T.M., *et al.* Pathogenesis of Bolivian Hemorrhagic Fever in Guinea Pigs. *Vet Pathol* **53**, 190-199 (2016).
41. Golden, J.W., *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* **7**, 4679 (2017).
42. Fels, J.M., *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell* **184**, 3486-3501 e3421 (2021).
43. Mishra, A.K., *et al.* Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies. *Science* **375**, 104-109 (2022).
44. Leventhal, S.S., *et al.* Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *EBioMedicine* **82**, 104188 (2022).
45. Negredo, A., *et al.* Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med* **377**, 154-161 (2017).
46. Laing, E.D., *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018).
47. Amaya, M., *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084 (2021).

48. Parrington, H.M., *et al.* Potently neutralizing human mAbs against the zoonotic pararubulavirus Sosuga virus. *JCI Insight* **8**(2023).

A. BACKGROUND AND SIGNIFICANCE. Paramyxoviruses, nairoviruses, and arenaviruses are widely distributed RNA viruses that cause severe human syndromes. Because of their geographical distribution and expansion, changes in human demographics and increased exposures to wild animals including rodents and bats, and their capacity for human-to-human spread, these viruses have the potential to cause pandemics. Together with the other investigators in **PABVAX**, we have selected several important prototype paramyxoviruses, nairoviruses, and arenaviruses to study to learn the rules governing immunity to these pathogens. The work on the pathogens selected is justified based on their prevalence and severity alone, but also because of their ability to serve as prototypes for genetically related viruses. We chose the viruses for focus in this RP4 on human antibody development because of their clinical relevance and because several will be studied as prototypes for vaccine development in **RP1** (arenaviruses, nairoviruses) and **RP2** (paramyxoviruses). Among the arenaviruses, we will target Lassa virus (LASV) and Machupo virus (MACV), and among the nairoviruses, we will study Crimean–Congo hemorrhagic fever virus (CCHFV). In tandem with the vaccine projects in **RP2** focused on paramyxoviruses, we will study Nipah virus (NiV) and Hendra virus (HeV) as the prototypes. Information about the principles we discover about critical neutralizing epitopes identified here in **RP4** with prototype viruses can feed back into all other **Projects** that address the subsequent plug and play test cases in later years (Lujo, Chapare, Kasokero, Langya).

LASV is an Old World arenavirus (OWAV) with a single-stranded RNA virus belonging to the Arenaviridae family. Infection in humans is often the result of exposure to infected excreta from the rodent reservoir, *Mastomys natalensis* where up to 500,000 cases and an estimated 5,000 deaths occur annually in West Africa[2]. While rodent infection is largely benign, human infection can result in severe hemorrhagic disease with neurological complications and long-term hearing and vision sequelae[3,4]. There are no approved vaccines or treatments for LASV infection. ~~The virus possesses a bi-segmented genome comprising large (L) and small (S) segments, both of which are enclosed within a lipid envelope. The L segment (~7.2 kb) encodes the RNA-dependent RNA polymerase (RdRp) and a zinc-binding protein (Z), while the S segment (~3.4 kb) codes for the nucleoprotein (NP) and the glycoprotein precursor (GPC). The GPC is further cleaved into two surface glycoproteins, GP1 and GP2, which play critical roles in viral entry. The genetic diversity of LASV can be classified into six lineages (I-VI), with lineages III and IV being the most prevalent[5]. Genetic variation arises mainly due to the error-prone nature of the viral RNA polymerase, leading to the emergence of quasi-species. This diversity contributes to the virus's ability to evade host immune responses and pose challenges to vaccine development[6]. The surface glycoproteins GP1 and GP2, are responsible for receptor binding and membrane fusion, respectively. While GP1 is highly variable among strains, GP2 is more conserved. The nucleoprotein (NP) is another major antigen, and its immunodominant epitopes are targeted by the host's immune response. Its genetic diversity and antigenic properties complicate diagnosis and vaccine development, emphasizing the need for continued research on medical countermeasures.~~

MACV is a New World Arenavirus (NWAV) and the causative agent of Bolivian Hemorrhagic fever. Humans are infected after exposure to excreta from the reservoir, *Calomys callosus,* the large vesper mouse. Disease in humans has a slow onset, but can progress to severe hemorrhagic fever coupled to insidious neurological complications including tremors, seizures, and paralysis[7]. ~~MACV has a similar genomic organization and contains a GPC, NP, Z, and L polymerase proteins. Genetic diversity of MACV is delineated into 8 lineages (I-VIII) where there is growing concern of an expanding host range of *C. callosus* is responsible for outbreaks outside the known endemic region[8]. While the host entry receptor for NWAV is not shared with OWAV, the role for GP to drive immunity as a major antigen alongside NP. The better studied Junin has a regionally approved vaccine[9] and convalescent plasma program[10] available to prevent and treat disease, respectively.~~ There are no approved vaccines or therapies approved for MACV.~~any other NWAV, including MACV. Further, it is not well established whether these countermeasures have potential for cross protect other NWAV such as MACV, underscoring the value of continued efforts to develop broadly active countermeasures to safeguard against lethal disease.~~

CCHFV is a highly pathogenic tick-borne virus classified under the Nairovirus genus within the Bunyaviridae family[11]. CCHF outbreaks have a case fatality rate of up to 40% and there are no vaccines or treatments approved for human use.~~It has a negative-sense, single-stranded RNA genome comprising three segments: Small (S), Medium (M), and Large (L). These segments are enclosed within a lipid envelope and code for various structural and non-structural proteins. The S segment (~1.7 kb) encodes the nucleocapsid protein (N), which is essential for viral replication and assembly. The M segment (~5.3 kb) codes for the glycoprotein precursor (GPC), which is post-translationally cleaved into two surface glycoproteins, Gn and Gc. These glycoproteins are involved in viral entry and fusion. The L segment (~12.1 kb) encodes the RNA-dependent RNA polymerase (RdRp), responsible for viral RNA synthesis. CCHFV exhibits significant genetic diversity, with at least seven distinct clades (Europe 1, Europe 2, Africa 1-3, and Asia 1-2) based on the partial S segment sequences[12]. This diversity is attributed to the segmented genome, geographical distribution, and error-prone viral RNA polymerase, leading~~

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~~to the emergence of various quasi-species. The major antigens of CCHFV include the surface glycoproteins Gn and Gc, as well as the nucleocapsid protein (N)[13,14]. These antigens are targeted by the host immune response. CCHFV is genetically diverse, so breadth of reactivity will be a focus of our discovery efforts.~~

NiV/HeV. Bat borne**,** NiV and HeV are enveloped, negative-sense, single-stranded RNA viruses belonging to the Henipavirus genus within the Paramyxoviridae family[15]. These emerging zoonotic viruses pose a significant threat to human and animal health due to their high fatality rates and broad host range. ~~The genomes of NiV and HeV are approximately 18.2 kb or 18.4 kb in length, respectively, and share a similar gene organization. They encode six major structural proteins: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment glycoprotein (G), and large polymerase (L) proteins. The N, P, and L proteins form the viral RNA-dependent RNA polymerase (RdRp) complex necessary for transcription and replication, whereas the M, F, and G proteins are essential for virus assembly and entry. Genetic diversity in henipaviruses is relatively low compared to other RNA viruses, with NiV strains showing 91-100% nucleotide similarity and HeV strains displaying 83-100% similarity[16]. However, the error-prone nature of the RdRp can still generate genetic variability, leading to the emergence of distinct lineages. The major surface antigens of Nipah and Hendra viruses are the attachment glycoprotein (G) and fusion protein (F). The G protein facilitates receptor binding, while the F protein mediates viral membrane fusion. Both antigens are involved in host immune responses and are potential targets for vaccine development, and the neutralizing antibody approaches we propose here. NiV and HeV exhibit relatively low genetic diversity, and in preliminary studies, we have identified human mAbs that neutralize HeV, NiV Bangladesh and NiV Malaysia strains[17-19]. Antibodies to the major surface antigens, the G and F proteins, are therefore, promising candidates for development as prophylactic or therapeutic molecules.~~As with the other prototype virus in the **PABAX** Center there are no vaccines or treatments against henipaviruses licensed for human use.

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Antibodies (Abs) are important correlates of protection for paramyxoviruses, nairoviruses, and arenaviruses. Immune sera against members of each of these groups of viruses has been shown to exhibit protective efficacy *in vivo*, which supports the use for Abs as treatment options. However, the specificity, functional properties, and other attributes of protective antibodies are poorly defined for most of these viruses. MAbs to the attachment and fusion surface proteins of paramyxoviruses (such as the G and F proteins of Hendra/Nipah[18,20] and the GP proteins of the arenaviruses like LASV[21,22]) can neutralize and protect against infection in rodent models. CCHFV is a bit of a conundrum in that ultrapotent human neutralizing antibodies to the related Rift Valley fever virus surface proteins are highly protective *in vivo*[23,24], but CCHFV neutralizing antibodies are not protective. In contrast, some non-neutralizing antibodies do protect partially for CCHFV[25]. Clearly, there is a lot to learn about the rules of immunity to these viruses, and the genetic, molecular, and structural basis of antibody-mediated protection for viruses like CCHFV. Based on these data, we propose the use of mAbs as treatment options to learn the rule of how to protect against emerging paramyxoviruses, nairoviruses, and arenaviruses and to develop candidate antibody countermeasures for translational development with **Core D**. To do so, we will further develop our technical methods for rapidly discovering mAbs, in the case of a future unexpected epidemic caused by viruses of these families. Most importantly, we will define the principles governing combination antibody therapy to prevent virus escape from treatment or prevention and to optimize the efficacy of the beneficial effects conferred by passive immunity.

B. SCIENTIFIC PREMISE. The work proposed here in **RP4** is based on the extensive basic, translational, and clinical trial data on the role of neutralizing Abs in protection against infection and disease caused by paramyxoviruses, nairoviruses, and arenaviruses. Although a significant amount of data supports the use of mAbs to prevent or control paramyxovirus, nairovirus, and arenavirus infection, many questions remain, such as the ideal epitopes to target with mAb combination therapy to improve upon neutralization potency, *in vivo* efficacy, and resistance to viral escape. Identifying these epitopes can help inform vaccine design to elicit optimal protective polyclonal responses (in **RP1** and **RP2**, especially with designs for test cases in years 4 and 5). Here we aim to refine our understanding of the correlates of mAb protection against paramyxoviruses, nairoviruses, and arenaviruses to enable the design of a modular "plug-and-play" workflow to rapidly respond to potential pandemics by generating optimized combinations of vaccine-like mAbs with an extended half-life that could be used for long-term prophylaxis (> 6 months) and therapy.

C. INNOVATION. RP4 has many innovative conceptual and technical features including: Conceptual. **(1)** determining the principles that govern optimal combinations of human mAbs against paramyxoviruses, nairoviruses, and arenaviruses for protection and resistance to viral escape; **(2)** the development of a clear workflow that generates therapeutic or preventative antibodies (long half-life mAbs that serve as vaccine surrogates) against emerging or new paramyxoviruses, nairoviruses, and arenaviruses with pandemic potential. Our goal is to define the principles and features, including epitope, combination ratios, and mechanisms of action, that result in optimal efficacy of mAbs against paramyxoviruses, nairoviruses, and arenaviruses in animal models and create a higher barrier for resistance. These studies will serve as paradigms for rapid antibody discovery in response to future paramyxovirus, nairovirus, and arenavirus pandemic threats. Technical. **(3)** large-scale B cell repertoire sequencing with customized methods and software; **(4**) use of humanized (Alloy) mice and novel immunogens from **RP1** and **RP2** and **Core D**; **(5)** single B cell functional (neutralization) assays performed on a Beacon instrument; **(6)** development of high-throughput real-time label-free virus neutralization assays (xCELLigence platform); and **(7)** use of *simultaneous* in parallel screening with several different high-throughput virus-specific B cell isolation approaches (Beacon, 10X Genomics, Rhapsody) to generate and validate best-in-class human mAbs as candidate medical countermeasures.

D. LINKAGE TO OTHER PABVAX PROJECTS AND CORES. The group will focus on developing a modular test case platform for rapid selection of highly neutralizing and/or protective mAbs against paramyxoviruses, nairoviruses, and arenaviruses. Members of our group have active collaborations with most members of the **PABVAX Center** as reflected in previous collaborations and publications (see **Overall** document). We will interact extensively with the Animal Model and Preclinical Evaluation Core (**Core E)** to study neutralization against authentic viruses and to test for protection in small and large animals as well as with **RP3** for testing paramyxovirus mAbs. The knowledge gained from our studies on the structure-function relationships of neutralizing mAb combinations can be applied to rational vaccine design for **RP 1** and **RP2** in later years of the effort when we pivot to apply the lessons learned to the same test case targets as **RP1** and **RP2.** Numerous antigens will be made available to our RP. Finally, long-half-life Fc mutations are now available based on engineering of human IgG1 for enhanced binding to the neonatal Fc receptor (FcRn) such as LS (M428L /N434S)[26], or YTE (M252Y/S254T/T256E)[27] mutations. With ≥ 90 days half-life in humans, our approach will generate mAbs with vaccine-like properties that remain effective for months during a virus epidemic and thus provides a platform for response to future paramyxovirus, nairovirus, and arenavirus pandemic threats, a key goal of our **PABVAX Center.** *Our laboratory previously validated this vaccine surrogate concept when we isolated the human mAbs that formed the basis for Evusheld with YTE long-half-life Fc mutations (used in 70 countries and millions of people) that exhibited > 6 months protective levels in humans after single IM injections*.

E. APPROACH

E.1. Specific Aim 1. Discover potently neutralizing arenavirus combination therapies. For the prototypes, we will focus on LASV, an Old World virus and MACV, a New World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[28,29]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV or MACV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.

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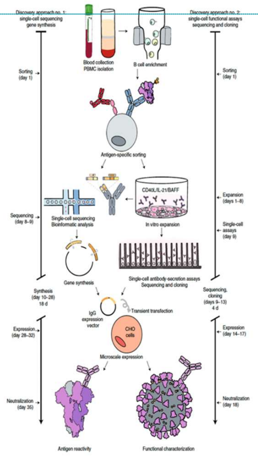

Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion allows us to further assess the functionality of the B cells or for antibody sequencing. Recombinant mAb expression validates the mAb sequence and functional activity. Figure is adapted from [1].

~~**E.1. Specific Aim 1. Discover potently neutralizing arenavirus combination therapies.** For the prototypes, we will focus on LASV, an Old World virus and MACV, a New World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[28,29]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV or MACV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.~~

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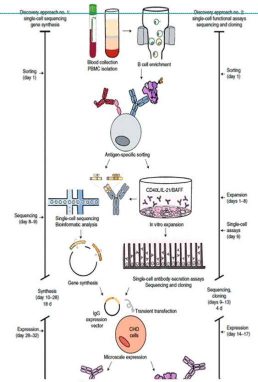

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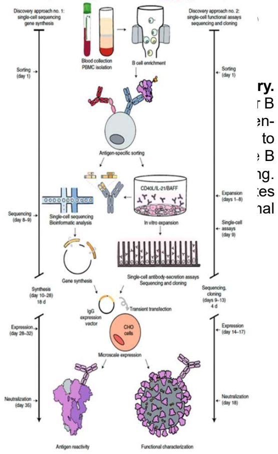


Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion allows us to further assess the functionality of the B cells or for antibody sequencing. Recombinant mAb expression validates the mAb sequence and functional activity. Figure is adapted from [1].

__Preliminary Data__. ***(a) Potently neutralizing human anti-LASV and anti-MACV mAbs.*** The Crowe laboratory has previously characterized panels of neutralizing human anti-viral human mAbs from virus-immune or vaccinated individuals for about 50 viruses, mostly RNA viruses. Isolated peripheral blood mononuclear cells (PBMCs) are used to generate hybridomas or alternatively antigen-sorted at single B cell level and antibody variable genes sequenced to generate mAbs recombinantly. Panels of human anti-viral mAbs are produced rapidly (we recently reported isolation of 15,000 mAb gene pairs from single Ebola-virus-specific human B cells from a single blood sample of an immune individual[30]. Previous studies by others clearly show that representative neutralizing and protective human antibodies can be isolated from LASV-immune subjects[31-35]. In this Aim, we will refine the approaches we have developed previously for other RNA viruses in a proof-of-concept study by identifying potently neutralizing combinations of anti-LASV and anti-MACV human mAbs. We will rapidly discover human anti-LASV mAbs from LASV-immune individuals and human anti-MACV mAbs by immunizing Alloy transgenic mice. Synergy studies will be performed to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify optimal anti-LASV and anti-MACV mAb combinations and further advance the discovery platform for rapid response against emerging arenaviruses.

(b) Advanced mAb technologies. The Crowe laboratory has developed some of the highest yield systems to isolate naturally occurring human mAbs using human hybridoma or advanced single-cell RNA-seq methods (**Fig 1**). We have used sorting of antigen-specific cells with recombinant viral surface protein antigens (like the GP1 and GP2 proteins we will use here), cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. One variation we will use is to capture Ab genes directly from viral glycoprotein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), as we have done for Ebola virus[30,36] and SARS-CoV-2[1,37-42]. These single-cell techniques enhance the capture of rare antigen-specific B cells.

E.1.1. Rapid isolation of human anti-LASV and anti-MACV mAbs. *(a) Immune cells.* We have obtained human PBMCs from one previously LASV-infected individual who was medically managed previously by Emory University in their infectious diseases containment unit. This person has a documented robust neutralizing serum antibody response, suggesting isolation of neutralizing human mAbs from these PBMC aliquots will be straightforward for us. Our colleagues in **RP1** have ongoing field operations for over a decade in Sierra Leone, Liberia, and Nigeria. This work is currently in association with the NIH-Centers for Research on Emerging Infectious Diseases (CREID) with a heavy focus on the study of LASV ecology, epidemiology, and host responses. **RP4** is currently working with **RP1** on obtaining PBMCs from ~10 individuals with prior laboratory-confirmed cases of LASV. The acquisition of these samples is being managed by the Vanderbilt Vaccine Center Biorepository with separate institutional funding to be de-identified prior to use in the studies proposed here. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies have been approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples will be deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded. For anti-MACV mAbs, as a human PBMC source is not readily available, we will immunize Alloy humanized mice. These animals yield PBMCs that secrete fully human mAbs. Alloy mice are used routinely in the Crowe lab workflow.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-LASV and anti-MACV mAbs in a rapid manner to mimic a pandemic response to an outbreak. We will single-cell sort antigen-specific B cells using recombinant LASV or MACV proteins (*e.g.*, **Table 1)** or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including LASV and MACV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe GP protein or virus-particle binding to single B cells. These cells are exported for sequencing of the variable gene regions and analysis using the PyIR software developed in the Crowe laboratory. We will select a panel of up to 500 neutralizing anti-LASV mAbs and a similar number of anti-MACV mAbs to express recombinantly and advance for further characterization. We can easily conduct multiple approaches simultaneously in parallel[1,43].

Table 1. Antigens from Core D
Lassa Josiah NP
Lassa Josiah GPe
Machupo GPe
Machupo NP

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using a custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors.

(d) Micro-scale mAb expression. The Crowe laboratory has the capacity for large-throughput micro-scale mAb expression and purification. The lower end of this range allows expression of thousands of mAbs, which will feed into automated mAb purification platforms. This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based LASV and MACV neutralization assay using RTCA. We have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of cytopathic effect (CPE). Here, we will apply this method to assess neutralization of LASV. Recently, we validated the inclusion of simultaneous live-cell fluorescence microscopy into this real-time CPE-based screening platform (xCelligence RTCA eSight). These capabilities expand the platform to screen viruses that may or may not induce CPE, allowing us to also use a replication-competent vesicular stomatitis virus (VSV) LASV surrogate that expresses LASV surface proteins and causes CPE[44]. Currently, we have a VSV-LASV-Josiah (lineage IV)[44] and a Sauerwald version (lineage II) of these recombinants. Making additional LASV VSV-LASVs is not difficult, and **RP1** will make the other missing lineages or needed arenaviruses based on the same approaches as previously published. **RP1** also can easily make versions of these VSVs that express the green fluorescent protein (GFP) reporter gene. Existing VSV-CCHFV, VSV-NiV, and VSV-HeV vectors[45-47] will be provided by Dr. Geisbert overall PI and Lead of **Core E**. We already have Vanderbilt IBC approval for use of these constructs. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression if used will identify mAbs with neutralizing activity against LASV. These mAbs will be moved forward for additional studies to identify an optimal anti-LASV mAb combination. Similar processes will be followed to determine neutralization capacity of MACV mAb candidates.

E.1.2. Identifying LASV and MACV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind LASV or MACV and validate their epitopes. We will perform competition-binding studies with GP or subunit proteins (as above) or virus-like particles (VLPs)[48] available from **Core E** at UTMB. **Core E** will also inactivate LASV and MACV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from previously reported antibodies for the identification of mAbs that can concurrently bind LASV or MACV with a goal of finding more potent clones at those sites or clones that complement these inhibitory mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-LASV or anti-MACV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations for each virus, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key residues binding residues of human anti-LASV and anti-MACV mAbs.*** To determine the key residues for binding of down-selected mAbs, we will perform deep mutational scanning[49]. Briefly, all amino acids of the GP proteins will be mutated to the other 19 amino acids with a unique barcode using PCR-based mutagenesis. The LASV or MACV GP protein library will be incubated with the appropriate mAbs to identify residues with loss-of-binding phenotypes. A sequence 'logo' showing the relative contributions of each amino acid mutation will identify residues critical for binding. Multiple amino acid substitutions can then be assessed by reverse genetics at UTMB to confirm the epitopes recognized by the down-selected mAbs. This method also serves as a surrogate for *in vitro* neutralization escape studies. ***(d) Neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-LASV or VSV-MACV constructs at BSL2 in the presence of saturating concentrations of mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Confirmatory reverse genetics studies will be performed by introducing mutations into an infectious cDNA clone of LASV or MACV by **RP1** to test neutralization. We hypothesize that escape will be less likely in the presence of mAb combinations than single mAbs. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of escape variants in the presence of mAbs.

E.1.3. Structural basis of neutralization by mAb combinations. The studies above should identify optimal anti-LASV and anti-MACV mAb combinations that simultaneously bind, neutralize, and increase the barrier for viral escape under mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for arenavirus by identifying key epitope targets (**RP1**).

E.1.4. Protective efficacy of human anti-arenavirus mAbs. ***(a) Studies in guinea pigs***. MAbs that satisfy the criteria stated above and that display stage-appropriate manufacturability characteristics will advance to *in vivo* testing. We will use established models of LASV[22,50] and MACV[51,52] infection in guinea pigs. PABVAX **Core E** has access to many strains from different LASV and MACV lineages, which will be tested. For prioritization, we will focus on LASV lineage II, III, and IV strains and MACV lineage I, II, and VIII strains for which uniformly lethal models are available. Groups of 6 outbred Hartley guinea pigs will be treated with low, medium, and high doses of single or combinations of anti-LASV or anti-MACVs mAb, or isotype controls at day +1 after inoculation with the LASVs or MACVs This approach allows us to determine whether combinations provide advantage over the best monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-LASV and anti-MACV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements (blood) at day 5 or 6 after LASV or MACV challenge and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal LASV and MACV mAb pairs that are comparable to or outperform previously reported mAbs for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-LASV and anti-MACV mAb combination against LASV or MACV in NHPs. As a final test of an optimal anti-LASV and anti-MACV mAb combinations, we will assess protective efficacy of the lead anti-LASV mAb pair in NHPs as well as the anti-MACV mAb pair in NHPs. These anti-LASV and anti-MACV mAb pairs will be selected based on the *in vitro* and guinea pig data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys will only be considered pending success in the guinea pig studies and consultation with the external Scientific Advisory Board (SAB) and NIAID program staff.

E.1.5. Applying lessons learned in LASV and MACV research with the selected test case pathogens for mAb generation: Lujo and Chapare mAbs. Once we have completed the LASV and MACV discovery campaigns, will turn to the test case pathogens to see if we can use the prototype pathogen approach to related viruses, Lujo (LUJV) and Chapare (CHAPV). We do not have access to human PBMCs from LUJV - or CHAPV-immune donors. As an alternative approach, we will use humanized mouse models as a source of B cells encoding fully human monoclonal antibodies. The Vanderbilt core investigators have an established fully executed agreement to use Alloy human antibody mice for this purpose (See Letter of Support). The Alloy ATX-Gx™ mouse is one of the most effective *in vivo* human antibody discovery platforms, with over 140 partners and counting. This mouse system was originally invented and validated inside a major pharma company and then further developed by Alloy. This foundational suite of highly immunocompetent transgenic mice is engineered to drive the greatest potential diversity of unique human antibodies binding to the viral target of interest, with broad epitopic coverage. The comprehensive functional human antibody repertoire in these mice is optimized for human Ab sequence developability and diversity. Alloy and Vanderbilt have already executed a simple licensing process for use of the ATX-Gx platform, and as an established partner the Vanderbilt core team will access the expanding portfolio of transgenic Alloy strains designed to address a range of discovery challenges (including diverse Ab isotypes). Mice will be immunized with conformationally correct recombinant protein antigens (**Table 2**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for the target of interest. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for LASV and MACV.

Table 2. Antigens available from RP1
LUJV NP NTD
LUJV GPe
LUJV NP NTD +GPe
CHAPV NP NTD
CHAPV GPe
CHAPV NP NTD +GPe

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-LASV and anti-MACV mAb combinations with optimal neutralizing and protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging LASV, MACV, or related arenaviruses. Given our previous ability to generate human antiviral mAbs, we do not anticipate problems in isolating anti-LASV or anti-MACV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently neutralizing anti-LASV or anti-MACV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody$^{\text{TM}}$) on enriched B cells (*i.e.*, memory B cells) isolated from human LASV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in LASV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-LASV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-LASV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-LASV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-LASV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the high-throughput Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E2. Specific Aim 2. Discover a protective CCHF virus mAb combination therapy. Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[25,53,54]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E).** As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.

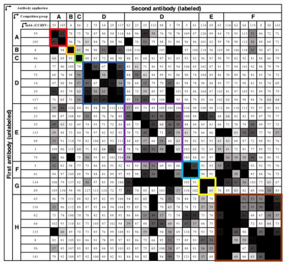


Figure 2. Competition assessment of human Abs to CCHFV using M-segment expressing cells. We tested 28 mAbs in competition assays. MAbs are displayed in 8 groups (A-H) based on their ability to compete for binding. Values shown are the % of binding that occurred during competition compared to non-competed binding of the mAb. This value was normalized to 100%. The values are also indicated by the box fill color; darker colors toward black indicate higher competition and lighter colors toward white indicate less competition, on a gradient scale.

__Preliminary Data__. ***__(a) Potently neutralizing and non-neutralizing human anti-CCHFV mAbs.__*** In this Aim, we will rapidly discover human anti-CCHFV mAbs from CCHFV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify an optimal anti-CCHFV mAb combination and further advance the discovery platform for rapid response against emerging nairoviruses. In preliminary experiments in collaboration with investigators in **RP1** and **Core E**, the Crowe laboratory has previously isolated and characterized panels of neutralizing human anti-CCHFV human mAbs from individuals who were convalescent after naturally-acquired laboratory-confirmed infection in Spain or Turkey. We isolated a panel of antibodies against CCHFV that recognize the M-segment in transfected cells, and they exhibit a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 2**). This panel contains cross-binding and cross-neutralizing antibodies for diverse strains. We observed a human mAb designated CCHF-82 competing with competition group 1 and group 2 antibodies from the previously isolated GP38-reactive murine antibodies. CCHF-82 showed some protection in a STAT1$^{-/-}$ mouse model of infection conducted by investigators in **RP1 (Fig 3)**. Further investigation with this antibody in the IbAr10200 prophylaxis and therapeutic models is warranted. In contrast, the CCHFV-neutralizing mAbs that we isolated and tested *in vivo* did not perform well in the Turkish strain STAT1$^{-/-}$ mouse model. We now know protection in this model is possible, given the work from Fels *et al*., 2021 who showed that potent antibodies against various sites on the Gc surface can afford protection in this model[53]. However, in their study they did not test GP38-reactive antibodies. To our knowledge, our preliminary data is the first indication that a human GP38-targeting antibody can provide some protection in this mouse model.

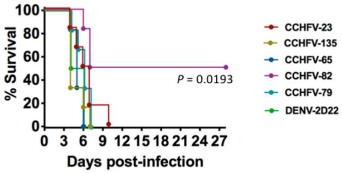


Figure 3. Human mAb CCHF-82 provides partial protection as a post-exposure prophylaxis against the Turkish strain of CCHFV in the STAT1$^{-/-}$ mouse model. A single dose of mAb was administered by the IP route to mice (n=6 per group (3 male, 3 female)) at 30 min post infections. A lethal dose of Turkish strain of CCHFV was administered. CCHF mAbs or DENV-2D22 (an isotype-matched negative control mAb) were tested in a single dose of 250 µg per mouse. Kaplan-Meier survival curves were statistically analyzed using a log-rank (Mantel-Cox) test where mAb-treated animals (*P* value shown in figure) were compared to animals treated with the DENV-2D22 negative control mAb. Unpublished data.

Non-neutralizing murine mAbs protect in mouse models of infection[25], but it is unknown if the human antibody response could provide similar levels of protection. Murine mAb 13G8 binds to GP38 and does not neutralize CCHFV. This mAb provides protection against lethal challenge as a prophylaxis and a therapeutic against CCHFV. In preliminary experiments we sought to understand if human survivors of CCHFV infection possess B cells encoding mAbs that bind a similar epitope as 13G8 and provide protection. CCHF-82 competes for binding with 13G8 to preGn-transfected cells containing GP38. This antibody offered some protection as a post-exposure prophylaxis against the Turkish strain of CCHFV (**Fig 3**). Human antibodies like this should be further studied and the frequency of these clones should be elucidated to understand if this is a feasible strategy for future vaccine efforts for humans. The epitope that 13G8 and CCHF-82 identifies could inform vaccine design if these antibodies offer protection in larger animal models. Understanding how non-neutralizing antibodies function is critical in efforts to validate the GP38-binding class of antibodies. Golden *et al.* undertook a study to understand the mechanisms 13G8 functioned and observed that complement activation contributes to the protective capacity of this antibody[25]. Here, in studies of CCHF-82, we also will assess complement activation in mouse models of infection to fully understand the function of this mAb *in vivo*. Furthermore, if this mAb protects in the CCHFV IbAr10200 strain challenge model, assessing its mechanism is warranted. Together with **Core D**, we will assess

IgG molecules with complement knockout (“KA”) and Fc gamma receptor knockout (LALA-PG) mutations in the Fc domain to study this matter. Human mAb CCHF-82 displayed limited cross-protection against experimental infection with the Turkish strain of CCHFV. Our antibody isolation here will aim to identify cross-protective non-neutralizing antibodies reactive with GP38. Recently, vaccinating with NP was shown to be another way to elicit non- neutralizing antibodies[55]. The NP of CCHFV is more conserved than the glycoproteins and might also be a non-canonical target for the identification of cross-protective non-neutralizing antibodies[55].

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant viral surface protein antigens, cloning of Ab variable genes, and then expression of mAbs as as described above in Specific Aim 1.

E.2.1. Rapid isolation of human anti-CCHFV mAbs. *(a) Immune cells.* We have obtained human PBMCs from a number of previously CCHFV-infected individuals. One was a health care survivor who was infected while caring for a patient in Spain[56]. We also collected a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Turkey, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples were only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies were approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples were deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-CCHFV mAbs rapidly, as if we had to respond to a nairovirus outbreak in real time. Our ability to respond rapidly in the setting of outbreaks is well-documented in the lay press (60 Minutes features and others) and the scientific literature, e.g., 78 days from sample to protection of NHPs for Zika virus[43], and 25 days from PBMC sample receipt to delivery of the antibody genes for the approved COVID-19 drug Evusheld to AstraZeneca[1]. Here, we will single-cell sort antigen-specific B cells using recombinant CCHFV proteins (GP38, Gc, Gn, Gc/Gn) or non-infectious particles as bait as described in Specific Aim 1. We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D**, (**Table 3**) or by **RP1 (Table 4)**.

Table 3. Core D CCHFV antigens
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF NP

(c) Recombinant mAb cloning. As described in Specific Aim 1 above.

(d) Micro-scale mAb expression. As described in Specific Aim 1 above.

(e) A CPE-based CCHFV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of CCHFV. We will use a rVSV CCHFV surrogate that expresses CCHFV surface proteins and causes CPE[46]. Investigators in **RP1** have the VSV-CCHFV-GFP versions of clades 1 through 7 based on this publication. We will obtain these VSV constructs from investigators in **PABVAX**. Existing VSV vectors will be provided by investigators in **RP1 and Core E**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against CCHFV. These mAbs will be moved forward for additional studies to identify an optimal anti-CCHFV mAb combination. GP38-reactive clones will be advanced based on avidity ranking since they are not likely to be neutralizing but may be protective *in vivo*.

Table 4. Antigens available from RP1
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF Turkey NP
CCHF Turkey GnGc+GP38+NP
CCHF Turkey GnGc+GP38
CCHF Turkey GP38+NP
CCHF Turkey GnGc+NP

E.2.2. Identifying CCHFV mAb combinations. *(a) Epitope mapping through competition-binding studies.* Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind CCHFV surface proteins and validate their epitopes. We will perform competition-binding studies with CCHFV Gc/Gn or subunit proteins (as above) or CCHFV virus-like particles (VLPs) made from a replicon system in hand. **Core E** will inactivate CCHFV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site. Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from some of the mAbs shown in the unpublished preliminary data above for the identification of mAbs that can concurrently bind CCHFV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-CCHFV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. GP-38-reactive clones will be screened for synergistic binding as they are not expected to be neutralizing. ***(c) Identification of key binding residues of human anti-CCHFV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-CCHFV constructs at BSL2 in the presence of saturating concentrations of CCHFV mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of VSV-CCHFV escape variants in the presence of mAbs.

E.2.3. Structural basis of neutralization by anti-CCHFV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.2.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies in $STAT1^{-/-}$ mouse models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (Core D), will advance to *in vivo* testing. We will use an established $STAT1^{-/-}$ mouse model of CCHFV infection that the **RP1** and **Core E** investigators have deployed[46]. To benchmark our mAbs, a separate set of $STAT1^{-/-}$ mice will be treated with CCHF-82, which we showed in unpublished preliminary data above confers substantial but incomplete protection (**Fig 3**). The **Core E** laboratory has access to strains representing all 6 clades that can be tested. Groups of 6-8 $STAT1^{-/-}$ mice will be treated with low, medium, and high doses) of single or combinations of anti-CCHFV mAb, or isotype controls at day +1 after inoculation with CCHFVs. This approach allows us to determine whether combinations provide advantage over the best CCHFV monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after CCHFV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal CCHFV mAb pairs that are comparable to or outperform the mAbs we reported above. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-CCHFV mAb combination against CCHFV in NHPs. As a final test of an optimal anti-CCHFV mAb combination, we will assess efficacy of the lead anti-CCHFV mAb pair in NHPs (**Core E**), to be selected based on the *in vitro,* $STAT1^{-/-}$ mice, and manufacturability data (**Core D**). Down-selection for testing in cynomolgus monkeys would only be considered with success in the $STAT1^{-/-}$ mouse studies and in consultation with the external SAB and NIAID program staff.

E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs. Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human mAbs, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.

~~**E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs.** Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human mAbs, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.~~

Table 5. Antigens available from RP1
Kasokero GnGC
Kasokero GP38
Kasokero NP
Kasokero GnGc +GP38 +NP
Kasokero GnGc +GP38
Kasokero GnGc +NP
Kasokero GP38 +NP

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-CCHFV mAb combinations with optimal protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging CCHFV or related nairoviruses. Given our previous ability to generate human anti-CCHFV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-CCHFV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-

CCHFV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (i.e., memory B cells) isolated from human CCHFV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in CCHFV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-CCHFV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-CCHFV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-CCHFV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-CCHFV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E3. Specific Aim 3. Establish optimal mAb combination therapies against henipaviruses Hendra (HeV) and Nipah (NiV) viruses. We will use a large panel of potent anti-henipavirus mAbs that we previously made from the memory B cells of immune individuals and generate new antibodies targeting the fusion (F) protein. **RP2** will provide new antigens for mAb selection and BSL-2-compatible viruses for neutralization screens including Cedar virus constructs displaying HeV and NiV F and G proteins. We will identify optimal mAb combinations to enhance henipavirus therapy, minimize viral escape, and improve treatment efficacy through synergy. *In vitro* competition-binding, neutralization, and mechanistic studies will be conducted, including authentic virus neutralization with **PABVAX Core E**. We will refine the recognized epitopes and investigate the structural basis for optimal combination neutralization of henipaviruses using HeV and NiV as prototypes. *In vivo* assessment of mAb combinations will determine the minimal effective dose for protection and the ability to prevent *in vivo* escape. Protective efficacy of lead mAb combinations will be assessed in hamster models of NiV and HeV infection with **Core E**. Leads will be transferred to **Core D**. As a final test of optimal henipavirus mAb combinations, we will assess protective efficacy of the lead henipavirus mAb pairs in NHPs, to be selected based on the *in vitro* and hamster data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in African green monkey (AGM) henipavirus model studies would only be considered pending success in the hamster studies and consultation with the external SAB and NIAID program staff. The HeV and NiV studies will be conducted in years 1-3 and then applied to the plug-and-play test case Langya virus in years 2-5. If **RP2** and **Core E** cannot rescue recombinant Langya virus, the lessons learned in Years 1-3 will be applied to the alternate “plug and play” test viruses Angavokely or Sosuga (SOSV) virus. Preliminary data on SOSV mAbs are provided.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-NIV/HeV mAbs.*** In this Aim, we will further adapt approaches we have developed in preliminary experiments with henipavirus mAb studies by identifying a potently protective combination of anti-NiV/HeV human mAbs. Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups. This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains. In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and outstanding potency anti-G antibodies with extensive characterization, including synergistic neutralization.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant henipavirus F protein antigens, as described above in Specific Aim 1.

E.3.1. Rapid isolation of human anti-HeV/NiV mAbs. ***(a) Immune cells.*** We are obtaining human PBMCs from a number of previously NiV-infected individuals who acquired immunity following natural infection in collaboration with the **icddr,b**, an international health research institute based in Dhaka, Bangladesh. We are collecting a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Bangladesh, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies are approved by the icddr,b and Vanderbilt University Medical Center IRBs. PBMCs isolated from the blood samples are deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their use in this **RP4,** if funded. ***(b) Applying mAb technologies for new mAb isolation***. We will use sorting of antigen-specific B cells, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer antigen-specific B cells. We will sort soluble fusion (sF) or attachment (G) protein-specific cells with recombinant viral surface protein antigens from **Core D** (**Table 6**).

(c) Ab screening and sequence analysis. Our goal is to identify human anti-NiV/HeV mAbs rapidly, as if we had to respond to a paramyxovirus outbreak in real time. We will single-cell sort antigen-specific B cells using recombinant HeV and NiV proteins (sF or G) as bait as described in Specific Aim 1 above.

(d) Recombinant mAb cloning. As described in Specific Aim 1 above.

(e) Micro-scale mAb expression. As described in Specific Aim 1 above.

(f) CPE-based HeV/NiV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of HeV and NiV. We will use rVSV HeV and NiV surrogates that expresses henipaviurs surface proteins and cause CPE. Investigators in **RP2 and RP3** have VSV-NiV F and VSV-NiV G with GFP[45], VSV-NiV G without GFP[47] and VSV-HeVG (Geisbert and Cross, unpublished). We will obtain these all these VSV constructs from investigators in **PABVAX**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against HeV and NiV. We also can use Cedar virus constructs for neutralization assays as the have reduced pathogenicity[57] and can be used in high-throughput screening assays for henipavirus antiviral discovery[58]. Cedar viruses will be provided by Dr. Broder of **RP2**. The mAbs with most potent activity will be moved forward for additional studies to identify an optimal anti-HeV/NiV mAb combination.

Table 6. Core D Henipavirus antigens
Nipah sF
Hendra sF
NiV G

E.3.2. Identifying HeV/NiV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind HeV/NiV surface proteins and validate their epitopes. We will perform competition-binding studies with HeV/NiV sF or G proteins (as above). We will include positive controls made recombinantly from some of the mAbs shown in the preliminary data above for the identification of mAbs that can concurrently bind HeV/NiV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti- HeV/NiV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key binding residues of human anti-HeV/NiV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-NiV F, VSV-NiV G and VSV-HeV G constructs at BSL-2 in the presence of saturating concentrations of HeV/NiV mAbs (single or combination) using RTCA to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test the level of infection of VSV-HeV/NiV escape variants in the presence of mAbs.

E.3.3. Structural basis of neutralization by anti-HeV/NiV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.3.4. Protective efficacy of human anti-henipavirus mAbs. ***(a) Studies in hamsters***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (**Core D**), will advance to *in vivo* testing. We will use an established hamster model of NiV or HeV infection that the **Core E** investigators have deployed. To benchmark our mAbs, a separate set of hamsters will be treated with G mAbs HENV-103+HENV-117, which we showed in previous data above confers substantial protection[18]. Groups of 6-8 hamsters will be treated with low, medium, or high IgG doses) of single or combinations of anti-HeV/NiV mAbs, or isotype controls at day +1 after inoculation with HeV or NiV. This approach allows us to determine whether combinations provide advantage over the best HeV or NiV mAb monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-HeV and anti-NiV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after HeV or NiV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal HeV/NiV mAb pairs that are comparable to or outperform the mAbs we reported previously. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-HeV/NiV mAb combination against HeV and NiV in NHPs. As a final test of an optimal anti-HeV/NiV mAb combination, we may assess efficacy of the lead anti-HeV/NiV mAb pair in NHPs (**Core E**), selected based on the *in vitro,* hamster data, and manufacturability data (**Core D**). Down-selection for testing in AGMs would only be considered with success in the hamster studies and in consultation with the external SAB and NIAID program staff.

E.3.5. Applying lessons learned in HeV/NiV research to Langya, Angavokely and Sosuga viruses. Once we have completed the HeV/NiV discovery campaigns, will turn to the test case pathogen Langya (or alternate Angavokely and Sosuga (SOSV) viruses) to see if we can use the prototype pathogen approach for this virus. We have plenty of human PBMCs from the sole identified SOSV-immune individual and have already shown that it is possible to obtain HN- and F-specific neutralizing antibodies from this individual's B cells[59]. We also have already prepared recombinant SOSV prefusion F and HN proteins[59]. We do not have access to human PBMCs from Langya- or Angavokely-immune donors. For those mAb discovery campaigns, we will use Alloy humanized mouse models as a source of B cells encoding human mAbs, as above. Mice will be immunized with recombinant protein antigens (**Table 7**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Langya or Angavokely. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for Hev/NiV described above.

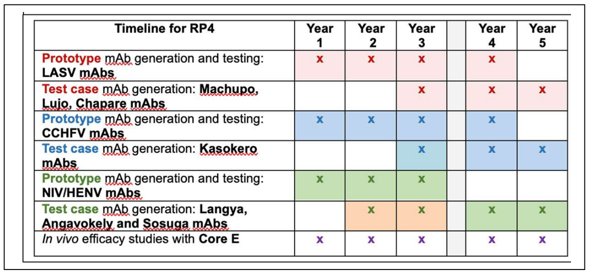
Table 7. Paramyxovirus antigens from Core D
Mojang sF
Angavokely sF
Langya sF

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-HeV/NiV mAb combinations with optimal protective activity. These mAb combinations will be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging HeV/NiV or related paramyxoviruses. Given our previous ability to generate human anti-HeV/NiV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-HeV/NiV mAbs. We may observe that new mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-HeV/NiV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells *(i.e., memory B cells) isolated from human NiV-immune PBMC samples. In addition, we can deep sequence the B* cell repertoires present in NiV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-HeV/NiV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-HeV/NiV mAb combinations that can inform vaccine design (**RP2**) for related viruses being used as test cases in years 2 through 5.

F. Scientific rigor. *Experimental design.* Experiments will include biological and technical replicates, and results will be analyzed by parametric or non-parametric analyses (depending on data distribution) and corrections for multiple comparisons. EC_{50} (binding) and IC_{50} (neutralization) values will be reported only following at least 3 identically conducted experiments. All mAb screening will incorporate appropriate + and - controls. Positive signals in binding assays will be defined as >3 SD above background. ***Protein quality.*** We have a high capacity to purify proteins using a Protein Maker workstation that allows purification chromatography on 24 parallel columns. We perform QC on protein reagents using a Maurice instrument with capillary electrophoresis technology to automate our protein profiling either by size or charge, providing cIEF and CE-SDS data. Animal ***studies*** will be performed blinded with independent replicates and include negative (*e.g.*, placebo), and positive (when available) controls. We will use power calculations (**Data Management Core B**) to determine sample sizes. For virological studies, power analysis was performed using accepted values for type I error (0.05) and power (80%). The values for population variance (15×) are based on existing data sets and our experience with rodent challenge studies. We will perform experiments at least 2-3 independent times (n = 4-5 each) to attain n = 12-15 per arm. Smaller group sizes can be used if differences are larger than expected.

G. Relevant biological variables. The human mAbs are derived from immune human donors or Alloy mice. Humans. Human Abs will be isolated for biological, structural, and functional studies at a clonal level. The sample size is not powered for large scale epidemiologic or clinical study conclusions. There is no evidence that sex, age, body mass index, or underlying health conditions other than immunodeficiency or immunosuppressive treatments alters the activity of cloned Abs in structure/function studies. The samples are de-identified without patient specific identifiers to protect the health information of the donors; thus, research with these samples is considered *Not Human Subjects Research* category activity. Mice, hamsters, guinea pigs, NHP, and viruses are also biological systems used in this application. Conventional animal housing conditions will be used because there is no evidence that altered room temperatures and light/dark cycles influence paramyxovirus, nairovirus, or arenavirus pathogenesis. Mice, hamsters, guinea pigs, and NHPs of both sexes will be used, and phenotypes will be monitored for sex-based differences. Virus stocks will be either low passage seed stocks from natural isolates or generated from existing cDNA clones and sequence-confirmed prior to use for infection studies.

H. Milestones and quantitative Go/No-Go criteria. Through **Aims 1-3**, we have proposed experiments that will advance our knowledge of the mAb-mediated mechanisms of action against paramyxoviruses, nairoviruses, and arenaviruses. In each Aim, we will use down-selection criteria (as below) to advance mAb combinations or variants for additional studies. By year 3, we will have defined the principles that govern optimal mAb combinations for paramyxoviruses, nairoviruses, and arenaviruses and the rational approaches needed to select mAbs for potency and breadth. We will use the following metrics to advance mAbs: ***(a) Neutralizing activity*.** All mAbs will have neutralizing potency < 10 ng/mL, EC_{50} value; ***(b) Binding affinity***. MAbs will be of high affinity to surface proteins or virions (K_D < 5 nM). ***(c) Protein stability and production quality*.** MAbs must produce at high levels without optimization (> 100 mg/L) and show biochemical/functional stability at 4°C or -80°C (>90% retention of binding and neutralization activity at 30 d). ***(d) Synergy***. Combinations of 2 mAbs against a given virus ideally will show synergy of neutralization *in vitro* or at a minimum, additive inhibition (no antagonism); ***(e) Epitopes***. To avoid resistance, mAbs in combination will bind 2 epitopes (determined by competition binding (<20% reduction in binding), mapping, and structural studies. ***(f) Resistance*** against mAbs will be assessed for virus escape. Deep sequencing of viruses that emerge from cells or animals treated with mAbs will inform prioritization. Combinations of mAbs with high genetic barriers to resistance (<10× loss in EC_{50}) will be advanced. ***(g) Protection in mice, hamsters, guinea pigs, or NHPs.*** The following metrics can be used: (i) Protection against lethal challenge by homologous virus (>90% survival; (ii) Protection against clinical disease by homologous virus (>80%); (iii) Protection against infection by homologous virus (>100-fold reduction in viral titers in multiple organs). ***(h) pK measurements***. For lead protective mAbs with mutations that should confer extended half-life, we will perform pK studies in NHPs (with **Core D** and **E**) to assess levels over time with a goal of a human predicted serum half-life of >6 months.



Timeline for RP4	**Year 1**	**Year 2**	**Year 3**		**Year 4**	**Year 5**
Prototype mAb generation and testing: **LASV mAbs**	x	x	x		x	
Test case mAb generation: **Machupo, Lujo, Chapare mAbs**			x		x	x
Prototype mAb generation and testing: **CCHFV mAbs**	x	x	x		x	
Test case mAb generation: **Kasokero mAbs**			x		x	x
Prototype mAb generation and testing: **NIV/HENV mAbs**	x	x	x			
Test case mAb generation: **Langya, Angavokely and Sosuga mAbs**		x	x		x	x
In vivo efficacy studies with **Core E**	x	x	x		x	x

LITERATURE CITED – RP4

1. Zost, S.J*., et al.* Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* **26**, 1422-1427 (2020).
2. Fischer, W.A., II & Wohl, D.A. Moving Lassa Fever Research and Care Into the 21st Century. *The Journal of Infectious Diseases* **215**, 1779-1781 (2017).
3. Ezeomah, C*., et al.* Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. *Open Forum Infect Dis* **6**, ofz512 (2019).
4. Li, A.L*., et al.* Ophthalmic manifestations and vision impairment in Lassa fever survivors. *PloS one* **15**, e0243766 (2020).
5. Whitmer, S.L.M*., et al.* New Lineage of Lassa Virus, Togo, 2016. *Emerging infectious diseases* **24**, 599-602 (2018).
6. Ibukun, F.I. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. *Viruses* **12**(2020).
7. Webb, P.A., Johnson, K.M., Mackenzie, R.B. & Kuns, M.L. Some characteristics of Machupo virus, causative agent of Bolivian hemorrhagic fever. *Am J Trop Med Hyg* **16**, 531-538 (1967).
8. Aguilar, P.V*., et al.* Reemergence of Bolivian hemorrhagic fever, 2007-2008. *Emerging infectious diseases* **15**, 1526-1528 (2009).
9. Enria, D.A., Ambrosio, A.M., Briggiler, A.M., Feuillade, M.R. & Crivelli, E. [Candid#1 vaccine against Argentine hemorrhagic fever produced in Argentina. Immunogenicity and safety]. *Medicina (B Aires)* **70**, 215-222 (2010).
10. Enria, D.A. & Maiztegui, J.I. Antiviral treatment of Argentine hemorrhagic fever. *Antiviral research* **23**, 23-31 (1994).
11. Hawman, D.W. & Feldmann, H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology* (2023).
12. Chinikar, S*., et al.* Genetic Diversity of Crimean Congo Hemorrhagic Fever Virus Strains from Iran. *J Arthropod Borne Dis* **10**, 127-140 (2016).
13. Shepherd, A.J., Swanepoel, R. & Leman, P.A. Antibody response in Crimean-Congo hemorrhagic fever. *Rev Infect Dis* **11 Suppl 4**, S801-806 (1989).
14. Lombe, B.P*., et al.* Mapping of Antibody Epitopes on the Crimean-Congo Hemorrhagic Fever Virus Nucleoprotein. *Viruses* **14**(2022).
15. Mackenzie, J., Wang, L. & Broder, C.C. Henipaviruses. in *Fields Virology*, Vol. 2 1070-1085 (2013).
16. Whitmer, S.L.M*., et al.* Inference of Nipah virus evolution, 1999–2015. *Virus Evolution* **7**(2020).
17. Dong, J*., et al.* Potent Henipavirus Neutralization by Antibodies Recognizing Diverse Sites on Hendra and Nipah Virus Receptor Binding Protein. *Cell* **183**, 1536-1550 e1517 (2020).
18. Doyle, M.P*., et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021).
19. Mire, C.E*., et al.* A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis* **221**, S471-S479 (2020).
20. Dang, H.V*., et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021).
21. Cross, R.W*., et al.* Antibody therapy for Lassa fever. *Curr Opin Virol* **37**, 97-104 (2019).
22. Cross, R.W*., et al.* Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral research* **133**, 218-222 (2016).
23. Cartwright, H.N., Barbeau, D.J. & McElroy, A.K. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere* **6**, e0055621 (2021).

24. Gutjahr, B., *et al.* Two monoclonal antibodies against glycoprotein Gn protect mice from Rift Valley Fever challenge by cooperative effects. *PLoS Negl Trop Dis* **14**, e0008143 (2020).
25. Golden, J.W., *et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv* **5**, eaaw9535 (2019).
26. Zalevsky, J., *et al.* Enhanced antibody half-life improves in vivo activity. *Nat Biotechnol* **28**, 157-159 (2010).
27. Dall'Acqua, W.F., *et al.* Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* **169**, 5171-5180 (2002).
28. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Levis, S.C. & Maiztegui, J.I. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* **2**, 255-256 (1984).
29. Jahrling, P.B. & Peters, C.J. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infection and immunity* **44**, 528-533 (1984).
30. Chen, E.C., *et al.* Systematic analysis of human antibody response to ebolavirus glycoprotein shows high prevalence of neutralizing public clonotypes. *Cell Rep* **42**, 112370 (2023).
31. Buck, T.K., *et al.* Neutralizing Antibodies against Lassa Virus Lineage I. *mBio* **13**, e0127822 (2022).
32. Enriquez, A.S., *et al.* Delineating the mechanism of anti-Lassa virus GPC-A neutralizing antibodies. *Cell Rep* **39**, 110841 (2022).
33. Hastie, K.M., *et al.* Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell* **178**, 1004-1015 e1014 (2019).
34. Hastie, K.M., *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).
35. Li, H., *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991 (2022).
36. Gilchuk, P., *et al.* Pan-ebolavirus protective therapy by two multifunctional human antibodies. *Cell* **184**, 5593-5607 e5518 (2021).
37. Chen, E.C., *et al.* Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* **36**, 109604 (2021).
38. Dong, J., *et al.* Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-1244 (2021).
39. Greaney, A.J., *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).
40. Kramer, K.J., *et al.* Potent neutralization of SARS-CoV-2 variants of concern by an antibody with an uncommon genetic signature and structural mode of spike recognition. *Cell Rep* **37**, 109784 (2021).
41. Suryadevara, N., *et al.* Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell* **184**, 2316-2331 e2315 (2021).
42. Zost, S.J., *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449 (2020).
43. Gilchuk, P., *et al.* Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics. *Nat Biomed Eng* **4**, 1030-1043 (2020).
44. Cross, R.W., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep* **40**, 111094 (2022).
45. Mire, C.E., *et al.* Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J* **10**, 353 (2013).
46. Rodriguez, S.E., *et al.* Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep* **9**, 7755 (2019).
47. Foster, S.L., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. *Proc Natl Acad Sci U S A* **119**, e2200065119 (2022).

48. Branco, L.M., *et al.* Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* **7**, 279 (2010).
49. Starr, T.N., *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
50. Deschambault, Y., *et al.* An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. *Viruses* **15**(2023).
51. Bell, T.M., *et al.* Pathogenesis of Bolivian Hemorrhagic Fever in Guinea Pigs. *Vet Pathol* **53**, 190-199 (2016).
52. Golden, J.W., *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* **7**, 4679 (2017).
53. Fels, J.M., *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell* **184**, 3486-3501 e3421 (2021).
54. Mishra, A.K., *et al.* Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies. *Science* **375**, 104-109 (2022).
55. Leventhal, S.S., *et al.* Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *EBioMedicine* **82**, 104188 (2022).
56. Negredo, A., *et al.* Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med* **377**, 154-161 (2017).
57. Laing, E.D., *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018).
58. Amaya, M., *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084 (2021).
59. Parrington, H.M., *et al.* Potently neutralizing human mAbs against the zoonotic pararubulavirus Sosuga virus. *JCI Insight* **8**(2023).

A. BACKGROUND AND SIGNIFICANCE. Paramyxoviruses, nairoviruses, and arenaviruses are widely distributed RNA viruses that cause severe human syndromes. Because of their geographical distribution and expansion, changes in human demographics and increased exposures to wild animals including rodents and bats, and their capacity for human-to-human spread, these viruses have the potential to cause pandemics. Together with the other investigators in **PABVAX**, we have selected several important prototype paramyxoviruses, nairoviruses, and arenaviruses to study to learn the rules governing immunity to these pathogens. The work on the pathogens selected is justified based on their prevalence and severity alone, but also because of their ability to serve as prototypes for genetically related viruses. We chose the viruses for focus in this RP4 on human antibody development because of their clinical relevance and because several will be studied as prototypes for vaccine development in **RP1** (arenaviruses, nairoviruses) and **RP2** (paramyxoviruses). Among the arenaviruses, we will target Lassa virus (LASV) and Machupo virus (MACV), and among the nairoviruses, we will study Crimean–Congo hemorrhagic fever virus (CCHFV). In tandem with the vaccine projects in **RP2** focused on paramyxoviruses, we will study Nipah virus (NiV) and Hendra virus (HeV) as the prototypes. Information about the principles we discover about critical neutralizing epitopes identified here in **RP4** with prototype viruses can feed back into all other **Projects** that address the subsequent plug and play test cases in later years (Lujo, Chapare, Kasokero, Langya).

LASV is an Old World arenavirus (OWAV) with a single-stranded RNA virus belonging to the Arenaviridae family. Infection in humans is often the result of exposure to infected excreta from the rodent reservoir, *Mastomys natalensis* where up to 500,000 cases and an estimated 5,000 deaths occur annually in West Africa[2]. While rodent infection is largely benign, human infection can result in severe hemorrhagic disease with neurological complications and long-term hearing and vision sequelae[3,4]. There are no approved vaccines or treatments for LASV infection. ~~The virus possesses a bi-segmented genome comprising large (L) and small (S) segments, both of which are enclosed within a lipid envelope. The L segment (~7.2 kb) encodes the RNA-dependent RNA polymerase (RdRp) and a zinc-binding protein (Z), while the S segment (~3.4 kb) codes for the nucleoprotein (NP) and the glycoprotein precursor (GPC). The GPC is further cleaved into two surface glycoproteins, GP1 and GP2, which play critical roles in viral entry. The genetic diversity of LASV can be classified into six lineages (I-VI), with lineages III and IV being the most prevalent[5]. Genetic variation arises mainly due to the error-prone nature of the viral RNA polymerase, leading to the emergence of quasi-species. This diversity contributes to the virus's ability to evade host immune responses and pose challenges to vaccine development[6]. The surface glycoproteins GP1 and GP2, are responsible for receptor binding and membrane fusion, respectively. While GP1 is highly variable among strains, GP2 is more conserved. The nucleoprotein (NP) is another major antigen, and its immunodominant epitopes are targeted by the host's immune response. Its genetic diversity and antigenic properties complicate diagnosis and vaccine development, emphasizing the need for continued research on medical countermeasures.~~

MACV is a New World Arenavirus (NWAV) and the causative agent of Bolivian Hemorrhagic fever. Humans are infected after exposure to excreta from the reservoir, *Calomys callosus,* the large vesper mouse. Disease in humans has a slow onset, but can progress to severe hemorrhagic fever coupled to insidious neurological complications including tremors, seizures, and paralysis[7]. ~~MACV has a similar genomic organization and contains a GPC, NP, Z, and L polymerase proteins. Genetic diversity of MACV is delineated into 8 lineages (I-VIII) where there is growing concern of an expanding host range of *C. callosus* is responsible for outbreaks outside the known endemic region[8]. While the host entry receptor for NWAV is not shared with OWAV, the role for GP to drive immunity as a major antigen alongside NP. The better studied Junin has a regionally approved vaccine[9] and convalescent plasma program[10] available to prevent and treat disease, respectively.~~ There are no approved vaccines or therapies approved for MACV.~~any other NWAV, including MACV. Further, it is not well established whether these countermeasures have potential for cross protect other NWAV such as MACV, underscoring the value of continued efforts to develop broadly active countermeasures to safeguard against lethal disease.~~

CCHFV is a highly pathogenic tick-borne virus classified under the Nairovirus genus within the Bunyaviridae family[11]. CCHF outbreaks have a case fatality rate of up to 40% and there are no vaccines or treatments approved for human use.~~It has a negative-sense, single-stranded RNA genome comprising three segments: Small (S), Medium (M), and Large (L). These segments are enclosed within a lipid envelope and code for various structural and non-structural proteins. The S segment (~1.7 kb) encodes the nucleocapsid protein (N), which is essential for viral replication and assembly. The M segment (~5.3 kb) codes for the glycoprotein precursor (GPC), which is post-translationally cleaved into two surface glycoproteins, Gn and Gc. These glycoproteins are involved in viral entry and fusion. The L segment (~12.1 kb) encodes the RNA-dependent RNA polymerase (RdRp), responsible for viral RNA synthesis. CCHFV exhibits significant genetic diversity, with at least seven distinct clades (Europe 1, Europe 2, Africa 1-3, and Asia 1-2) based on the partial S segment sequences[12]. This diversity is attributed to the segmented genome, geographical distribution, and error-prone viral RNA polymerase, leading~~

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~~to the emergence of various quasi-species. The major antigens of CCHFV include the surface glycoproteins Gn and Gc, as well as the nucleocapsid protein (N)[13,14]. These antigens are targeted by the host immune response. CCHFV is genetically diverse, so breadth of reactivity will be a focus of our discovery efforts.~~

NiV/HeV. Bat borne**,** NiV and HeV are enveloped, negative-sense, single-stranded RNA viruses belonging to the Henipavirus genus within the Paramyxoviridae family[15]. These emerging zoonotic viruses pose a significant threat to human and animal health due to their high fatality rates and broad host range. ~~The genomes of NiV and HeV are approximately 18.2 kb or 18.4 kb in length, respectively, and share a similar gene organization. They encode six major structural proteins: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment glycoprotein (G), and large polymerase (L) proteins. The N, P, and L proteins form the viral RNA-dependent RNA polymerase (RdRp) complex necessary for transcription and replication, whereas the M, F, and G proteins are essential for virus assembly and entry. Genetic diversity in henipaviruses is relatively low compared to other RNA viruses, with NiV strains showing 91-100% nucleotide similarity and HeV strains displaying 83-100% similarity[16]. However, the error-prone nature of the RdRp can still generate genetic variability, leading to the emergence of distinct lineages. The major surface antigens of Nipah and Hendra viruses are the attachment glycoprotein (G) and fusion protein (F). The G protein facilitates receptor binding, while the F protein mediates viral membrane fusion. Both antigens are involved in host immune responses and are potential targets for vaccine development, and the neutralizing antibody approaches we propose here. NiV and HeV exhibit relatively low genetic diversity, and in preliminary studies, we have identified human mAbs that neutralize HeV, NiV Bangladesh and NiV Malaysia strains[17-19]. Antibodies to the major surface antigens, the G and F proteins, are therefore, promising candidates for development as prophylactic or therapeutic molecules.~~As with the other prototype virus in the **PABAX** Center there are no vaccines or treatments against henipaviruses licensed for human use.

Antibodies (Abs) are important correlates of protection for paramyxoviruses, nairoviruses, and arenaviruses. Immune sera against members of each of these groups of viruses has been shown to exhibit protective efficacy *in vivo*, which supports the use for Abs as treatment options. However, the specificity, functional properties, and other attributes of protective antibodies are poorly defined for most of these viruses. MAbs to the attachment and fusion surface proteins of paramyxoviruses (such as the G and F proteins of Hendra/Nipah[18,20] and the GP proteins of the arenaviruses like LASV[21,22]) can neutralize and protect against infection in rodent models. CCHFV is a bit of a conundrum in that ultrapotent human neutralizing antibodies to the related Rift Valley fever virus surface proteins are highly protective *in vivo*[23,24], but CCHFV neutralizing antibodies are not protective. In contrast, some non-neutralizing antibodies do protect partially for CCHFV[25]. Clearly, there is a lot to learn about the rules of immunity to these viruses, and the genetic, molecular, and structural basis of antibody-mediated protection for viruses like CCHFV. Based on these data, we propose the use of mAbs as treatment options to learn the rule of how to protect against emerging paramyxoviruses, nairoviruses, and arenaviruses and to develop candidate antibody countermeasures for translational development with **Core D**. To do so, we will further develop our technical methods for rapidly discovering mAbs, in the case of a future unexpected epidemic caused by viruses of these families. Most importantly, we will define the principles governing combination antibody therapy to prevent virus escape from treatment or prevention and to optimize the efficacy of the beneficial effects conferred by passive immunity.

B. SCIENTIFIC PREMISE. The work proposed here in **RP4** is based on the extensive basic, translational, and clinical trial data on the role of neutralizing Abs in protection against infection and disease caused by paramyxoviruses, nairoviruses, and arenaviruses. Although a significant amount of data supports the use of mAbs to prevent or control paramyxovirus, nairovirus, and arenavirus infection, many questions remain, such as the ideal epitopes to target with mAb combination therapy to improve upon neutralization potency, *in vivo* efficacy, and resistance to viral escape. Identifying these epitopes can help inform vaccine design to elicit optimal protective polyclonal responses (in **RP1** and **RP2**, especially with designs for test cases in years 4 and 5). Here we aim to refine our understanding of the correlates of mAb protection against paramyxoviruses, nairoviruses, and arenaviruses to enable the design of a modular "plug-and-play" workflow to rapidly respond to potential pandemics by generating optimized combinations of vaccine-like mAbs with an extended half-life that could be used for long-term prophylaxis (> 6 months) and therapy.

C. INNOVATION. RP4 has many innovative conceptual and technical features including: Conceptual. **(1)** determining the principles that govern optimal combinations of human mAbs against paramyxoviruses, nairoviruses, and arenaviruses for protection and resistance to viral escape; **(2)** the development of a clear workflow that generates therapeutic or preventative antibodies (long half-life mAbs that serve as vaccine surrogates) against emerging or new paramyxoviruses, nairoviruses, and arenaviruses with pandemic potential. Our goal is to define the principles and features, including epitope, combination ratios, and mechanisms of action, that result in optimal efficacy of mAbs against paramyxoviruses, nairoviruses, and arenaviruses in animal models and create a higher barrier for resistance. These studies will serve as paradigms for rapid antibody discovery in response to future paramyxovirus, nairovirus, and arenavirus pandemic threats. Technical. **(3)** large-scale B cell

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repertoire sequencing with customized methods and software; **(4**) use of humanized (Alloy) mice and novel immunogens from **RP1** and **RP2** and **Core D**; **(5)** single B cell functional (neutralization) assays performed on a Beacon instrument; **(6)** development of high-throughput real-time label-free virus neutralization assays (xCELLigence platform); and **(7)** use of *simultaneous* in parallel screening with several different high-throughput virus-specific B cell isolation approaches (Beacon, 10X Genomics, Rhapsody) to generate and validate best-in-class human mAbs as candidate medical countermeasures.

D. LINKAGE TO OTHER PABVAX PROJECTS AND CORES. The group will focus on developing a modular test case platform for rapid selection of highly neutralizing and/or protective mAbs against paramyxoviruses, nairoviruses, and arenaviruses. Members of our group have active collaborations with most members of the **PABVAX Center** as reflected in previous collaborations and publications (see **Overall** document). We will interact extensively with the Animal Model and Preclinical Evaluation Core (**Core E)** to study neutralization against authentic viruses and to test for protection in small and large animals as well as with **RP3** for testing paramyxovirus mAbs. The knowledge gained from our studies on the structure-function relationships of neutralizing mAb combinations can be applied to rational vaccine design for **RP 1** and **RP2** in later years of the effort when we pivot to apply the lessons learned to the same test case targets as **RP1** and **RP2.** Numerous antigens will be made available to our RP. Finally, long-half-life Fc mutations are now available based on engineering of human IgG1 for enhanced binding to the neonatal Fc receptor (FcRn) such as LS (M428L /N434S)[26], or YTE (M252Y/S254T/T256E)[27] mutations. With ≥ 90 days half-life in humans, our approach will generate mAbs with vaccine-like properties that remain effective for months during a virus epidemic and thus provides a platform for response to future paramyxovirus, nairovirus, and arenavirus pandemic threats, a key goal of our **PABVAX Center.** *Our laboratory previously validated this vaccine surrogate concept when we isolated the human mAbs that formed the basis for Evusheld with YTE long-half-life Fc mutations (used in 70 countries and millions of people) that exhibited > 6 months protective levels in humans after single IM injections*.

E. APPROACH

E.1. Specific Aim 1. Discover potently neutralizing arenavirus combination therapies. For the prototypes, we will focus on LASV, an Old World virus and MACV, a New World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[28,29]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV or MACV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.

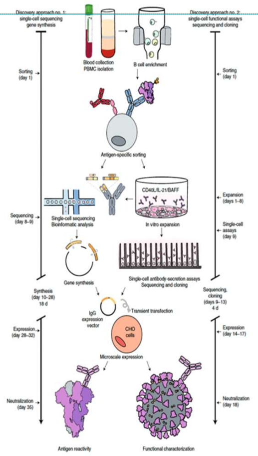

Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion allows us to further assess the functionality of the B cells or for antibody sequencing. Recombinant mAb expression validates the mAb sequence and functional activity. Figure is adapted from [1].

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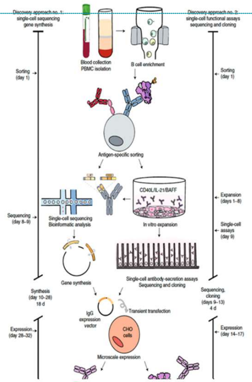

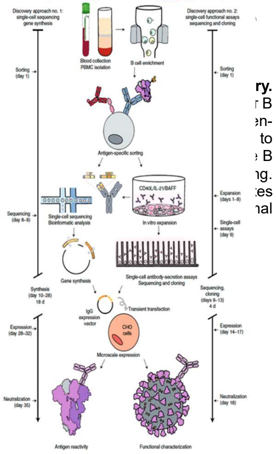

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__Preliminary Data__. ***(a) Potently neutralizing human anti-LASV and anti-MACV mAbs.*** The Crowe laboratory has previously characterized panels of neutralizing human anti-viral human mAbs from virus-immune or vaccinated individuals for about 50 viruses, mostly RNA viruses. Isolated peripheral blood mononuclear cells (PBMCs) are used to generate hybridomas or alternatively antigen-sorted at single B cell level and antibody variable genes sequenced to generate mAbs recombinantly. Panels of human anti-viral mAbs are produced rapidly (we recently reported isolation of 15,000 mAb gene pairs from single Ebola-virus-specific human B cells from a single blood sample of an immune individual[30]. Previous studies by others clearly show that representative neutralizing and protective human antibodies can be isolated from LASV-immune subjects[31-35]. In this Aim, we will refine the approaches we have developed previously for other RNA viruses in a proof-of-concept study by identifying potently neutralizing combinations of anti-LASV and anti-MACV human mAbs. We will rapidly discover human anti-LASV mAbs from LASV-immune individuals and human anti-MACV mAbs by immunizing Alloy transgenic mice. Synergy studies will be performed to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify optimal anti-LASV and anti-MACV mAb combinations and further advance the discovery platform for rapid response against emerging arenaviruses.

(b) Advanced mAb technologies. The Crowe laboratory has developed some of the highest yield systems to isolate naturally occurring human mAbs using human hybridoma or advanced single-cell RNA-seq methods (**Fig 1**). We have used sorting of antigen-specific cells with recombinant viral surface protein antigens (like the GP1 and GP2 proteins we will use here), cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. One variation we will use is to capture Ab genes directly from viral glycoprotein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), as we have done for Ebola virus[30,36] and SARS-CoV-2[1,37-42]. These single-cell techniques enhance the capture of rare antigen-specific B cells.

E.1.1. Rapid isolation of human anti-LASV and anti-MACV mAbs. *(a) Immune cells.* We have obtained human PBMCs from one previously LASV-infected individual who was medically managed previously by Emory University in their infectious diseases containment unit. This person has a documented robust neutralizing serum antibody response, suggesting isolation of neutralizing human mAbs from these PBMC aliquots will be straightforward for us. Our colleagues in **RP1** have ongoing field operations for over a decade in Sierra Leone, Liberia, and Nigeria. This work is currently in association with the NIH-Centers for Research on Emerging Infectious Diseases (CREID) with a heavy focus on the study of LASV ecology, epidemiology, and host responses. **RP4** is currently working with **RP1** on obtaining PBMCs from ~10 individuals with prior laboratory-confirmed cases of LASV. The acquisition of these samples is being managed by the Vanderbilt Vaccine Center Biorepository with separate institutional funding to be de-identified prior to use in the studies proposed here. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies have been approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples will be deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded. For anti-MACV mAbs, as a human PBMC source is not readily available, we will immunize Alloy humanized mice. These animals yield PBMCs that secrete fully human mAbs. Alloy mice are used routinely in the Crowe lab workflow.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-LASV and anti-MACV mAbs in a rapid manner to mimic a pandemic response to an outbreak. We will single-cell sort antigen-specific B cells using recombinant LASV or MACV proteins (*e.g.*, **Table 1)** or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including LASV and MACV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe GP protein or virus-particle binding to single B cells. These cells are exported for sequencing of the variable gene regions and analysis using the PyIR software developed in the Crowe laboratory. We will select a panel of up to 500 neutralizing anti-LASV mAbs and a similar number of anti-MACV mAbs to express recombinantly and advance for further characterization. We can easily conduct multiple approaches simultaneously in parallel[1,43].

Table 1. Antigens from Core D
Lassa Josiah NP
Lassa Josiah GPe
Machupo GPe
Machupo NP

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using a custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors.

(d) Micro-scale mAb expression. The Crowe laboratory has the capacity for large-throughput micro-scale mAb expression and purification. The lower end of this range allows expression of thousands of mAbs, which will feed into automated mAb purification platforms. This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based LASV and MACV neutralization assay using RTCA. We have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of cytopathic effect (CPE). Here, we will apply this method to assess neutralization of LASV. Recently, we validated the inclusion of simultaneous live-cell fluorescence microscopy into this real-time CPE-based screening platform (xCelligence RTCA eSight). These capabilities expand the platform to screen viruses that may or may not induce CPE, allowing us to also use a replication-competent vesicular stomatitis virus (VSV) LASV surrogate that expresses LASV surface proteins and causes CPE[44]. Currently, we have a VSV-LASV-Josiah (lineage IV)[44] and a Sauerwald version (lineage II) of these recombinants. Making additional LASV VSV-LASVs is not difficult, and **RP1** will make the other missing lineages or needed arenaviruses based on the same approaches as previously published. **RP1** also can easily make versions of these VSVs that express the green fluorescent protein (GFP) reporter gene. Existing VSV-CCHFV, VSV-NiV, and VSV-HeV vectors[45-47] will be provided by Dr. Geisbert overall PI and Lead of **Core E**. We already have Vanderbilt IBC approval for use of these constructs. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression if used will identify mAbs with neutralizing activity against LASV. These mAbs will be moved forward for additional studies to identify an optimal anti-LASV mAb combination. Similar processes will be followed to determine neutralization capacity of MACV mAb candidates.

E.1.2. Identifying LASV and MACV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind LASV or MACV and validate their epitopes. We will perform competition-binding studies with GP or subunit proteins (as above) or virus-like particles (VLPs)[48] available from **Core E** at UTMB. **Core E** will also inactivate LASV and MACV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from previously reported antibodies for the identification of mAbs that can concurrently bind LASV or MACV with a goal of finding more potent clones at those sites or clones that complement these inhibitory mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-LASV or anti-MACV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations for each virus, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key residues binding residues of human anti-LASV and anti-MACV mAbs.*** To determine the key residues for binding of down-selected mAbs, we will perform deep mutational scanning[49]. Briefly, all amino acids of the GP proteins will be mutated to the other 19 amino acids with a unique barcode using PCR-based mutagenesis. The LASV or MACV GP protein library will be incubated with the appropriate mAbs to identify residues with loss-of-binding phenotypes. A sequence 'logo' showing the relative contributions of each amino acid mutation will identify residues critical for binding. Multiple amino acid substitutions can then be assessed by reverse genetics at UTMB to confirm the epitopes recognized by the down-selected mAbs. This method also serves as a surrogate for *in vitro* neutralization escape studies. ***(d) Neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-LASV or VSV-MACV constructs at BSL2 in the presence of saturating concentrations of mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Confirmatory reverse genetics studies will be performed by introducing mutations into an infectious cDNA clone of LASV or MACV by **RP1** to test neutralization. We hypothesize that escape will be less likely in the presence of mAb combinations than single mAbs. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of escape variants in the presence of mAbs.

E.1.3. Structural basis of neutralization by mAb combinations. The studies above should identify optimal anti-LASV and anti-MACV mAb combinations that simultaneously bind, neutralize, and increase the barrier for viral escape under mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for arenavirus by identifying key epitope targets (**RP1**).

E.1.4. Protective efficacy of human anti-arenavirus mAbs. ***(a) Studies in guinea pigs***. MAbs that satisfy the criteria stated above and that display stage-appropriate manufacturability characteristics will advance to *in vivo* testing. We will use established models of LASV[22,50] and MACV[51,52] infection in guinea pigs. PABVAX **Core E** has access to many strains from different LASV and MACV lineages, which will be tested. For prioritization, we will focus on LASV lineage II, III, and IV strains and MACV lineage I, II, and VIII strains for which uniformly lethal models are available. Groups of 6 outbred Hartley guinea pigs will be treated with low, medium, and high doses of single or combinations of anti-LASV or anti-MACVs mAb, or isotype controls at day +1 after inoculation with the LASVs or MACVs This approach allows us to determine whether combinations provide advantage over the best monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-LASV and anti-MACV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements (blood) at day 5 or 6 after LASV or MACV challenge and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal LASV and MACV mAb pairs that are comparable to or outperform previously reported mAbs for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-LASV and anti-MACV mAb combination against LASV or MACV in NHPs. As a final test of an optimal anti-LASV and anti-MACV mAb combinations, we will assess protective efficacy of the lead anti-LASV mAb pair in NHPs as well as the anti-MACV mAb pair in NHPs. These anti-LASV and anti-MACV mAb pairs will be selected based on the *in vitro* and guinea pig data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys will only be considered pending success in the guinea pig studies and consultation with the external Scientific Advisory Board (SAB) and NIAID program staff.

E.1.5. Applying lessons learned in LASV and MACV research with the selected test case pathogens for mAb generation: Lujo and Chapare mAbs. Once we have completed the LASV and MACV discovery campaigns, will turn to the test case pathogens to see if we can use the prototype pathogen approach to related viruses, Lujo (LUJV) and Chapare (CHAPV). We do not have access to human PBMCs from LUJV - or CHAPV-immune donors. As an alternative approach, we will use humanized mouse models as a source of B cells encoding fully human monoclonal antibodies. The Vanderbilt core investigators have an established fully executed agreement to use Alloy human antibody mice for this purpose (See Letter of Support). The Alloy ATX-Gx™ mouse is one of the most effective *in vivo* human antibody discovery platforms, with over 140 partners and counting. This mouse system was originally invented and validated inside a major pharma company and then further developed by Alloy. This foundational suite of highly immunocompetent transgenic mice is engineered to drive the greatest potential diversity of unique human antibodies binding to the viral target of interest, with broad epitopic coverage. The comprehensive functional human antibody repertoire in these mice is optimized for human Ab sequence developability and diversity. Alloy and Vanderbilt have already executed a simple licensing process for use of the ATX-Gx platform, and as an established partner the Vanderbilt core team will access the expanding portfolio of transgenic Alloy strains designed to address a range of discovery challenges (including diverse Ab isotypes). Mice will be immunized with conformationally correct recombinant protein antigens (**Table 2**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for the target of interest. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for LASV and MACV.

Table 2. Antigens available from RP1
LUJV NP NTD
LUJV GPe
LUJV NP NTD +GPe
CHAPV NP NTD
CHAPV GPe
CHAPV NP NTD +GPe

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-LASV and anti-MACV mAb combinations with optimal neutralizing and protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging LASV, MACV, or related arenaviruses. Given our previous ability to generate human antiviral mAbs, we do not anticipate problems in isolating anti-LASV or anti-MACV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently neutralizing anti-LASV or anti-MACV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody$^{\text{TM}}$) on enriched B cells (*i.e.*, memory B cells) isolated from human LASV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in LASV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-LASV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-LASV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-LASV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-LASV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the high-throughput Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E2. Specific Aim 2. Discover a protective CCHF virus mAb combination therapy. Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[25,53,54]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E).** As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.

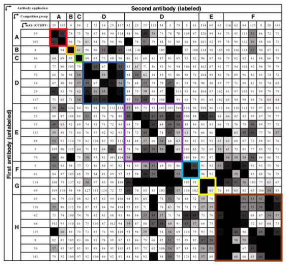


Figure 2. Competition assessment of human Abs to CCHFV using M-segment expressing cells. We tested 28 mAbs in competition assays. MAbs are displayed in 8 groups (A-H) based on their ability to compete for binding. Values shown are the % of binding that occurred during competition compared to non-competed binding of the mAb. This value was normalized to 100%. The values are also indicated by the box fill color; darker colors toward black indicate higher competition and lighter colors toward white indicate less competition, on a gradient scale.

<u>**Preliminary Data**</u>**.** ***<u>(a) Potently neutralizing and non-neutralizing human anti-CCHFV mAbs.</u>*** In this Aim, we will rapidly discover human anti-CCHFV mAbs from CCHFV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify an optimal anti-CCHFV mAb combination and further advance the discovery platform for rapid response against emerging nairoviruses. In preliminary experiments in collaboration with investigators in **RP1** and **Core E**, the Crowe laboratory has previously isolated and characterized panels of neutralizing human anti-CCHFV human mAbs from individuals who were convalescent after naturally-acquired laboratory-confirmed infection in Spain or Turkey. We isolated a panel of antibodies against CCHFV that recognize the M-segment in transfected cells, and they exhibit a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 2**). This panel contains cross-binding and cross-neutralizing antibodies for diverse strains. We observed a human mAb designated CCHF-82 competing with competition group 1 and group 2 antibodies from the previously isolated GP38-reactive murine antibodies. CCHF-82 showed some protection in a STAT1$^{-/-}$ mouse model of infection conducted by investigators in **RP1 (Fig 3)**. Further investigation with this antibody in the IbAr10200 prophylaxis and therapeutic models is warranted. In contrast, the CCHFV-neutralizing mAbs that we isolated and tested *in vivo* did not perform well in the Turkish strain STAT1$^{-/-}$ mouse model. We now know protection in this model is possible, given the work from Fels *et al*., 2021 who showed that potent antibodies against various sites on the Gc surface can afford protection in this model[53]. However, in their study they did not test GP38-reactive antibodies. To our knowledge, our preliminary data is the first indication that a human GP38-targeting antibody can provide some protection in this mouse model.

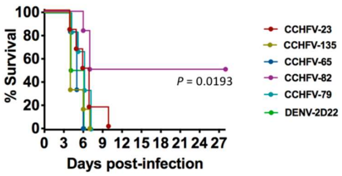


Figure 3. Human mAb CCHF-82 provides partial protection as a post-exposure prophylaxis against the Turkish strain of CCHFV in the STAT1$^{-/-}$ mouse model. A single dose of mAb was administered by the IP route to mice (n=6 per group (3 male, 3 female)) at 30 min post infections. A lethal dose of Turkish strain of CCHFV was administered. CCHF mAbs or DENV-2D22 (an isotype-matched negative control mAb) were tested in a single dose of 250 µg per mouse. Kaplan-Meier survival curves were statistically analyzed using a log-rank (Mantel-Cox) test where mAb-treated animals (*P* value shown in figure) were compared to animals treated with the DENV-2D22 negative control mAb. Unpublished data.

Non-neutralizing murine mAbs protect in mouse models of infection[25], but it is unknown if the human antibody response could provide similar levels of protection. Murine mAb 13G8 binds to GP38 and does not neutralize CCHFV. This mAb provides protection against lethal challenge as a prophylaxis and a therapeutic against CCHFV. In preliminary experiments we sought to understand if human survivors of CCHFV infection possess B cells encoding mAbs that bind a similar epitope as 13G8 and provide protection. CCHF-82 competes for binding with 13G8 to preGn-transfected cells containing GP38. This antibody offered some protection as a post-exposure prophylaxis against the Turkish strain of CCHFV (**Fig 3**). Human antibodies like this should be further studied and the frequency of these clones should be elucidated to understand if this is a feasible strategy for future vaccine efforts for humans. The epitope that 13G8 and CCHF-82 identifies could inform vaccine design if these antibodies offer protection in larger animal models. Understanding how non-neutralizing antibodies function is critical in efforts to validate the GP38-binding class of antibodies. Golden *et al.* undertook a study to understand the mechanisms 13G8 functioned and observed that complement activation contributes to the protective capacity of this antibody[25]. Here, in studies of CCHF-82, we also will assess complement activation in mouse models of infection to fully understand the function of this mAb *in vivo*. Furthermore, if this mAb protects in the CCHFV IbAr10200 strain challenge model, assessing its mechanism is warranted. Together with **Core D**, we will assess

IgG molecules with complement knockout (“KA”) and Fc gamma receptor knockout (LALA-PG) mutations in the Fc domain to study this matter. Human mAb CCHF-82 displayed limited cross-protection against experimental infection with the Turkish strain of CCHFV. Our antibody isolation here will aim to identify cross-protective non-neutralizing antibodies reactive with GP38. Recently, vaccinating with NP was shown to be another way to elicit non- neutralizing antibodies[55]. The NP of CCHFV is more conserved than the glycoproteins and might also be a non-canonical target for the identification of cross-protective non-neutralizing antibodies[55].

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant viral surface protein antigens, cloning of Ab variable genes, and then expression of mAbs as as described above in Specific Aim 1.

E.2.1. Rapid isolation of human anti-CCHFV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from a number of previously CCHFV-infected individuals. One was a health care survivor who was infected while caring for a patient in Spain[56]. We also collected a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Turkey, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples were only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies were approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples were deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-CCHFV mAbs rapidly, as if we had to respond to a nairovirus outbreak in real time. Our ability to respond rapidly in the setting of outbreaks is well-documented in the lay press (60 Minutes features and others) and the scientific literature, e.g., 78 days from sample to protection of NHPs for Zika virus[43], and 25 days from PBMC sample receipt to delivery of the antibody genes for the approved COVID-19 drug Evusheld to AstraZeneca[1]. Here, we will single-cell sort antigen-specific B cells using recombinant CCHFV proteins (GP38, Gc, Gn, Gc/Gn) or non-infectious particles as bait as described in Specific Aim 1. We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D**, (**Table 3**) or by **RP1 (Table 4)**.

Table 3. Core D CCHFV antigens
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF NP

(c) Recombinant mAb cloning. As described in Specific Aim 1 above.

(d) Micro-scale mAb expression. As described in Specific Aim 1 above.

Table 4. Antigens available from RP1
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF Turkey NP
CCHF Turkey GnGc+GP38+NP
CCHF Turkey GnGc+GP38
CCHF Turkey GP38+NP
CCHF Turkey GnGc+NP

(e) A CPE-based CCHFV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of CCHFV. We will use a rVSV CCHFV surrogate that expresses CCHFV surface proteins and causes CPE[46]. Investigators in **RP1** have the VSV-CCHFV-GFP versions of clades 1 through 7 based on this publication. We will obtain these VSV constructs from investigators in **PABVAX**. Existing VSV vectors will be provided by investigators in **RP1 and Core E**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against CCHFV. These mAbs will be moved forward for additional studies to identify an optimal anti-CCHFV mAb combination. GP38-reactive clones will be advanced based on avidity ranking since they are not likely to be neutralizing but may be protective *in vivo*.

E.2.2. Identifying CCHFV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind CCHFV surface proteins and validate their epitopes. We will perform competition-binding studies with CCHFV Gc/Gn or subunit proteins (as above) or CCHFV virus-like particles (VLPs) made from a replicon system in hand. **Core E** will inactivate CCHFV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site. Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from some of the mAbs shown in the unpublished preliminary data above for the identification of mAbs that can concurrently bind CCHFV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-CCHFV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. GP-38-reactive clones will be screened for synergistic binding as they are not expected to be neutralizing. ***(c) Identification of key binding residues of human anti-CCHFV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-CCHFV constructs at BSL2 in the presence of saturating concentrations of CCHFV mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of VSV-CCHFV escape variants in the presence of mAbs.

E.2.3. Structural basis of neutralization by anti-CCHFV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.2.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies in STAT1$^{-/-}$ mouse models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (Core D), will advance to *in vivo* testing. We will use an established STAT1$^{-/-}$ mouse model of CCHFV infection that the **RP1** and **Core E** investigators have deployed[46]. To benchmark our mAbs, a separate set of STAT1$^{-/-}$ mice will be treated with CCHF-82, which we showed in unpublished preliminary data above confers substantial but incomplete protection (**Fig 3**). The **Core E** laboratory has access to strains representing all 6 clades that can be tested. Groups of 6-8 STAT1$^{-/-}$ mice will be treated with low, medium, and high doses) of single or combinations of anti-CCHFV mAb, or isotype controls at day +1 after inoculation with CCHFVs. This approach allows us to determine whether combinations provide advantage over the best CCHFV monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after CCHFV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal CCHFV mAb pairs that are comparable to or outperform the mAbs we reported above. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-CCHFV mAb combination against CCHFV in NHPs. As a final test of an optimal anti-CCHFV mAb combination, we will assess efficacy of the lead anti-CCHFV mAb pair in NHPs (**Core E**), to be selected based on the *in vitro,* STAT1$^{-/-}$ mice, and manufacturability data (**Core D**). Down-selection for testing in cynomolgus monkeys would only be considered with success in the STAT1$^{-/-}$ mouse studies and in consultation with the external SAB and NIAID program staff.

E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs. Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human mAbs, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.

~~**E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs.** Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human mAbs, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.~~

Table 5. Antigens available from RP1
Kasokero GnGC
Kasokero GP38
Kasokero NP
Kasokero GnGc +GP38 +NP
Kasokero GnGc +GP38
Kasokero GnGc +NP
Kasokero GP38 +NP

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-CCHFV mAb combinations with optimal protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging CCHFV or related nairoviruses. Given our previous ability to generate human anti-CCHFV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-CCHFV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-

CCHFV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (i.e., memory B cells) isolated from human CCHFV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in CCHFV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-CCHFV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-CCHFV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-CCHFV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-CCHFV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E3. Specific Aim 3. Establish optimal mAb combination therapies against henipaviruses Hendra (HeV) and Nipah (NiV) viruses. We will use a large panel of potent anti-henipavirus mAbs that we previously made from the memory B cells of immune individuals and generate new antibodies targeting the fusion (F) protein. **RP2** will provide new antigens for mAb selection and BSL-2-compatible viruses for neutralization screens including Cedar virus constructs displaying HeV and NiV F and G proteins. We will identify optimal mAb combinations to enhance henipavirus therapy, minimize viral escape, and improve treatment efficacy through synergy. *In vitro* competition-binding, neutralization, and mechanistic studies will be conducted, including authentic virus neutralization with **PABVAX Core E**. We will refine the recognized epitopes and investigate the structural basis for optimal combination neutralization of henipaviruses using HeV and NiV as prototypes. *In vivo* assessment of mAb combinations will determine the minimal effective dose for protection and the ability to prevent *in vivo* escape. Protective efficacy of lead mAb combinations will be assessed in hamster models of NiV and HeV infection with **Core E**. Leads will be transferred to **Core D**. As a final test of optimal henipavirus mAb combinations, we will assess protective efficacy of the lead henipavirus mAb pairs in NHPs, to be selected based on the *in vitro* and hamster data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in African green monkey (AGM) henipavirus model studies would only be considered pending success in the hamster studies and consultation with the external SAB and NIAID program staff. The HeV and NiV studies will be conducted in years 1-3 and then applied to the plug-and-play test case Langya virus in years 2-5. If **RP2** and **Core E** cannot rescue recombinant Langya virus, the lessons learned in Years 1-3 will be applied to the alternate “plug and play” test viruses Angavokely or Sosuga (SOSV) virus. Preliminary data on SOSV mAbs are provided.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-NIV/HeV mAbs.*** In this Aim, we will further adapt approaches we have developed in preliminary experiments with henipavirus mAb studies by identifying a potently protective combination of anti-NiV/HeV human mAbs. Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups. This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains. In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and outstanding potency anti-G antibodies with extensive characterization, including synergistic neutralization.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant henipavirus F protein antigens, as described above in Specific Aim 1.

E.3.1. Rapid isolation of human anti-HeV/NiV mAbs. ***(a) Immune cells.*** We are obtaining human PBMCs from a number of previously NiV-infected individuals who acquired immunity following natural infection in collaboration with the **icddr,b**, an international health research institute based in Dhaka, Bangladesh. We are collecting a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Bangladesh, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies are approved by the icddr,b and Vanderbilt University Medical Center IRBs. PBMCs isolated from the blood samples are deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their use in this **RP4,** if funded. ***(b) Applying mAb technologies for new mAb isolation***. We will use sorting of antigen-specific B cells, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer antigen-specific B cells. We will sort soluble fusion (sF) or attachment (G) protein-specific cells with recombinant viral surface protein antigens from **Core D** (**Table 6**).

(c) Ab screening and sequence analysis. Our goal is to identify human anti-NiV/HeV mAbs rapidly, as if we had to respond to a paramyxovirus outbreak in real time. We will single-cell sort antigen-specific B cells using recombinant HeV and NiV proteins (sF or G) as bait as described in Specific Aim 1 above.

(d) Recombinant mAb cloning. As described in Specific Aim 1 above.

(e) Micro-scale mAb expression. As described in Specific Aim 1 above.

(f) CPE-based HeV/NiV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of HeV and NiV. We will use rVSV HeV and NiV surrogates that expresses henipaviurs surface proteins and cause CPE. Investigators in **RP2 and RP3** have VSV-NiV F and VSV-NiV G with GFP[45], VSV-NiV G without GFP[47] and VSV-HeVG (Geisbert and Cross, unpublished). We will obtain these all these VSV constructs from investigators in **PABVAX**. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against HeV and NiV. We also can use Cedar virus constructs for neutralization assays as the have reduced pathogenicity[57] and can be used in high-throughput screening assays for henipavirus antiviral discovery[58]. Cedar viruses will be provided by Dr. Broder of **RP2**. The mAbs with most potent activity will be moved forward for additional studies to identify an optimal anti-HeV/NiV mAb combination.

Table 6. Core D Henipavirus antigens
Nipah sF
Hendra sF
NiV G

E.3.2. Identifying HeV/NiV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind HeV/NiV surface proteins and validate their epitopes. We will perform competition-binding studies with HeV/NiV sF or G proteins (as above). We will include positive controls made recombinantly from some of the mAbs shown in the preliminary data above for the identification of mAbs that can concurrently bind HeV/NiV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti- HeV/NiV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key binding residues of human anti-HeV/NiV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-NiV F, VSV-NiV G and VSV-HeV G constructs at BSL-2 in the presence of saturating concentrations of HeV/NiV mAbs (single or combination) using RTCA to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test the level of infection of VSV-HeV/NiV escape variants in the presence of mAbs.

E.3.3. Structural basis of neutralization by anti-HeV/NiV mAb combinations. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.

E.3.4. Protective efficacy of human anti-henipavirus mAbs. ***(a) Studies in hamsters***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (**Core D**), will advance to *in vivo* testing. We will use an established hamster model of NiV or HeV infection that the **Core E** investigators have deployed. To benchmark our mAbs, a separate set of hamsters will be treated with G mAbs HENV-103+HENV-117, which we showed in previous data above confers substantial protection[18]. Groups of 6-8 hamsters will be treated with low, medium, or high IgG doses) of single or combinations of anti-HeV/NiV mAbs, or isotype controls at day +1 after inoculation with HeV or NiV. This approach allows us to determine whether combinations provide advantage over the best HeV or NiV mAb monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-HeV and anti-NiV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after HeV or NiV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal HeV/NiV mAb pairs that are comparable to or outperform the mAbs we reported previously. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-HeV/NiV mAb combination against HeV and NiV in NHPs. As a final test of an optimal anti-HeV/NiV mAb combination, we may assess efficacy of the lead anti-HeV/NiV mAb pair in NHPs (**Core E**), selected based on the *in vitro,* hamster data, and manufacturability data (**Core D**). Down-selection for testing in AGMs would only be considered with success in the hamster studies and in consultation with the external SAB and NIAID program staff.

E.3.5. Applying lessons learned in HeV/NiV research to Langya, Angavokely and Sosuga viruses. Once we have completed the HeV/NiV discovery campaigns, will turn to the test case pathogen Langya (or alternate Angavokely and Sosuga (SOSV) viruses) to see if we can use the prototype pathogen approach for this virus. We have plenty of human PBMCs from the sole identified SOSV-immune individual and have already shown that it is possible to obtain HN- and F-specific neutralizing antibodies from this individual's B cells[59]. We also have already prepared recombinant SOSV prefusion F and HN proteins[59]. We do not have access to human PBMCs from Langya- or Angavokely-immune donors. For those mAb discovery campaigns, we will use Alloy humanized mouse models as a source of B cells encoding human mAbs, as above. Mice will be immunized with recombinant protein antigens (**Table 7**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Langya or Angavokely. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for Hev/NiV described above.

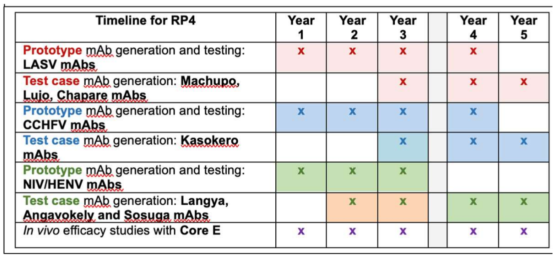
Table 7. Paramyxovirus antigens from Core D
Mojang sF
Angavokely sF
Langya sF

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-HeV/NiV mAb combinations with optimal protective activity. These mAb combinations will be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging HeV/NiV or related paramyxoviruses. Given our previous ability to generate human anti-HeV/NiV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-HeV/NiV mAbs. We may observe that new mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-HeV/NiV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells *(i.e., memory B cells) isolated from human NiV-immune PBMC samples. In addition, we can deep sequence the B* cell repertoires present in NiV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-HeV/NiV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-HeV/NiV mAb combinations that can inform vaccine design (**RP2**) for related viruses being used as test cases in years 2 through 5.

F. Scientific rigor. ***Experimental design.*** Experiments will include biological and technical replicates, and results will be analyzed by parametric or non-parametric analyses (depending on data distribution) and corrections for multiple comparisons. EC_{50} (binding) and IC_{50} (neutralization) values will be reported only following at least 3 identically conducted experiments. All mAb screening will incorporate appropriate + and - controls. Positive signals in binding assays will be defined as >3 SD above background. ***Protein quality.*** We have a high capacity to purify proteins using a Protein Maker workstation that allows purification chromatography on 24 parallel columns. We perform QC on protein reagents using a Maurice instrument with capillary electrophoresis technology to automate our protein profiling either by size or charge, providing cIEF and CE-SDS data. Animal ***studies*** will be performed blinded with independent replicates and include negative (*e.g.*, placebo), and positive (when available) controls. We will use power calculations (**Data Management Core B**) to determine sample sizes. For virological studies, power analysis was performed using accepted values for type I error (0.05) and power (80%). The values for population variance (15×) are based on existing data sets and our experience with rodent challenge studies. We will perform experiments at least 2-3 independent times (n = 4-5 each) to attain n = 12-15 per arm. Smaller group sizes can be used if differences are larger than expected.

G. Relevant biological variables. The human mAbs are derived from immune human donors or Alloy mice. Humans. Human Abs will be isolated for biological, structural, and functional studies at a clonal level. The sample size is not powered for large scale epidemiologic or clinical study conclusions. There is no evidence that sex, age, body mass index, or underlying health conditions other than immunodeficiency or immunosuppressive treatments alters the activity of cloned Abs in structure/function studies. The samples are de-identified without patient specific identifiers to protect the health information of the donors; thus, research with these samples is considered *Not Human Subjects Research* category activity. Mice, hamsters, guinea pigs, NHP, and viruses are also biological systems used in this application. Conventional animal housing conditions will be used because there is no evidence that altered room temperatures and light/dark cycles influence paramyxovirus, nairovirus, or arenavirus pathogenesis. Mice, hamsters, guinea pigs, and NHPs of both sexes will be used, and phenotypes will be monitored for sex-based differences. Virus stocks will be either low passage seed stocks from natural isolates or generated from existing cDNA clones and sequence-confirmed prior to use for infection studies.

H. Milestones and quantitative Go/No-Go criteria. Through **Aims 1-3**, we have proposed experiments that will advance our knowledge of the mAb-mediated mechanisms of action against paramyxoviruses, nairoviruses, and arenaviruses. In each Aim, we will use down-selection criteria (as below) to advance mAb combinations or variants for additional studies. By year 3, we will have defined the principles that govern optimal mAb combinations for paramyxoviruses, nairoviruses, and arenaviruses and the rational approaches needed to select mAbs for potency and breadth. We will use the following metrics to advance mAbs: ***(a) Neutralizing activity***. All mAbs will have neutralizing potency < 10 ng/mL, EC_{50} value; ***(b) Binding affinity***. MAbs will be of high affinity to surface proteins or virions (K_D < 5 nM). ***(c) Protein stability and production quality***. MAbs must produce at high levels without optimization (> 100 mg/L) and show biochemical/functional stability at 4°C or -80°C (>90% retention of binding and neutralization activity at 30 d). ***(d) Synergy***. Combinations of 2 mAbs against a given virus ideally will show synergy of neutralization *in vitro* or at a minimum, additive inhibition (no antagonism); ***(e) Epitopes***. To avoid resistance, mAbs in combination will bind 2 epitopes (determined by competition binding (<20% reduction in binding), mapping, and structural studies. ***(f) Resistance*** against mAbs will be assessed for virus escape. Deep sequencing of viruses that emerge from cells or animals treated with mAbs will inform prioritization. Combinations of mAbs with high genetic barriers to resistance (<10× loss in EC_{50}) will be advanced. ***(g) Protection in mice, hamsters, guinea pigs, or NHPs.*** The following metrics can be used: (i) Protection against lethal challenge by homologous virus (>90% survival; (ii) Protection against clinical disease by homologous virus (>80%); (iii) Protection against infection by homologous virus (>100-fold reduction in viral titers in multiple organs). ***(h) pK measurements***. For lead protective mAbs with mutations that should confer extended half-life, we will perform pK studies in NHPs (with **Core D** and **E**) to assess levels over time with a goal of a human predicted serum half-life of >6 months.



Timeline for RP4	**Year 1**	**Year 2**	**Year 3**		**Year 4**	**Year 5**
Prototype mAb generation and testing: **LASV mAbs**	x	x	x		x	
Test case mAb generation: **Machupo, Lujo, Chapare mAbs**			x		x	x
Prototype mAb generation and testing: **CCHFV mAbs**	x	x	x		x	
Test case mAb generation: **Kasokero mAbs**			x		x	x
Prototype mAb generation and testing: **NIV/HENV mAbs**	x	x	x			
Test case mAb generation: **Langya, Angavokely and Sosuga mAbs**		x	x		x	x
In vivo efficacy studies with **Core E**	x	x	x		x	x

LITERATURE CITED – RP4

1. Zost, S.J*., et al.* Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* **26**, 1422-1427 (2020).
2. Fischer, W.A., II & Wohl, D.A. Moving Lassa Fever Research and Care Into the 21st Century. *The Journal of Infectious Diseases* **215**, 1779-1781 (2017).
3. Ezeomah, C*., et al.* Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. *Open Forum Infect Dis* **6**, ofz512 (2019).
4. Li, A.L*., et al.* Ophthalmic manifestations and vision impairment in Lassa fever survivors. *PloS one* **15**, e0243766 (2020).
5. Whitmer, S.L.M*., et al.* New Lineage of Lassa Virus, Togo, 2016. *Emerging infectious diseases* **24**, 599-602 (2018).
6. Ibukun, F.I. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. *Viruses* **12**(2020).
7. Webb, P.A., Johnson, K.M., Mackenzie, R.B. & Kuns, M.L. Some characteristics of Machupo virus, causative agent of Bolivian hemorrhagic fever. *Am J Trop Med Hyg* **16**, 531-538 (1967).
8. Aguilar, P.V*., et al.* Reemergence of Bolivian hemorrhagic fever, 2007-2008. *Emerging infectious diseases* **15**, 1526-1528 (2009).
9. Enria, D.A., Ambrosio, A.M., Briggiler, A.M., Feuillade, M.R. & Crivelli, E. [Candid#1 vaccine against Argentine hemorrhagic fever produced in Argentina. Immunogenicity and safety]. *Medicina (B Aires)* **70**, 215-222 (2010).
10. Enria, D.A. & Maiztegui, J.I. Antiviral treatment of Argentine hemorrhagic fever. *Antiviral research* **23**, 23-31 (1994).
11. Hawman, D.W. & Feldmann, H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology* (2023).
12. Chinikar, S*., et al.* Genetic Diversity of Crimean Congo Hemorrhagic Fever Virus Strains from Iran. *J Arthropod Borne Dis* **10**, 127-140 (2016).
13. Shepherd, A.J., Swanepoel, R. & Leman, P.A. Antibody response in Crimean-Congo hemorrhagic fever. *Rev Infect Dis* **11 Suppl 4**, S801-806 (1989).
14. Lombe, B.P*., et al.* Mapping of Antibody Epitopes on the Crimean-Congo Hemorrhagic Fever Virus Nucleoprotein. *Viruses* **14**(2022).
15. Mackenzie, J., Wang, L. & Broder, C.C. Henipaviruses. in *Fields Virology*, Vol. 2 1070-1085 (2013).
16. Whitmer, S.L.M*., et al.* Inference of Nipah virus evolution, 1999–2015. *Virus Evolution* **7**(2020).
17. Dong, J*., et al.* Potent Henipavirus Neutralization by Antibodies Recognizing Diverse Sites on Hendra and Nipah Virus Receptor Binding Protein. *Cell* **183**, 1536-1550 e1517 (2020).
18. Doyle, M.P*., et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021).
19. Mire, C.E*., et al.* A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis* **221**, S471-S479 (2020).
20. Dang, H.V*., et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021).
21. Cross, R.W*., et al.* Antibody therapy for Lassa fever. *Curr Opin Virol* **37**, 97-104 (2019).
22. Cross, R.W*., et al.* Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral research* **133**, 218-222 (2016).
23. Cartwright, H.N., Barbeau, D.J. & McElroy, A.K. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere* **6**, e0055621 (2021).

24. Gutjahr, B., *et al.* Two monoclonal antibodies against glycoprotein Gn protect mice from Rift Valley Fever challenge by cooperative effects. *PLoS Negl Trop Dis* **14**, e0008143 (2020).
25. Golden, J.W., *et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv* **5**, eaaw9535 (2019).
26. Zalevsky, J., *et al.* Enhanced antibody half-life improves in vivo activity. *Nat Biotechnol* **28**, 157-159 (2010).
27. Dall'Acqua, W.F., *et al.* Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* **169**, 5171-5180 (2002).
28. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Levis, S.C. & Maiztegui, J.I. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* **2**, 255-256 (1984).
29. Jahrling, P.B. & Peters, C.J. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infection and immunity* **44**, 528-533 (1984).
30. Chen, E.C., *et al.* Systematic analysis of human antibody response to ebolavirus glycoprotein shows high prevalence of neutralizing public clonotypes. *Cell Rep* **42**, 112370 (2023).
31. Buck, T.K., *et al.* Neutralizing Antibodies against Lassa Virus Lineage I. *mBio* **13**, e0127822 (2022).
32. Enriquez, A.S., *et al.* Delineating the mechanism of anti-Lassa virus GPC-A neutralizing antibodies. *Cell Rep* **39**, 110841 (2022).
33. Hastie, K.M., *et al.* Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell* **178**, 1004-1015 e1014 (2019).
34. Hastie, K.M., *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).
35. Li, H., *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991 (2022).
36. Gilchuk, P., *et al.* Pan-ebolavirus protective therapy by two multifunctional human antibodies. *Cell* **184**, 5593-5607 e5518 (2021).
37. Chen, E.C., *et al.* Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* **36**, 109604 (2021).
38. Dong, J., *et al.* Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-1244 (2021).
39. Greaney, A.J., *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).
40. Kramer, K.J., *et al.* Potent neutralization of SARS-CoV-2 variants of concern by an antibody with an uncommon genetic signature and structural mode of spike recognition. *Cell Rep* **37**, 109784 (2021).
41. Suryadevara, N., *et al.* Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell* **184**, 2316-2331 e2315 (2021).
42. Zost, S.J., *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449 (2020).
43. Gilchuk, P., *et al.* Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics. *Nat Biomed Eng* **4**, 1030-1043 (2020).
44. Cross, R.W., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep* **40**, 111094 (2022).
45. Mire, C.E., *et al.* Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J* **10**, 353 (2013).
46. Rodriguez, S.E., *et al.* Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep* **9**, 7755 (2019).
47. Foster, S.L., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. *Proc Natl Acad Sci U S A* **119**, e2200065119 (2022).

48. Branco, L.M., *et al.* Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* **7**, 279 (2010).
49. Starr, T.N., *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
50. Deschambault, Y., *et al.* An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. *Viruses* **15**(2023).
51. Bell, T.M., *et al.* Pathogenesis of Bolivian Hemorrhagic Fever in Guinea Pigs. *Vet Pathol* **53**, 190-199 (2016).
52. Golden, J.W., *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* **7**, 4679 (2017).
53. Fels, J.M., *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell* **184**, 3486-3501 e3421 (2021).
54. Mishra, A.K., *et al.* Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies. *Science* **375**, 104-109 (2022).
55. Leventhal, S.S., *et al.* Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *EBioMedicine* **82**, 104188 (2022).
56. Negredo, A., *et al.* Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med* **377**, 154-161 (2017).
57. Laing, E.D., *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018).
58. Amaya, M., *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084 (2021).
59. Parrington, H.M., *et al.* Potently neutralizing human mAbs against the zoonotic pararubulavirus Sosuga virus. *JCI Insight* **8**(2023).

A. BACKGROUND AND SIGNIFICANCE. Paramyxoviruses, nairoviruses, and arenaviruses are widely distributed RNA viruses that cause severe human syndromes. Because of their geographical distribution and expansion, changes in human demographics and increased exposures to wild animals including rodents and bats, and their capacity for human-to-human spread, these viruses have the potential to cause pandemics. Together with the other investigators in **PABVAX**, we have selected several important prototype paramyxoviruses, nairoviruses, and arenaviruses to study to learn the rules governing immunity to these pathogens. The work on the pathogens selected is justified based on their prevalence and severity alone, but also because of their ability to serve as prototypes for genetically related viruses. We chose the viruses for focus in this RP4 on human antibody development because of their clinical relevance and because several will be studied as prototypes for vaccine development in **RP1** (arenavirus and nairovirus vaccines) and **RP2** (paramyxovirus vaccines). Among the arenaviruses, we will target *Lassa mammarenavirus* (LASV) and *Machupo mammarenavirus* (MACV)*, and* among the family Nairoviridae of RNA viruses, we will study Crimean–Congo hemorrhagic fever orthonairovirus (CCHFV). In tandem with the vaccine projects in **RP2** focused on paramyxoviruses, we study Nipah virus (NiV) and Hendra virus (HeV) as the prototypes, and already have preliminary data with Sosuga virus as a test case. Information about the principles we discover about critical neutralizing epitopes identified here in **RP4** with prototype viruses can feed back into all other **Projects** that address the subsequent test cases in later years (Lujo, Chapare, Kasokero, Langya, Sosuga).

LASV is an Old World arenavirus (OWAV) with a single-stranded RNA virus belonging to the Arenaviridae family. Infection in humans is often the result of exposure to infected excreta from the rodent reservoir, *Mastomys natalensis* where up to 500,000 cases and an estimated 5,000 deaths occur annually in West Africa[2]. While rodent infection is largely benign, human infection can result in severe hemorrhagic disease with neurological complications and long-term hearing and vision sequelae[3,4]. The virus possesses a bi-segmented genome comprising large (L) and small (S) segments, both of which are enclosed within a lipid envelope. The L segment (~7.2 kb) encodes the RNA-dependent RNA polymerase (RdRp) and a zinc-binding protein (Z), while the S segment (~3.4 kb) codes for the nucleoprotein (NP) and the glycoprotein precursor (GPC). The GPC is further cleaved into two surface glycoproteins, GP1 and GP2, which play critical roles in viral entry. The genetic diversity of LASV can be classified into six lineages (I-VI), with lineages III and IV being the most prevalent[5]. Genetic variation arises mainly due to the error-prone nature of the viral RNA polymerase, leading to the emergence of quasi-species. This diversity contributes to the virus's ability to evade host immune responses and pose challenges to vaccine development[6]. ~~LASV antigens include~~T~~ t~~he surface glycoproteins GP1 and GP2, are responsible for receptor binding and membrane fusion, respectively. While GP1 is highly variable among strains, GP2 is more conserved. The nucleoprotein (NP) is another major antigen, and its immunodominant epitopes are targeted by the host's immune response. Its genetic diversity and antigenic properties complicate diagnosis and vaccine development, emphasizing the need for continued research on medical countermeasures.

MACV is a New World Arenavirus (NWAV) and the causative agent of Bolivian Hemorrhagic fever. Humans are infected after exposure to excreta from the reservoir, *Calomys callosus,* the large vesper mouse. Disease in humans has a slow onset, but can progress to severe hemorrhagic fever coupled to insidious neurological complications including tremors, seizures, and paralysis[7]. MACV has a similar genomic organization and contains a GPC, NP, Z, and L polymerase proteins. Genetic diversity of MACV is delineated into 8 lineages (I-VIII) where there is growing concern of an expanding host range of *C. callosus* is responsible for outbreaks outside the known endemic region[8]. While the host entry receptor for NWAV is not shared with OWAV, the role for GP to drive immunity as a major antigen alongside NP. The better studied Junin has a regionally approved vaccine[9] and convalescent plasma program[10] available to prevent and treat disease, respectively. There are no approved vaccines or therapies approved for any other NWAV, including MACV. Further, itis not well established whether these countermeasures have potential for cross protect other NWAV such as MACV, underscoring the value of continued efforts to develop broadly active countermeasures to safeguard against lethal disease.

CCHFV is a highly pathogenic tick-borne virus classified under the Nairovirus genus within the Bunyaviridae family[11]. It has a negative-sense, single-stranded RNA genome comprising three segments: Small (S), Medium (M), and Large (L). These segments are enclosed within a lipid envelope and code for various structural and non-structural proteins. The S segment (~1.7 kb) encodes the nucleocapsid protein (N), which is essential for viral replication and assembly. The M segment (~5.3 kb) codes for the glycoprotein precursor (GPC), which is post-translationally cleaved into two surface glycoproteins, Gn and Gc. These glycoproteins are involved in viral entry and fusion. The L segment (~12.1 kb) encodes the RNA-dependent RNA polymerase (RdRp), responsible for viral RNA synthesis. CCHFV exhibits significant genetic diversity, with at least seven distinct clades (Europe 1, Europe 2, Africa 1-3, and Asia 1-2) based on the partial S segment sequences[12]. This diversity is attributed to the segmented genome, geographical distribution, and error-prone viral RNA polymerase, leading to the emergence of various quasi-species. The major antigens of CCHFV include the surface glycoproteins Gn and

Gc, as well as the nucleocapsid protein (N)[13,14]. These antigens are targeted by the host immune response. CCHFV is genetically diverse, so breadth of reactivity will be a focus of our discovery efforts.

NiV/HeV. Bat borne**,** NiV and HeV are enveloped, negative-sense, single-stranded RNA viruses belonging to the Henipavirus genus within the Paramyxoviridae family[15]. These emerging zoonotic viruses pose a significant threat to human and animal health due to their high fatality rates and broad host range. The genomes of NiV and HeV are approximately 18.2 kb or 18.4 kb in length, respectively, and share a similar gene organization. They encode six major structural proteins: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment glycoprotein (G), and large polymerase (L) proteins. The N, P, and L proteins form the viral RNA-dependent RNA polymerase (RdRp) complex necessary for transcription and replication, whereas the M, F, and G proteins are essential for virus assembly and entry. Genetic diversity in Henipaviruses is relatively low compared to other RNA viruses, with NiV strains showing 91-100% nucleotide similarity and HeV strains displaying 83-100% similarity[16]. However, the error-prone nature of the RdRp can still generate genetic variability, leading to the emergence of distinct lineages. The major surface antigens of Nipah and Hendra viruses are the attachment glycoprotein (G) and fusion protein (F). The G protein facilitates receptor binding, while the F protein mediates viral membrane fusion. Both antigens are involved in host immune responses and are potential targets for vaccine development, and the neutralizing antibody approaches we propose here. NiV and HeV exhibit relatively low genetic diversity, and in preliminary studies, we have identified human mAbs that neutralize HeV, NiV Bangladesh and NiV Malaysia strains[17-19]. Antibodies to the major surface antigens, the G and F proteins, are therefore, promising candidates for development as prophylactic or therapeutic molecules.

Antibodies (Abs) are important correlates of protection for paramyxoviruses, nairoviruses, and arenaviruses. Immune sera against members of each of these groups of viruses has been shown to exhibit protective efficacy *in vivo*, which supports the use for Abs as treatment options. However, the specificity, functional properties, and other attributes of protective antibodies are poorly defined for most of these viruses. MAbs to the attachment and fusion surface proteins of paramyxoviruses (such as the G and F proteins of Hendra/Nipah[18,20] and the GP proteins of the arenaviruses like LASV[21,22] can neutralize and protect against infection in rodent models. CCHFV is a bit of a conundrum in that ultrapotent human neutralizing antibodies to the related Rift Valley fever virus surface proteins are highly protective *in vivo*[23,24], but CCHFV neutralizing antibodies are not protective. In contrast, some non-neutralizing antibodies do protect partially for CCHFV[25]. Clearly, there is a lot to learn about the rules of immunity to these viruses, and the genetic, molecular, and structural basis of antibody-mediated protection for viruses like CCHFV. Based on these data, we propose the use of mAbs as treatment options to learn the rule of how to protect against emerging paramyxoviruses, nairoviruses, and arenaviruses and to develop candidate antibody countermeasures for translational development with **Core D**. To do so, we will further develop our technical methods for rapidly discovering mAbs, in the case of a future unexpected epidemic caused by viruses of these families. Most importantly, we will define the principles governing combination antibody therapy to prevent virus escape from treatment or prevention and to optimize the efficacy of the beneficial effects conferred by passive immunity.

B. SCIENTIFIC PREMISE. The work proposed here in **RP4** is based on the extensive basic, translational, and clinical trial data on the role of neutralizing Abs in protection against infection and disease caused by paramyxoviruses, nairoviruses, and arenaviruses. Although a significant amount of data supports the use of mAbs to prevent or control paramyxovirus, nairovirus, and arenavirus infection, many questions remain, such as the ideal epitopes to target with mAb combination therapy to improve upon neutralization potency, *in vivo* efficacy, and resistance to viral escape. Identifying these epitopes can help inform vaccine design to elicit optimal protective polyclonal responses (in **RP1** and **RP2**, especially with designs for test cases in years 4 and 5). Here we aim to refine our understanding of the correlates of mAb protection against paramyxoviruses, nairoviruses, and arenaviruses to enable the design of a modular ‘plug-and-play’ workflow to rapidly respond to potential pandemics by generating optimized combinations of vaccine-like mAbs with an extended half-life that could be used for long-term prophylaxis (> 6 months) and therapy.

C. INNOVATION. RP4 has many innovative conceptual and technical features including: Conceptual. **(1)** determining the principles that govern optimal combinations of human mAbs against paramyxoviruses, nairoviruses, and arenaviruses for protection and resistance to viral escape; **(2)** the development of a clear workflow that generates therapeutic or preventative antibodies (long half-life mAbs that serve as vaccine surrogates) against emerging or new paramyxoviruses, nairoviruses, and arenaviruses with pandemic potential. Our goal is to define the principles and features, including epitope, combination ratios, and mechanisms of action, that result in optimal efficacy of mAbs against paramyxoviruses, nairoviruses, and arenaviruses in animal models and create a higher barrier for resistance. These studies will serve as paradigms for rapid antibody discovery in response to future paramyxovirus, nairovirus, and arenavirus pandemic threats. Technical. **(3)** large-scale B cell repertoire sequencing with customized methods and software; **(4**) use of humanized (Alloy) mice and novel immunogens from **RP1** and **RP2** and **Core D**; **(5)** single B cell functional (neutralization) assays performed on a

Beacon instrument; **(6)** development of high-throughput real-time label-free virus neutralization assays (xCELLigence platform); and **(7)** use of *simultaneous* in parallel screening with several different high-throughput virus-specific B cell isolation approaches (Beacon, 10X Genomics, Rhapsody) to generate and validate best-in-class human mAbs as candidate medical countermeasures.

D. LINKAGE TO OTHER PABVAX PROJECTS AND CORES. The group will focus on developing a modular test case platform for rapid selection of highly neutralizing and/or protective mAbs against paramyxoviruses, nairoviruses, and arenaviruses. Members of our group have active collaborations with most members of the **PABVAX Center** as reflected in previous collaborations and publications (see **Overall** document). We will interact extensively with the Animal Model and Preclinical Evaluation Core (**Core E)** to study neutralization against authentic viruses and to test for protection in small and large animals as well as with **RP3** for testing paramyxovirus mAbs. The knowledge gained from our studies on the structure-function relationships of neutralizing mAb combinations can be applied to rational vaccine design for **RP 1** and **RP2** in later years of the effort when we pivot to apply the lessons learned to the same test case targets as **RP1** and **RP2.** Numerous antigens will be made available to our RP. Finally, long-half-life Fc mutations are now available based on engineering of human IgG1 for enhanced binding to the neonatal Fc receptor (FcRn) such as LS (M428L /N434S)[26], or YTE (M252Y/S254T/T256E)[27] mutations. With ≥ 90 days half-life in humans, our approach will generate mAbs with vaccine-like properties that remain effective for months during a virus epidemic and thus provides a platform for response to future paramyxovirus, nairovirus, and arenavirus pandemic threats, a key goal of our **PABVAX Center.** *Our laboratory previously validated this vaccine surrogate concept when we isolated the human mAbs that formed the basis for Evusheld with YTE long-half-life Fc mutations (used in 70 countries and millions of people) that exhibited > 6 months protective levels in humans after single IM injections*.

E. APPROACH

E.1. Specific Aim 1. Discover potently neutralizing arenavirus combination therapies. For the prototypes, we will focus on Lassa virus (LASV), an Old World virus and Machupo virus (MACV), a New World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[28,29]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.

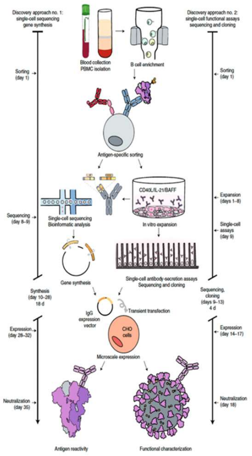


Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion allows us~~of these B cells is done~~ to further assess the functionality of the B cells or for antibody sequencing~~ to determine the variable genes contributing to antigen reactivity~~. Recombinant mAb

<u>Preliminary Data</u>. ***(a) Potently neutralizing human anti-LASV and anti-MACV mAbs.*** The Crowe laboratory has previously characterized panels of neutralizing human anti-viral human mAbs from virus-immune or vaccinated individuals for about 50 viruses, mostly RNA viruses. Isolated peripheral blood mononuclear cells (PBMCs) are used to generate hybridomas or alternatively antigen-sorted at single B cell level and antibody variable genes sequenced to generate mAbs recombinantly. Panels of human anti-viral mAbs are produced rapidly (we recently reported isolation of 15,000 mAb gene pairs from single Ebola-virus-specific human B cells from a single blood sample of an immune individual[30]. Previous studies by others clearly show that representative neutralizing and protective human antibodies can be isolated from LASV-immune subjects[31-35]. In this Aim, we will refine the approaches we have developed previously for other RNA viruses in a proof-of-concept study by identifying potently neutralizing combinations of anti-LASV and anti-MACV human mAbs. We will rapidly discover human anti-LASV mAbs from LASV-immune individuals and human anti-MACV mAbs by immunizing Alloy transgenic mice. Synergy studies will be performed to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify optimal anti-LASV and anti-MACV mAb combinations and further advance the discovery platform for rapid response against emerging arenaviruses.

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(b) Advanced mAb technologies. The Crowe laboratory has developed some of the highest yield systems to isolate naturally occurring human mAbs using human hybridoma or advanced single-cell RNA-seq methods (**Fig 1**). We have used sorting of antigen-specific cells with recombinant viral surface protein antigens (like the GP1 and GP2 proteins we will use here), cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. One variation we will use is to capture Ab genes directly from viral glycoprotein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), as we have done for Ebola virus[30,36] and SARS-CoV-2[1,37-42]. These single-cell techniques enhance the capture of rare antigen-specific B cells.

E.1.1. Rapid isolation of human anti-LASV and anti-MACV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from one previously LASV-infected individual who was medically managed previously by Emory University in their infectious diseases containment unit. This person has a documented robust neutralizing serum antibody response, suggesting isolation of neutralizing human mAbs from these PBMC aliquots will be straightforward for us. Our colleagues in **RP1** have ongoing field operations for over a decade in Sierra Leone, Liberia, and Nigeria. This work is currently in association with the NIH-Centers for Research on Emerging Infectious Diseases (CREID) with a heavy focus on the study of LASV ecology, epidemiology, and host responses. **RP4** is currently working with **RP1** on obtaining PBMCs from ~10 individuals with prior laboratory-confirmed cases of LASV. The acquisition of these samples is being managed by the Vanderbilt Vaccine Center Biorepository with separate institutional funding to be de-identified prior to use in the studies proposed here. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies have been approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples will be deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded. For anti-MACV mAbs, as a human PBMC source is not readily available, we will immunize Alloy humanized mice. These animals yield PBMCs that secrete fully human mAbs. Alloy mice are used routinely in the Crowe lab workflow.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-LASV and anti-MACV mAbs in a rapid manner to mimic a pandemic response to an outbreak. We will single-cell sort antigen-specific B cells using recombinant LASV or MACV proteins (*e.g.* **Table 1**) or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including LASV and MACV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe GP protein or virus-particle binding to single B cells. These cells are exported for sequencing of the variable gene regions and analysis using the PyIR software developed in the Crowe laboratory. We will select a panel of up to 500 neutralizing anti-LASV mAbs and a similar number of anti-MACV mAbs to express recombinantly and advance for further characterization. We can easily conduct multiple approaches simultaneously in parallel[1,43].

Table 1. Antigens from Core D
Lassa Josiah NP
Lassa Josiah GPe
Machupo GPe
Machupo NP

~~Table 1. Antigens from Core D~~
~~Lassa Josiah NP~~
~~Lassa Josiah GPe~~
~~Machupo GPe~~
~~Machupo NP~~

~~We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D** listed in **Table 1**.~~

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using a custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors.

(d) Micro-scale mAb expression. The Crowe laboratory has the capacity for large-throughput micro-scale mAb expression and purification. The lower end of this range allows expression of thousands of mAbs, which will feed into automated mAb purification platforms. This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based LASV and MACV neutralization assay using RTCA. We have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of cytopathic effect (CPE). Here, we will apply this method to assess neutralization of LASV. Recently, we validated the inclusion of simultaneous live-cell fluorescence microscopy into this real-time CPE-based screening platform (xCelligence RTCA eSight). These capabilities expand the platform to screen viruses that may or may not induce CPE, allowing us to also use a replication-competent vesicular stomatitis virus (VSV) LASV surrogate that expresses LASV surface proteins and causes CPE[44]. Currently, we have a VSV-LASV-Josiah (lineage IV)[44] and a Sauerwald version (lineage II) of these recombinants. Making additional LASV VSV-LASVs is not difficult, and RP1 will make the other missing lineages or needed NWAV (MACV and CHAPV for test case evaluation) based on the same approaches as previously published. RP1 also can easily also make versions of these VSVs that express the

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green fluorescent protein (GFP) reporter gene. We will obtain these all these VSV constructs from investigators in **PABVAX**. Existing VSV-CCHFV, VSV-NiV, and VSV-HeV vectors[45-47] will be provided by Dr. Geisbert overall PI and Lead of **Core E**. We already have Vanderbilt Institutional Biosafety Committee approval for use of these constructs in this format and assay. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression if used will identify mAbs with neutralizing activity against LASV. These mAbs will be moved forward for additional studies to identify an optimal anti-LASV mAb combination. Similar processes will be followed to determine neutralization capacity of MACV mAb candidates.

E.1.2. Identifying LASV and MACV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind LASV or MACV and validate their epitopes. We will perform competition-binding studies with GP or subunit proteins (as above) or virus-like particles (VLPs)[48] available from **Core E** at UTMB. **Core E** will also inactivate LASV and MACV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from previously reported antibodies for the identification of mAbs that can concurrently bind LASV or MACV with a goal of finding more potent clones at those sites or clones that complement these inhibitory mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-LASV or anti-MACV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations for each virus, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key residues binding residues of human anti-LASV and anti-MACV mAbs.*** To determine the key residues for binding of down-selected mAbs, we will perform deep mutational scanning[49]. Briefly, all amino acids of the GP proteins will be mutated to the other 19 amino acids with a unique barcode using PCR-based mutagenesis. The LASV or MACV GP protein library will be incubated with the appropriate mAbs to identify residues with loss-of-binding phenotypes. A sequence 'logo' showing the relative contributions of each amino acid mutation will identify residues critical for binding. Multiple amino acid substitutions can then be assessed by reverse genetics at UTMB to confirm the epitopes recognized by the down-selected mAbs. This method also serves as a surrogate for *in vitro* neutralization escape studies. ***(d) Neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-LASV or VSV-MACV constructs at BSL2 in the presence of saturating concentrations of mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Confirmatory reverse genetics studies will be performed by introducing mutations into an infectious cDNA clone of LASV or MACV by **RP1** to test neutralization. We hypothesize that escape will be less likely in the presence of mAb combinations than single mAbs. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of escape variants in the presence of mAbs.

E.1.3. Structural basis of neutralization by mAb combinations. The studies above should identify optimal anti-LASV and anti-MACV mAb combinations that simultaneously bind, neutralize, and increase the barrier for viral escape under mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for arenavirus by identifying key epitope targets (**RP1**).

E.1.4. Protective efficacy of human anti-arenavirus mAbs. ***(a) Studies in guinea pigs***. MAbs that satisfy the criteria stated above and that display stage-appropriate manufacturability characteristics will advance to in vivo testing. We will use established models of LASV[22,50] and MACV[51,52] infection in guinea pigs. PABVAX **Core E** has access to many strains from different LASV and MACV lineages, which will be tested. For prioritization, we will focus on LASV lineage II, III, and IV strains and MACV lineage I, II, and VIII strainsfor which uniformly lethal models are available. Groups of 6 outbred Hartley guinea pigs will be treated with low, medium, and high doses) of single or combinations of anti-LASV or anti-MACVs mAb, or isotype controls at day +1 after inoculation with the LASVs or MACVs This approach allows us to determine whether combinations provide advantage over the best monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-LASV mAbs and 10 single mAbs and 5 pairs of anti-MACV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements (blood) at day 5 or 6 after LASV or MACV challenge and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal LASV mAb pairs and two additional MACV mAb pairs that are comparable to or outperform previously reported mAbs for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-LASV and anti-MACV mAb combination against LASV or MACV in NHPs. As a final test of an optimal anti-LASV and anti-MACV mAb combinations, we will assess protective efficacy of the lead anti-LASV mAb pair in NHPs as well as the anti-MACV mAb pair in NHPs. These anti-LASV and anti-MACV mAb pairs will be selected based on the *in vitro* and guinea pig data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys will only be considered pending success in the guinea pig studies and consultation with the external Scientific Advisory Board (SAB) and NIAID program staff.

E.1.5. Applying lessons learned in LASV and MACV research with the selected test case pathogens for mAb generation: Lujo and Chapare mAbs. Once we have completed the LASV and MACV discovery campaigns, will turn to the test case pathogens to see if we can use the prototype pathogen approach to related viruses, Lujo (LUJV) and Chapare (CHAPV). We do not have access to human PBMCs from LUJV - or CHAPV-immune donors. As an alternative approach, we will use humanized mouse models as a source of B cells encoding fully human monoclonal antibodies. The Vanderbilt core investigators have an established fully executed agreement to use Alloy human antibody mice for this purpose (See Letter of Support). The Alloy ATX-Gx™ mouse is one of the most effective *in vivo* human antibody discovery platforms, with over 140 partners and counting. This mouse system was originally invented and validated inside a major pharma company and then further developed by Alloy. This foundational suite of highly immunocompetent transgenic mice is engineered to drive the greatest potential diversity of unique human antibodies binding to the viral target of interest, with broad epitopic coverage. The comprehensive functional human antibody repertoire in these mice is optimized for human Ab sequence developability and diversity. Alloy and Vanderbilt have already executed a simple licensing process for use of the ATX-Gx platform, and as an established partner the Vanderbilt core team will access the expanding portfolio of transgenic Alloy strains designed to address a range of discovery challenges (including diverse Ab isotypes). Mice will be immunized with conformationally correct recombinant protein antigens (**Table 2**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for the target of interest. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for LASV and MACV.

Table 2. Antigens available from RP1
LUJV NP NTD
LUJV GPe
LUJV NP NTD +GPe
CHAPV NP NTD
CHAPV GPe
CHAPV NP NTD +GPe

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-LASV and anti-MACV mAb combinations with optimal neutralizing and protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging LASV, MACV, or related arenaviruses. Given our previous ability to generate human antiviral mAbs, we do not anticipate problems in isolating anti-LASV or anti-MACV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently neutralizing anti-LASV or anti-MACV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (*i.e.*, memory B cells) isolated from human LASV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in LASV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-LASV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-LASV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-LASV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-LASV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the high-throughput Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E2. Specific Aim 2. Discover a protective CCHF virus mAb combination therapy. Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[25,53,54]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E**). As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.

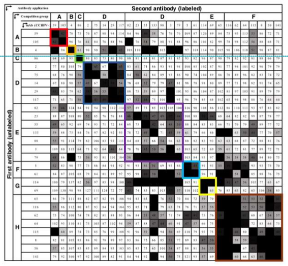

Figure 2. Competition assessment of human Abs to CCHFV using M-segment expressing cells. We tested 28 mAbs in competition assays. MAbs are displayed in 8 groups (A-H) based on their ability to compete for binding. Values shown are the % of binding that occurred during competition compared to non-competed binding of the mAb. This value was normalized to 100%. The values are also indicated by the box fill color; darker colors toward black indicate higher competition and lighter colors toward white indicate less competition, on a gradient scale.

~~**E2. Specific Aim 2. Discover a protective Crimean-Congo hemorrhagic fever (CCHF) virus mAb combination therapy.** Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[25,53,54]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E**). As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.~~

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Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-CCHFV mAbs.*** In this Aim, we will rapidly discover human anti-CCHFV mAbs from CCHFV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify an optimal anti-CCHFV mAb combination and further advance the discovery platform for rapid response against emerging nairoviruses. In preliminary experiments in collaboration with investigators in **RP1** and **Core E**, the Crowe laboratory has previously isolated and characterized panels of neutralizing human anti-CCHFV human mAbs from individuals who were convalescent after naturally-acquired laboratory-confirmed infection in Spain or Turkey. ~~PBMCs were used to generate hybridomas and antibody variable genes sequenced to generate mAbs recombinantly.~~ We isolated a panel of antibodies against CCHFV that recognize the M-segment in transfected cells, and they exhibit a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 2**). This panel contains cross-binding and cross-neutralizing antibodies for diverse strains. We observed a human mAb designated CCHF-82 competing with competition group 1 and group 2 antibodies from the previously isolated GP38-reactive murine antibodies. CCHF-82 showed some protection in a STAT1$^{-/-}$ mouse model of infection conducted by investigators in **RP1 (Fig 3)**. Further investigation with this antibody in the IbAr10200 prophylaxis and therapeutic models is warranted. In contrast, the CCHFV-neutralizing mAbs that we isolated and tested *in vivo* did not perform well in the Turkish strain STAT1$^{-/-}$ mouse model. We now know protection in this model is possible, given the work from Fels *et al*., 2021 who showed that potent antibodies against various sites on the Gc surface can afford protection in this model[53]. However, in their study they did not test GP38-reactive antibodies. To our knowledge, our preliminary data is the first indication that a human GP38-targeting antibody can provide some protection in this mouse model of infection.

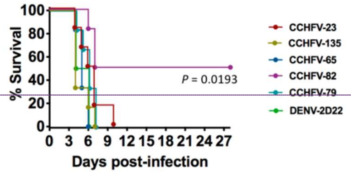

Figure 3. Human mAb CCHF-82 provides partial protection as a post-exposure prophylaxis against the Turkish strain of CCHFV in the STAT1$^{-/-}$ mouse model. A single dose of mAb was administered by the IP route to mice (n=6 per group (3 male, 3 female)) at 30 min post infections. A lethal dose of Turkish strain of CCHFV~~virus~~ was administered. CCHF mAbs~~-23, CCHF-135, CCHF-65, CCHF-82, CCHF-79,~~ or DENV-2D22 (an isotype-matched negative control mAb) were tested in a single dose of 250 µg per mouse ~~except for CCHFV-135 at 125 µg per mouse~~. Kaplan-Meier survival curves were statistically analyzed using a log-rank (Mantel-Cox) test where mAb-treated animals (*P* value shown in

Non-neutralizing murine mAbs protect in mouse models of infection[25], but it ~~has not been shown~~is unknown if the human antibody response could provide similar levels of protection. Murine mAb 13G8 binds to GP38 and does not neutralize CCHFV. This mAb provides protection against lethal challenge as a prophylaxis and a therapeutic against CCHFV. In preliminary experiments we sought to understand if human survivors of CCHFV infection possess B cells encoding mAbs that bind a similar epitope as 13G8 and provide protection. CCHF-82 competes for binding with 13G8 to preGn-transfected cells containing GP38. This antibody offered some protection as a post-exposure prophylaxis against the Turkish strain of CCHFV (**Fig 3**). Human antibodies like this should be further studied and the frequency of these clones should be elucidated to understand if this is a feasible strategy for future vaccine efforts for humans. The epitope that 13G8 and CCHF-82 identifies could inform vaccine design if these antibodies offer protection in larger animal models.

Understanding how non-neutralizing antibodies function is critical in efforts to validate the GP38-binding class of antibodies. Golden *et al.* undertook a study to understand the mechanisms 13G8 functioned and observed that complement activation contributes to the protective capacity of this antibody[25]. Here, in studies of CCHF-82, we also will assess complement activation in mouse models of infection to fully understand the function of this mAb *in vivo*. Furthermore, if this mAb protects in the CCHFV IbAr10200 strain challenge model, assessing its mechanism is warranted. Together with **Core D**, we will assess IgG molecules with complement knockout (“KA”) and Fc gamma receptor knockout (LALA-PG) mutations in the Fc domain to study this matter. Human mAb CCHF-82 displayed limited cross-protection against experimental infection with the Turkish strain of CCHFV. Our antibody isolation here will aim to identify cross-protective non-neutralizing antibodies reactive with GP38. Recently, vaccinating with NP was shown to be another way to elicit non- neutralizing antibodies[55]. The NP of CCHFV is more conserved than the glycoproteins and might also be a non-canonical target for the identification of cross-protective non-neutralizing antibodies[55].

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant viral surface protein antigens, cloning of Ab variable genes, and then expression of mAbs as ~~recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer GP-38-specific B cells~~as described above in Specific Aim 1.

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E.2.1. Rapid isolation of human anti-CCHFV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from a number of previously CCHFV-infected individuals. One was a health care survivor who was infected while caring for a patient in Spain[56]. We also collected a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Turkey, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples were only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies were approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples were deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-CCHFV mAbs rapidly, as if we had to respond to a nairovirus outbreak in real time. Our ability to respond rapidly in the setting of outbreaks is well-documented in the lay press (60 Minutes features and others) and the scientific literature, for ex., 78 days from sample to protection of NHPs for Zika virus[43], and 25 days from PBMC sample receipt to delivery of the antibody genes for the approved COVID-19 drug Evusheld to AstraZeneca[1]. Here, we will single-cell sort antigen-specific B cells using recombinant CCHFV proteins (GP38, Gc, Gn, Gc/Gn) or non-infectious viral particles as bait as described above in Specific Aim 1.~~.~~ ~~These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including VSV-CCHFV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe CCHFV protein or virus-particle binding to single B cells. These cells will be exported for sequencing of the variable gene regions and analysis using the PyIR software. We will select a panel of up to 500 neutralizing anti-CCHFV mAbs to express as recombinant mAbs and advance for further characterization. We have demonstrated the capacity to execute multiple approaches simultaneously in parallel[1,43], which enhances the diversity of clones obtained.~~ We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D**, listed in **Table 3** or by **RP1** **(Table 4)**.

Table 3. Core D CCHFV antigens ~~from Core D~~
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF NP

Table 4. Antigens available from RP1
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF Turkey NP
CCHF Turkey GnGc+GP38+NP
CCHF Turkey GnGc+GP38
CCHF Turkey GP38+NP
CCHF Turkey GnGc+NP

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(c) Recombinant mAb cloning. ~~We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using the custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson assembly-cloned on instrument into our custom full-length Ig expression vectors. We will design human anti-LASV mAbs as IgG1 with the Fc mutation, LALA-PG, to abrogate Fc effector functions to assess role of Fc-mediated effector functions to protection in animal models. We also will engineer IgG1 molecules with extended-half-life Fc mutations based on enhanced binding to FcRn including LS (M428L +N434S)[26] and YTE (M252Y +S254T +T256E)[27] mutations. These Fc engineering approaches will generate mAbs with vaccine-like properties. The LS and YTE mutations were developed in commercial settings (and have been used safely in the clinic), but the patent life of these mutations will expire prior to approval of antibodies developed here.~~As described in Specific Aim 1 above.

(d) Micro-scale mAb expression. ~~The Crowe laboratory will perform large-throughput micro-scale mAb expression and purification that, with our integrated approach allows, screening assays to identify candidate mAbs This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with diverse Fc mutants to assess the role of Fc-mediated effector functions *in vivo*~~ (~~**Core E**).~~As described in Specific Aim 1 above.

(e) A CPE-based CCHFV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of CCHFV. We will use a replication-competent vesicular stomatitis virus (VSV) CCHFV surrogate that expresses CCHFV surface proteins and causes CPE[46]. Investigators in **RP1** have the VSV-CCHFV-GFP versions of clades 1 through 7 based on this publication. We will obtain these all these VSV constructs from investigators in **PABVAX**. Existing VSV vectors will be provided by investigators in **RP1 and Core E**. We already have Vanderbilt Institutional Biosafety Committee approval for use of these constructs in this format and assay. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against CCHFV. These mAbs will be moved forward for additional studies to identify an optimal anti-CCHFV mAb combination. GP38-reactive clones will be advanced based on avidity ranking since they are not likely to be neutralizing but may be protective *in vivo*.

E.2.2. Identifying CCHFV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind CCHFV surface proteins and validate their epitopes. We will perform competition-binding studies with CCHFV Gc/Gn or subunit proteins (as above) or CCHFV virus-like particles (VLPs) made from a replicon system in hand. **Core E** will inactivate CCHFV virus particles that they have in the UTMB center by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from some of the mAbs shown in the unpublished preliminary data above for the identification of mAbs that can concurrently bind CCHFV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-CCHFV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. GP-38-reactive clones will be screened for synergistic binding as they are not expected to be neutralizing. ***(c) Identification of key binding residues of human anti-CCHFV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-CCHFV constructs at BSL2 in the presence of saturating concentrations of CCHFV mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of VSV-CCHFV escape variants in the presence of mAbs.

E.2.3. Structural basis of neutralization by anti-CCHFV mAb combinations. ~~The studies above should identify optimal anti-CCHFV mAb combinations that simultaneously bind, neutralize, and increase the barrier for CCHFV to escape mAb selective pressure.~~ For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above~~for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for nairoviruses by identifying key epitope targets (**RP1**)~~.

E.2.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies in STAT1$^{-/-}$ mouse models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (Core D), will advance to *in vivo* testing. We will use an established STAT1$^{-/-}$ mouse model of CCHFV infection that the **RP1** and **Core E** investigators have deployed[46]. To benchmark our mAbs, a separate set of STAT1$^{-/-}$ mice will be treated with CCHF-82, which we showed in unpublished preliminary data above confers substantial but incomplete protection (**Fig 3**). The **Core E** laboratory has access to strains representing all 6 clades that can be tested. Groups of 10 STAT1$^{-/-}$ mice will be treated with low, medium, and high doses) of single or combinations of anti-CCHFV mAb, or isotype controls at day +1 after inoculation with CCHFVs. This approach allows us to determine whether combinations provide advantage over the best CCHFV monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after CCHFV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal CCHFV mAb pairs that are comparable to or outperform the mAbs we reported above. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-CCHFV mAb combination against CCHFV in NHPs. As a final test of an optimal anti-CCHFV mAb combination, we will assess efficacy of the lead anti-CCHFV mAb pair in NHPs (**Core E**), to be selected based on the *in vitro,* STAT1$^{-/-}$ mice, and manufacturability data (**Core D**). Down-selection for testing in cynomolgus monkeys would only be considered with success in the STAT1$^{-/-}$ mouse studies and in consultation with the external SAB and NIAID program staff.

Table 5. Antigens available from RP1
Kasokero GnGC

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E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs. Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human monoclonal antibodies, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.

Kasokero GP38
Kasokero NP
Kasokero GnGc +GP38 +NP
Kasokero GnGc +GP38
Kasokero GnGc +NP
Kasokero GP38 +NP

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Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-CCHFV mAb combinations with optimal protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging CCHFV or related nairoviruses. Given our previous ability to generate human anti-CCHFV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-CCHFV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-CCHFV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (*i.e.*, memory B cells) isolated from human CCHFV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in CCHFV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-CCHFV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-CCHFV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-CCHFV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-CCHFV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E3. Specific Aim 3. Establish optimal mAb combination therapies against henipaviruses Hendra (HeV) and Nipah (NiV) viruses. We will use a large panel of potent anti-henipavirus mAbs that we previously made from the memory B cells of immune individuals and generate new antibodies targeting the fusion (F) protein. **RP2** will provide new antigens for mAb selection and BSL-2-compatible viruses for neutralization screens including Cedar virus constructs displaying HeV and NiV F and G proteins. We will identify optimal mAb combinations to enhance henipavirus therapy, minimize viral escape, and improve treatment efficacy through synergy. *In vitro* competition-binding, neutralization, and mechanistic studies will be conducted, including authentic virus neutralization with **PABVAX Core E**. We will refine the recognized epitopes and investigate the structural basis for optimal combination neutralization of henipaviruses using HeV and NiV as prototypes. *In vivo* assessment of mAb combinations will determine the minimal effective dose for protection and the ability to prevent *in vivo* escape. Protective efficacy of lead mAb combinations will be assessed in hamster models of NiV and HeV infection with **Core E**. Leads will be transferred to **Core D**. As a final test of optimal henipavirus mAb combinations, we will assess protective efficacy of the lead henipavirus mAb pairs in NHPs, to be selected based on the *in vitro* and hamster data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in African green monkey (AGM) henipavirus model studies would only be considered pending success in the hamster studies and consultation with the external SAB and NIAID program staff. The HeV+NiV studies will be conducted in years 1-3 and then applied to the plug-and-play test case Langya virus in years 2-5. If **RP2** and **Core E** cannot rescue recombinant Langya virus, the lessons learned in Years 1-3 will be applied to the alternate plug-and-play test viruses Angavokely or Sosuga (SOSV) virus. Preliminary data on SOSV mAbs are provided.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-NIV/HeV mAbs.*** In this Aim, we will further adapt approaches we have developed in preliminary experiments with henipavirus mAb studies by identifying a potently protective combination of anti-NiV/HeV human mAbs. ~~We already have in hand some~~~~perform synergy studies to identify optimal mAb combinations by pairing them with each other and with anti-G antibodies.~~ Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups ~~(**Fig 4**~~). This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains. In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and outstanding potency anti-G antibodies with extensive characterization, including synergistic neutralization. ~~In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them with each other and with anti-G antibodies. Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 4**). This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains.~~

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant henipavirus F protein antigens, ~~perform cloning of Ab variable genes, and then express mAbs as recombinant IgGs. Alternatively, F-specific B cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through 2 different single-cell RNA-seq approaches (10X Genomics & Rhapsody)~~as described above in Specific Aim 1.

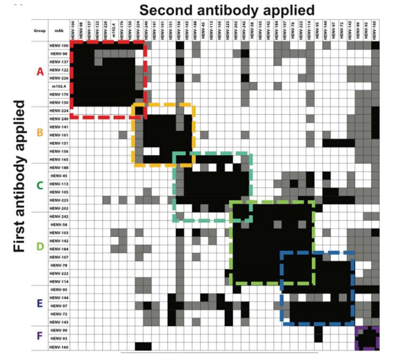

Figure 4. Identification of major antigenic sites for recognition of HeV RBP by human mAbs. SPR competition binding of human Abs against HeV-RBP. A first Ab was applied to a gold-coated sensor chip, and recombinant HeV-RBP head domain was associated to the coupled Ab. A second Ab was applied to the sensor chip to determine binding to RBP. Black boxes - a pairwise interaction in which the binding of the second Ab is blocked by the first. White - both Abs could bind simultaneously. Gray - intermediate competition phenotype.

E.3.1. Rapid isolation of human anti-HeV/NiV mAbs. *(a) Immune cells.* We are obtaining human PBMCs from a number of previously NiV-infected individuals who acquired immunity following natural infection in collaboration with the **icddr,b**, an international health research institute based in Dhaka, Bangladesh. We are collecting a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Bangladesh, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies are approved by the icddr,b and Vanderbilt University Medical Center IRBs. PBMCs isolated from the blood samples are deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their use in this **RP4,** if funded.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific B cells, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody^{TM}), and this may be helpful for rarer antigen-specific B cells. We will sort soluble fusion (sF) or attachment (G) protein-specific cells with recombinant viral surface protein antigens from **Core D** (**Table 6**).

Table 6. Core D Henipavirus antigens ~~from Core D~~
Nipah sF
Hendra sF
NiV G

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(c) Ab screening and sequence analysis. Our goal is to identify human anti-NiV/HeV mAbs rapidly, as if we had to respond to a paramyxovirus outbreak in real time. We will single-cell sort antigen-specific B cells using recombinant HeV and NiV proteins (sF or G) as bait as described in Specific Aim 1 above. ~~. These cells will be expanded on feeder layers expressing CD40L, IL-21, and BAFF for downstream applications, including VSV-CCHFV neutralization assays. We also can use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe CCHFV protein or virus-particle binding to single B cells. These cells will be exported for sequencing of the variable gene regions and analysis using the PyIR software. We will select a panel of up to 500 neutralizing anti-HeV/NiV mAbs to express as recombinant mAbs and advance for further characterization.~~

(d) Recombinant mAb cloning. As described in Specific Aim 1 above~~We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using the custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors. We will design human anti-LASV mAbs as IgG1 with the Fc mutation, LALA-PG, to abrogate Fc effector functions to assess role of Fc-mediated effector functions to protection in animal models. We also will engineer IgG1 molecules with extended half-life Fc mutations based on enhanced binding to FcRn including LS (M428L +N434S)[26] and YTE (M252Y +S254T +T256E)[27] mutations. These approaches will generate mAbs with vaccine-like properties~~.

(e) Micro-scale mAb expression. As described in Specific Aim 1 above~~The Crowe laboratory will perform large-throughput micro-scale mAb expression and purification that, with our integrated approach allows, screening assays to identify candidate mAbs. Early-stage assays will identify candidate mAbs that will then be produced at~~

~~larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**)~~.

(f) CPE-based HeV/NiV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of HeV and NiV. We will use replication-competent vesicular stomatitis virus (VSV) HeV and NiV surrogates that expresses henipaviurs surface proteins and cause CPE. Investigators in **RP2 and RP3** have VSV-NiV F and VSV-NiV G with GFP[45], VSV-NiV G without GFP[47] and VSV-HeVG (Geisbert and Cross, unpublished). We will obtain these all these VSV constructs from investigators in **PABVAX**. We already have Vanderbilt Institutional Biosafety Committee approval for use of these constructs in this format and assay. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against HeV and NiV. We also can use Cedar virus constructs for neutralization assays as the have reduced pathogenicity[57] and can be used in high-throughput screening assays for henipavirus antiviral discovery[58]. Cedar viruses will be provided by Dr. Broder of **RP3**. The mAbs with most potent activity will be moved forward for additional studies to identify an optimal anti-HeV/NiV mAb combination.

E.3.2. Identifying HeV/NiV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig** ~~2~~4) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind HeV/NiV surface proteins and validate their epitopes. We will perform competition-binding studies with HeV/NiV sF or G proteins (as above). We will include positive controls made recombinantly from some of the mAbs shown in the preliminary data above for the identification of mAbs that can concurrently bind HeV/NiV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti- HeV/NiV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key binding residues of human anti-HeV/NiV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-NiV F, VSV-NiV G and VSV-HeV G constructs at BSL2 in the presence of saturating concentrations of HeV/NiV mAbs (single or combination) using RTCA to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test the level of infection of VSV-HeV/NiV escape variants in the presence of mAbs.

E.3.3. Structural basis of neutralization by anti-HeV/NiV mAb combinations. ~~The studies above should identify optimal anti-HeV/NiV mAb combinations that simultaneously bind, neutralize, and increase the barrier for CCHFV to escape mAb selective pressure.~~ For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, as described in Specific Aim 1 above.~~for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for henipaviruses by identifying key epitope targets.~~

E.3.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies hamster models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (**Core D**), will advance to *in vivo* testing. We will use an established hamster model of NiV or HeV infection that the **Core E** investigators have deployed. To benchmark our mAbs, a separate set of hamsters will be treated with G mAbs HENV-103+HENV-117, which we showed in previous data above confers substantial protection[18]. Groups of 10 hamsters will be treated with low, medium, or high IgG doses) of single or combinations of anti-HeV/NiV mAbs, or isotype controls at day +1 after inoculation with HeV or NiV. This approach allows us to determine whether combinations provide advantage over the best HeV or NiV mAb monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after HeV or NiV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal HeV/NiV mAb pairs that are comparable to or outperform the mAbs we reported previously. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-HeV/NiV mAb combination against HeV and NiV in NHPs. As a final test of an optimal anti-HeV/NiV mAb combination, we may assess efficacy of the lead anti-HeV/NiV mAb pair in NHPs (**Core E**), selected based on the *in vitro,* hamster data, and manufacturability data (**Core D**). Down-selection for testing in African green monkeys would only be considered with success in the hamster studies and in consultation with the external SAB and NIAID program staff.

E.3.5. Applying lessons learned in HeV/NiV research to Langya, Angavokely and Sosuga viruses. Once we have completed the HeV/NiV discovery campaigns, will turn to the test case pathogen Langya, Angavokely and Sosuga (SOSV) viruses to see if we can use the prototype pathogen approach for this virus. We have plenty of human PBMCs from the sole identified SOSV-immune individual and have already shown that it is possible to obtain HN- and F-specific neutralizing antibodies from this individual's B cells[59]. We also have already prepared recombinant SOSV prefusion F and HN proteins[59]. We do not have access to human PBMCs from Langya- or Angavokely-immune donors. For those mAb discovery campaigns, we will use Alloy humanized mouse models as a source of B cells encoding human mAbs, as above. Mice will be immunized with recombinant protein antigens (**Table 7**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Langya or Angavokely. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for Hev/NiV described above.

Table 7. Paramyxovirus antigens from Core D
Mojiang sF
Angavokely sF
Langya sF

~~antigens from Core D~~
~~Mojiang sF~~
~~Angavokely sF~~
~~Langya sF~~

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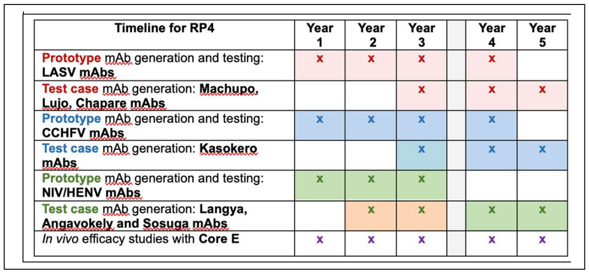
Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-HeV/NiV mAb combinations with optimal protective activity. These mAb combinations will be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging HeV/NiV or related paramyxoviruses. Given our previous ability to generate human anti-HeV/NiV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-HeV/NiV mAbs. We may observe that new mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-HeV/NiV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (*i.e.*, memory B cells) isolated from human NiV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in NiV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-HeV/NiV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-HeV/NiV mAb combinations that can inform vaccine design (**RP2**) for related viruses being used as test cases in years 2 through 5.

F. Scientific rigor. *Experimental design.* Experiments will include biological and technical replicates, and results will be analyzed by parametric or non-parametric analyses (depending on data distribution) and corrections for multiple comparisons. EC_{50} (binding) and IC_{50} (neutralization) values will be reported only following at least 3 identically conducted experiments. All mAb screening will incorporate appropriate + and - controls. Positive signals in binding assays will be defined as >3 SD above background. ***Protein quality.*** We have a high capacity to purify proteins using a Protein Maker workstation that allows purification chromatography on 24 parallel columns. We perform QC on protein reagents using a Maurice instrument with capillary electrophoresis technology to automate our protein profiling either by size or charge, providing cIEF and CE-SDS data. Animal ***studies*** will be performed blinded with independent replicates and include negative (*e.g.*, placebo), and positive (when available) controls. We will use power calculations (**Data Management Core B**) to determine sample sizes. For virological studies, power analysis was performed using accepted values for type I error (0.05) and power (80%). The values for population variance (15×) are based on existing data sets and our experience with rodent challenge studies. We will perform experiments at least 2-3 independent times (n = 4-5 each) to attain n = 12-15 per arm. Smaller group sizes can be used if differences are larger than expected.

G. Relevant biological variables. The human mAbs are derived from immune human donors or Alloy mice. Humans. Human Abs will be isolated for biological, structural, and functional studies at a clonal level. The sample size is not powered for large scale epidemiologic or clinical study conclusions. There is no evidence that sex, age, body mass index, or underlying health conditions other than immunodeficiency or immunosuppressive treatments alters the activity of cloned Abs in structure/function studies. The samples are de-identified without patient specific identifiers to protect the health information of the donors; thus, research with these samples is considered *Not Human Subjects Research* category activity. Mice, hamsters, guinea pigs, NHP, and viruses are also biological systems used in this application. Conventional animal housing conditions will be used because there is no evidence that altered room temperatures and light/dark cycles influence paramyxovirus, nairovirus, or arenavirus pathogenesis. Mice, hamsters, guinea pigs, and NHPs of both sexes will be used, and phenotypes will be monitored for sex-based differences. Virus stocks will be either low passage seed stocks from natural isolates or generated from existing cDNA clones and sequence-confirmed prior to use for infection studies.

H. Milestones and quantitative Go/No-Go criteria. Through **Aims 1-3**, we have proposed experiments that will advance our knowledge of the mAb-mediated mechanisms of action against paramyxoviruses, nairoviruses, and arenaviruses. In each Aim, we will use down-selection criteria (as below) to advance mAb combinations or variants for additional studies. By year 3, we will have defined the principles that govern optimal mAb combinations for paramyxoviruses, nairoviruses, and arenaviruses and the rational approaches needed to select mAbs for potency and breadth. We will use the following metrics to advance mAbs: ***(a) Neutralizing activity*.** All mAbs will have neutralizing potency < 10 ng/mL, EC_{50} value; ***(b) Binding affinity***. MAbs will be of high affinity to surface proteins or virions (K_D < 5 nM). ***(c) Protein stability and production quality*.** MAbs must produce at high levels without optimization (> 100 mg/L) and show biochemical/functional stability at 4°C or -80°C (>90% retention of binding and neutralization activity at 30 d). ***(d) Synergy***. Combinations of 2 mAbs against a given virus ideally will show synergy of neutralization *in vitro* or at a minimum, additive inhibition (no antagonism); ***(e) Epitopes***. To avoid resistance, mAbs in combination will bind 2 epitopes (determined by competition binding (<20% reduction in binding), mapping, and structural studies. ***(f) Resistance*** against mAbs will be assessed for virus escape. Deep sequencing of viruses that emerge from cells or animals treated with mAbs will inform prioritization. Combinations of mAbs with high genetic barriers to resistance (<10× loss in EC_{50}) will be advanced. ***(g) Protection in mice, hamsters, guinea pigs, or NHPs.*** The following metrics can be used: (i) Protection against lethal challenge by homologous virus (>90% survival; (ii) Protection against clinical disease by homologous virus (>80%); (iii) Protection against infection by homologous virus (>100-fold reduction in viral titers in multiple organs). ***(h) pK measurements***. For lead protective mAbs with mutations that should confer extended half-life, we will perform pK studies in NHPs (with **Core D** and **E**) to assess levels over time with a goal of a human predicted serum half-life of >6 months.



Timeline for RP4	**Year 1**	**Year 2**	**Year 3**		**Year 4**	**Year 5**
Prototype mAb generation and testing: **LASV mAbs**	x	x	x		x	
Test case mAb generation: **Machupo, Lujo, Chapare mAbs**			x		x	x
Prototype mAb generation and testing: **CCHFV mAbs**	x	x	x		x	
Test case mAb generation: **Kasokero mAbs**			x		x	x
Prototype mAb generation and testing: **NIV/HENV mAbs**	x	x	x			
Test case mAb generation: **Langya, Angavokely and Sosuga mAbs**		x	x		x	x
In vivo efficacy studies with **Core E**	x	x	x		x	x

LITERATURE CITED – RP4

1. Zost, S.J., *et al.* Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* **26**, 1422-1427 (2020).
2. Fischer, W.A., II & Wohl, D.A. Moving Lassa Fever Research and Care Into the 21st Century. *The Journal of Infectious Diseases* **215**, 1779-1781 (2017).
3. Ezeomah, C., *et al.* Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. *Open Forum Infect Dis* **6**, ofz512 (2019).
4. Li, A.L., *et al.* Ophthalmic manifestations and vision impairment in Lassa fever survivors. *PloS one* **15**, e0243766 (2020).
5. Whitmer, S.L.M., *et al.* New Lineage of Lassa Virus, Togo, 2016. *Emerging infectious diseases* **24**, 599-602 (2018).
6. Ibukun, F.I. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. *Viruses* **12**(2020).
7. Webb, P.A., Johnson, K.M., Mackenzie, R.B. & Kuns, M.L. Some characteristics of Machupo virus, causative agent of Bolivian hemorrhagic fever. *Am J Trop Med Hyg* **16**, 531-538 (1967).
8. Aguilar, P.V., *et al.* Reemergence of Bolivian hemorrhagic fever, 2007-2008. *Emerging infectious diseases* **15**, 1526-1528 (2009).
9. Enria, D.A., Ambrosio, A.M., Briggiler, A.M., Feuillade, M.R. & Crivelli, E. [Candid#1 vaccine against Argentine hemorrhagic fever produced in Argentina. Immunogenicity and safety]. *Medicina (B Aires)* **70**, 215-222 (2010).
10. Enria, D.A. & Maiztegui, J.I. Antiviral treatment of Argentine hemorrhagic fever. *Antiviral research* **23**, 23-31 (1994).
11. Hawman, D.W. & Feldmann, H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology* (2023).
12. Chinikar, S., *et al.* Genetic Diversity of Crimean Congo Hemorrhagic Fever Virus Strains from Iran. *J Arthropod Borne Dis* **10**, 127-140 (2016).
13. Shepherd, A.J., Swanepoel, R. & Leman, P.A. Antibody response in Crimean-Congo hemorrhagic fever. *Rev Infect Dis* **11 Suppl 4**, S801-806 (1989).
14. Lombe, B.P., *et al.* Mapping of Antibody Epitopes on the Crimean-Congo Hemorrhagic Fever Virus Nucleoprotein. *Viruses* **14**(2022).
15. Mackenzie, J., Wang, L. & Broder, C.C. Henipaviruses. in *Fields Virology*, Vol. 2 1070-1085 (2013).
16. Whitmer, S.L.M., *et al.* Inference of Nipah virus evolution, 1999–2015. *Virus Evolution* **7**(2020).
17. Dong, J., *et al.* Potent Henipavirus Neutralization by Antibodies Recognizing Diverse Sites on Hendra and Nipah Virus Receptor Binding Protein. *Cell* **183**, 1536-1550 e1517 (2020).
18. Doyle, M.P., *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021).
19. Mire, C.E., *et al.* A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis* **221**, S471-S479 (2020).
20. Dang, H.V., *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021).
21. Cross, R.W., *et al.* Antibody therapy for Lassa fever. *Curr Opin Virol* **37**, 97-104 (2019).
22. Cross, R.W., *et al.* Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral research* **133**, 218-222 (2016).
23. Cartwright, H.N., Barbeau, D.J. & McElroy, A.K. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere* **6**, e0055621 (2021).

24. Gutjahr, B., *et al.* Two monoclonal antibodies against glycoprotein Gn protect mice from Rift Valley Fever challenge by cooperative effects. *PLoS Negl Trop Dis* **14**, e0008143 (2020).
25. Golden, J.W., *et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv* **5**, eaaw9535 (2019).
26. Zalevsky, J., *et al.* Enhanced antibody half-life improves in vivo activity. *Nat Biotechnol* **28**, 157-159 (2010).
27. Dall'Acqua, W.F., *et al.* Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* **169**, 5171-5180 (2002).
28. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Levis, S.C. & Maiztegui, J.I. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* **2**, 255-256 (1984).
29. Jahrling, P.B. & Peters, C.J. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infection and immunity* **44**, 528-533 (1984).
30. Chen, E.C., *et al.* Systematic analysis of human antibody response to ebolavirus glycoprotein shows high prevalence of neutralizing public clonotypes. *Cell Rep* **42**, 112370 (2023).
31. Buck, T.K., *et al.* Neutralizing Antibodies against Lassa Virus Lineage I. *mBio* **13**, e0127822 (2022).
32. Enriquez, A.S., *et al.* Delineating the mechanism of anti-Lassa virus GPC-A neutralizing antibodies. *Cell Rep* **39**, 110841 (2022).
33. Hastie, K.M., *et al.* Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell* **178**, 1004-1015 e1014 (2019).
34. Hastie, K.M., *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).
35. Li, H., *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991 (2022).
36. Gilchuk, P., *et al.* Pan-ebolavirus protective therapy by two multifunctional human antibodies. *Cell* **184**, 5593-5607 e5518 (2021).
37. Chen, E.C., *et al.* Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* **36**, 109604 (2021).
38. Dong, J., *et al.* Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-1244 (2021).
39. Greaney, A.J., *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).
40. Kramer, K.J., *et al.* Potent neutralization of SARS-CoV-2 variants of concern by an antibody with an uncommon genetic signature and structural mode of spike recognition. *Cell Rep* **37**, 109784 (2021).
41. Suryadevara, N., *et al.* Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell* **184**, 2316-2331 e2315 (2021).
42. Zost, S.J., *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449 (2020).
43. Gilchuk, P., *et al.* Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics. *Nat Biomed Eng* **4**, 1030-1043 (2020).
44. Cross, R.W., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep* **40**, 111094 (2022).
45. Mire, C.E., *et al.* Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J* **10**, 353 (2013).
46. Rodriguez, S.E., *et al.* Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep* **9**, 7755 (2019).
47. Foster, S.L., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. *Proc Natl Acad Sci U S A* **119**, e2200065119 (2022).

48. Branco, L.M., *et al.* Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* **7**, 279 (2010).
49. Starr, T.N., *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
50. Deschambault, Y., *et al.* An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. *Viruses* **15**(2023).
51. Bell, T.M., *et al.* Pathogenesis of Bolivian Hemorrhagic Fever in Guinea Pigs. *Vet Pathol* **53**, 190-199 (2016).
52. Golden, J.W., *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* **7**, 4679 (2017).
53. Fels, J.M., *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell* **184**, 3486-3501 e3421 (2021).
54. Mishra, A.K., *et al.* Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies. *Science* **375**, 104-109 (2022).
55. Leventhal, S.S., *et al.* Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *EBioMedicine* **82**, 104188 (2022).
56. Negredo, A., *et al.* Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med* **377**, 154-161 (2017).
57. Laing, E.D., *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018).
58. Amaya, M., *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084 (2021).
59. Parrington, H.M., *et al.* Potently neutralizing human mAbs against the zoonotic pararubulavirus Sosuga virus. *JCI Insight* **8**(2023).

A. BACKGROUND AND SIGNIFICANCE. Paramyxoviruses, nairoviruses, and arenaviruses are widely distributed RNA viruses that cause severe human syndromes. Because of their geographical distribution and expansion, changes in human demographics and increased exposures to wild animals including rodents and bats, and their capacity for human-to-human spread, these viruses have the potential to cause pandemics. Together with the other investigators in **PABVAX**, we have selected several important prototype paramyxoviruses, nairoviruses, and arenaviruses to study to learn the rules governing immunity to these pathogens. The work on the pathogens selected is justified based on their prevalence and severity alone, but also because of their ability to serve as prototypes for genetically related viruses. We chose the viruses for focus in this RP4 on human antibody development because of their clinical relevance and because several will be studied as prototypes for vaccine development in **RP1** (arenavirus and nairovirus vaccines) and **RP2** (paramyxovirus vaccines). Among the arenaviruses, we will target *Lassa mammarenavirus* (LASV) and *Machupo mammarenavirus* (MACV)*, and* among the family Nairoviridae of RNA viruses, we will study Crimean–Congo hemorrhagic fever orthonairovirus (CCHFV). In tandem with the vaccine projects in **RP2** focused on paramyxoviruses, we study Nipah virus (NiV) and Hendra virus (HeV) as the prototypes, and already have preliminary data with Sosuga virus as a test case. Information about the principles we discover about critical neutralizing epitopes identified here in **RP4** with prototype viruses can feed back into all other **Projects** that address the subsequent test cases in later years (Lujo, Chapare, Kasokero, Langya, Sosuga).

LASV is an Old World arenavirus (OWAV) with a single-stranded RNA virus belonging to the Arenaviridae family. Infection in humans is often the result of exposure to infected excreta from the rodent reservoir, *Mastomys natalensis* where up to 500,000 cases and an estimated 5,000 deaths occur annually in West Africa[2]. While rodent infection is largely benign, human infection can result in severe hemorrhagic disease with neurological complications and long-term hearing and vision sequelae[3,4]. The virus possesses a bi-segmented genome comprising large (L) and small (S) segments, both of which are enclosed within a lipid envelope. The L segment (~7.2 kb) encodes the RNA-dependent RNA polymerase (RdRp) and a zinc-binding protein (Z), while the S segment (~3.4 kb) codes for the nucleoprotein (NP) and the glycoprotein precursor (GPC). The GPC is further cleaved into two surface glycoproteins, GP1 and GP2, which play critical roles in viral entry. The genetic diversity of LASV can be classified into six lineages (I-VI), with lineages III and IV being the most prevalent[5]. Genetic variation arises mainly due to the error-prone nature of the viral RNA polymerase, leading to the emergence of quasi-species. This diversity contributes to the virus's ability to evade host immune responses and pose challenges to vaccine development[6]. LASV antigens include the surface glycoproteins GP1 and GP2, responsible for receptor binding and membrane fusion, respectively. While GP1 is highly variable among strains, GP2 is more conserved. The nucleoprotein (NP) is another major antigen, and its immunodominant epitopes are targeted by the host's immune response. Its genetic diversity and antigenic properties complicate diagnosis and vaccine development, emphasizing the need for continued research on medical countermeasures.

MACV is a New World Arenavirus (NWAV) and the causative agent of Bolivian Hemorrhagic fever. Humans are infected after exposure to excreta from the reservoir, *Calomys callosus,* the large vesper mouse. Disease in humans has a slow onset, but can progress to severe hemorrhagic fever coupled to insidious neurological complications including tremors, seizures, and paralysis[7]. MACV has a similar genomic organization and contains a GPC, NP, Z, and L polymerase proteins. Genetic diversity of MACV is delineated into 8 lineages (I-VIII) where there is growing concern of an expanding host range of *C. callosus* is responsible for outbreaks outside the known endemic region[8]. While the host entry receptor for NWAV is not shared with OWAV, the role for GP to drive immunity as a major antigen alongside NP. The better studied Junin has a regionally approved vaccine[9] and convalescent plasma program[10] available to prevent and treat disease, respectively. There are no approved vaccines or therapies approved for any other NWAV, including MACV. Further, itis not well established whether these countermeasures have potential for cross protect other NWAV such as MACV, underscoring the value of continued efforts to develop broadly active countermeasures to safeguard against lethal disease.

CCHFV is a highly pathogenic tick-borne virus classified under the Nairovirus genus within the Bunyaviridae family[11]. It has a negative-sense, single-stranded RNA genome comprising three segments: Small (S), Medium (M), and Large (L). These segments are enclosed within a lipid envelope and code for various structural and non-structural proteins. The S segment (~1.7 kb) encodes the nucleocapsid protein (N), which is essential for viral replication and assembly. The M segment (~5.3 kb) codes for the glycoprotein precursor (GPC), which is post-translationally cleaved into two surface glycoproteins, Gn and Gc. These glycoproteins are involved in viral entry and fusion. The L segment (~12.1 kb) encodes the RNA-dependent RNA polymerase (RdRp), responsible for viral RNA synthesis. CCHFV exhibits significant genetic diversity, with at least seven distinct clades (Europe 1, Europe 2, Africa 1-3, and Asia 1-2) based on the partial S segment sequences[12]. This diversity is attributed to the segmented genome, geographical distribution, and error-prone viral RNA polymerase, leading to the emergence of various quasi-species. The major antigens of CCHFV include the surface glycoproteins Gn and

Gc, as well as the nucleocapsid protein (N)[13,14]. These antigens are targeted by the host immune response. CCHFV is genetically diverse, so breadth of reactivity will be a focus of our discovery efforts.

NiV/HeV. Bat borne, NiV) and HeV are enveloped, negative-sense, single-stranded RNA viruses belonging to the Henipavirus genus within the Paramyxoviridae family[15]. These emerging zoonotic viruses pose a significant threat to human and animal health due to their high fatality rates and broad host range. The genomes of NiV and HeV are approximately 18.2 kb or 18.4 kb in length, respectively, and share a similar gene organization. They encode six major structural proteins: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), attachment glycoprotein (G), and large polymerase (L) proteins. The N, P, and L proteins form the viral RNA-dependent RNA polymerase (RdRp) complex necessary for transcription and replication, whereas the M, F, and G proteins are essential for virus assembly and entry. Genetic diversity in Henipaviruses is relatively low compared to other RNA viruses, with NiV strains showing 91-100% nucleotide similarity and HeV strains displaying 83-100% similarity[16]. However, the error-prone nature of the RdRp can still generate genetic variability, leading to the emergence of distinct lineages. The major surface antigens of Nipah and Hendra viruses are the attachment glycoprotein (G) and fusion protein (F). The G protein facilitates receptor binding, while the F protein mediates viral membrane fusion. Both antigens are involved in host immune responses and are potential targets for vaccine development, and the neutralizing antibody approaches we propose here. NiV and HeV exhibit relatively low genetic diversity, and in preliminary studies, we have identified human mAbs that neutralize HeV, NiV Bangladesh and NiV Malaysia strains[17-19]. Antibodies to the major surface antigens, the G and F proteins, are therefore, promising candidates for development as prophylactic or therapeutic molecules.

Antibodies (Abs) are important correlates of protection for paramyxoviruses, nairoviruses, and arenaviruses. Immune sera against members of each of these groups of viruses has been shown to exhibit protective efficacy *in vivo*, which supports the use for Abs as treatment options. However, the specificity, functional properties, and other attributes of protective antibodies are poorly defined for most of these viruses. MAbs to the attachment and fusion surface proteins of paramyxoviruses (such as the G and F proteins of Hendra/Nipah[18,20] and the GP proteins of the arenaviruses like LASV[21,22] can neutralize and protect against infection in rodent models. CCHFV is a bit of a conundrum in that ultrapotent human neutralizing antibodies to the related Rift Valley fever virus surface proteins are highly protective *in vivo*[23,24], but CCHFV neutralizing antibodies are not protective. In contrast, some non-neutralizing antibodies do protect partially for CCHFV[25]. Clearly, there is a lot to learn about the rules of immunity to these viruses, and the genetic, molecular, and structural basis of antibody-mediated protection for viruses like CCHFV. Based on these data, we propose the use of mAbs as treatment options to learn the rule of how to protect against emerging paramyxoviruses, nairoviruses, and arenaviruses and to develop candidate antibody countermeasures for translational development with **Core D**. To do so, we will further develop our technical methods for rapidly discovering mAbs, in the case of a future unexpected epidemic caused by viruses of these families. Most importantly, we will define the principles governing combination antibody therapy to prevent virus escape from treatment or prevention and to optimize the efficacy of the beneficial effects conferred by passive immunity.

B. SCIENTIFIC PREMISE. The work proposed here in **RP4** is based on the extensive basic, translational, and clinical trial data on the role of neutralizing Abs in protection against infection and disease caused by paramyxoviruses, nairoviruses, and arenaviruses. Although a significant amount of data supports the use of mAbs to prevent or control paramyxovirus, nairovirus, and arenavirus infection, many questions remain, such as the ideal epitopes to target with mAb combination therapy to improve upon neutralization potency, *in vivo* efficacy, and resistance to viral escape. Identifying these epitopes can help inform vaccine design to elicit optimal protective polyclonal responses (in **RP1** and **RP2**, especially with designs for test cases in years 4 and 5). Here we aim to refine our understanding of the correlates of mAb protection against paramyxoviruses, nairoviruses, and arenaviruses to enable the design of a modular 'plug-and-play' workflow to rapidly respond to potential pandemics by generating optimized combinations of vaccine-like mAbs with an extended half-life that could be used for long-term prophylaxis (> 6 months) and therapy.

C. INNOVATION. RP4 has many innovative conceptual and technical features including: Conceptual. **(1)** determining the principles that govern optimal combinations of human mAbs against paramyxoviruses, nairoviruses, and arenaviruses for protection and resistance to viral escape; **(2)** the development of a clear workflow that generates therapeutic or preventative antibodies (long half-life mAbs that serve as vaccine surrogates) against emerging or new paramyxoviruses, nairoviruses, and arenaviruses with pandemic potential. Our goal is to define the principles and features, including epitope, combination ratios, and mechanisms of action, that result in optimal efficacy of mAbs against paramyxoviruses, nairoviruses, and arenaviruses in animal models and create a higher barrier for resistance. These studies will serve as paradigms for rapid antibody discovery in response to future paramyxovirus, nairovirus, and arenavirus pandemic threats. Technical. **(3)** large-scale B cell repertoire sequencing with customized methods and software; **(4)** use of humanized (Alloy) mice and novel immunogens from **RP1** and **RP2** and **Core D**; **(5)** single B cell functional (neutralization) assays performed on a

Beacon instrument; **(6)** development of high-throughput real-time label-free virus neutralization assays (xCELLigence platform); and **(7)** use of *simultaneous* in parallel screening with several different high-throughput virus-specific B cell isolation approaches (Beacon, 10X Genomics, Rhapsody) to generate and validate best-in-class human mAbs as candidate medical countermeasures.

D. LINKAGE TO OTHER PABVAX PROJECTS AND CORES. The group will focus on developing a modular test case platform for rapid selection of highly neutralizing and/or protective mAbs against paramyxoviruses, nairoviruses, and arenaviruses. Members of our group have active collaborations with most members of the **PABVAX Center** as reflected in previous collaborations and publications (see **Overall** document). We will interact extensively with the Animal Model and Preclinical Evaluation Core (**Core E)** to study neutralization against authentic viruses and to test for protection in small and large animals as well as with **RP3** for testing paramyxovirus mAbs. The knowledge gained from our studies on the structure-function relationships of neutralizing mAb combinations can be applied to rational vaccine design for **RP 1** and **RP2** in later years of the effort when we pivot to apply the lessons learned to the same test case targets as **RP1** and **RP2.** Numerous antigens will be made available to our RP. Finally, long-half-life Fc mutations are now available based on engineering of human IgG1 for enhanced binding to the neonatal Fc receptor (FcRn) such as LS (M428L /N434S)[26], or YTE (M252Y/S254T/T256E)[27] mutations. With ≥ 90 days half-life in humans, our approach will generate mAbs with vaccine-like properties that remain effective for months during a virus epidemic and thus provides a platform for response to future paramyxovirus, nairovirus, and arenavirus pandemic threats, a key goal of our **PABVAX Center.** *Our laboratory previously validated this vaccine surrogate concept when we isolated the human mAbs that formed the basis for Evusheld with YTE long-half-life Fc mutations (used in 70 countries and millions of people) that exhibited > 6 months protective levels in humans after single IM injections*.

E. APPROACH

E.1. Specific Aim 1. Discover potently neutralizing arenavirus combination therapies. For the prototypes, we will focus on Lassa virus (LASV), an Old World virus and Machupo virus (MACV), a New World virus. We will investigate the principles underlying antibody-mediated arenavirus protection. Preliminary evidence suggests that potent virus neutralization is a mechanistic correlate of protection for arenaviruses[28,29]. **RP1/Core D** will provide optimized antigens for mAb selection for the Prototype LASV and MACV (or subsequent Test Cases). We will use a workflow for rapid identification of potently neutralizing mAb combinations for LASV and MACV. We will generate mAbs from individuals with prior natural infection or use transgenic mice with human antibody repertoires. A large panel of human anti-LASV and anti-MACV mAbs will be generated using multiple approaches (immune human B cells and immunized humanized mice), and highly neutralizing mAbs will be evaluated to identify optimal combinations. The best mAb combinations will be assessed to determine the minimal effective dose for protection, and the lead mAb combination therapy(ies) will be transferred to **Core E** for further evaluation and ultimately testing in an NHP model of LASV infection. Leads will be transferred to **Core D** for further translational development as medical countermeasures. The prototype LASV and MACV will be studied in years 1 to 3, followed by mAb discovery efforts for Test Cases Lujo and Chapare viruses based on the established principles in years 4-5.

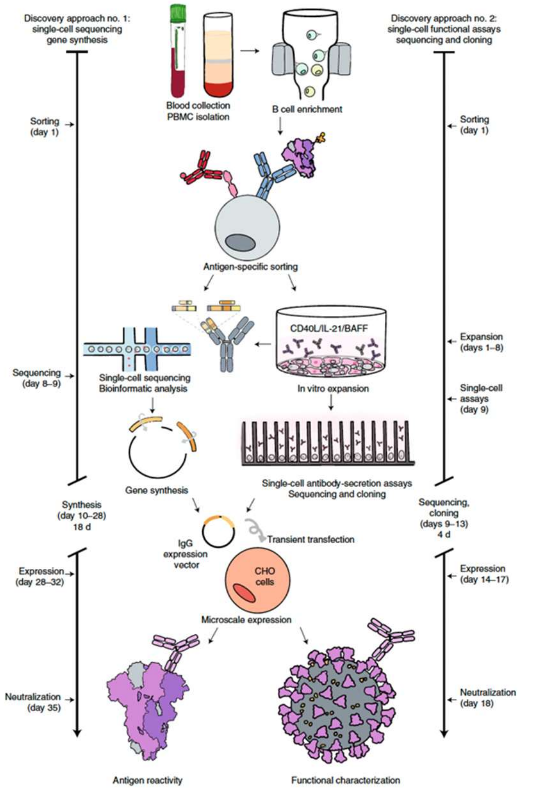


Figure 1. Single cell mAb discovery. PBMC samples are used to enrich for B cells, which can be sorted for antigen-specific B cells. Expansion of these B cells is done to further assess the functionality of the B cells or for antibody sequencing to determine the variable genes contributing to antigen reactivity. Recombinant mAb expression validates the mAb sequence and functional activity. Figure is adapted from [1].

<u>Preliminary Data</u>. ***(a) Potently neutralizing human anti-LASV and anti-MACV mAbs.*** The Crowe laboratory has previously characterized panels of neutralizing human anti-viral human mAbs from virus-immune or vaccinated individuals for about 50 viruses, mostly RNA viruses. Isolated peripheral blood mononuclear cells (PBMCs) are used to generate hybridomas or alternatively antigen-sorted at single B cell level and antibody variable genes sequenced to generate mAbs recombinantly. Panels of human anti-viral mAbs are produced rapidly (we recently reported isolation of 15,000 mAb gene pairs from single Ebola-virus-specific human B cells from a single blood sample of an immune individual[30]. Previous studies by others clearly show

that representative neutralizing and protective human antibodies can be isolated from LASV-immune subjects[31-35]. In this Aim, we will refine the approaches we have developed previously for other RNA viruses in a proof-of-concept study by identifying potently neutralizing combinations of anti-LASV and anti-MACV human mAbs. We will rapidly discover human anti-LASV mAbs from LASV-immune individuals and human anti-MACV mAbs by immunizing Alloy transgenic mice. Synergy studies will be performed to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify optimal anti-LASV and anti-MACV mAb combinations and further advance the discovery platform for rapid response against emerging arenaviruses.

(b) Advanced mAb technologies. The Crowe laboratory has developed some of the highest yield systems to isolate naturally occurring human mAbs using human hybridoma or advanced single-cell RNA-seq methods (**Fig 1**). We have used sorting of antigen-specific cells with recombinant viral surface protein antigens (like the GP1 and GP2 proteins we will use here), cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. One variation we will use is to capture Ab genes directly from viral glycoprotein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), as we have done for Ebola virus[30,36] and SARS-CoV-2[1,37-42]. These single-cell techniques enhance the capture of rare antigen-specific B cells.

E.1.1. Rapid isolation of human anti-LASV and anti-MACV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from one previously LASV-infected individual who was medically managed previously by Emory University in their infectious diseases containment unit. This person has a documented robust neutralizing serum antibody response, suggesting isolation of neutralizing human mAbs from these PBMC aliquots will be straightforward for us. Our colleagues in **RP1** have ongoing field operations for over a decade in Sierra Leone, Liberia, and Nigeria. This work is currently in association with the NIH-Centers for Research on Emerging Infectious Diseases (CREID) with a heavy focus on the study of LASV ecology, epidemiology, and host responses. **RP4** is currently working with **RP1** on obtaining PBMCs from ~10 individuals with prior laboratory-confirmed cases of LASV. The acquisition of these samples is being managed by the Vanderbilt Vaccine Center Biorepository with separate institutional funding to be de-identified prior to use in the studies proposed here. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies have been approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples will be deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded. For anti-MACV mAbs, as a human source is not readily available, we will immunize Alloy humanized mice. These animals yield PBMCs that secrete fully human mAbs. Alloy mice are used routinely in the Crowe lab workflow.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-LASV and anti-MACV mAbs in a rapid manner to mimic a pandemic response to an outbreak. We will single-cell sort antigen-specific B cells using recombinant LASV or MACV proteins or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including LASV and MACV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe GP protein or virus-particle binding to single B cells. These cells are exported for sequencing of the variable gene regions and analysis using the PyIR software developed in the Crowe laboratory. We will select a panel of up to 500 neutralizing anti-LASV mAbs and a similar number of anti-MACV mAbs to express recombinantly and advance for further characterization. We can easily conduct multiple approaches simultaneously in parallel[1,43].

We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D** listed in **Table 1**.

Table 1. Antigens from Core D

Antigen	Manufacturer (expression system)
Lassa Josiah NP	Mapp (bacteria)
Lassa Josiah GPe	Genovac (mammalian)
Machupo GPe	Genovac (mammalian)
Machupo NP	Mapp (bacteria)

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using a custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors.

(d) Micro-scale mAb expression. The Crowe laboratory has the capacity for large-throughput micro-scale mAb expression and purification. The lower end of this range allows expression of thousands of mAbs, which will feed into automated mAb purification platforms. This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based LASV and MACV neutralization assay using RTCA. We have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of cytopathic effect (CPE). Here, we will apply this method to assess neutralization of LASV. Recently, we validated the inclusion of simultaneous live-cell fluorescence microscopy into this real-time CPE-based screening platform (xCelligence RTCA eSight). These capabilities expand the platform to screen viruses that may or may not induce CPE, allowing us to also use a replication-competent vesicular stomatitis virus (VSV) LASV surrogate that expresses LASV surface proteins and causes CPE[44]. Currently, we have a VSV-LASV-Josiah (lineage IV)[44] and a Sauerwald version (lineage II) of these recombinants. Making additional LASV VSV-LASVs is not difficult, and RP1 will make the other missing lineages or needed NWAV (MACV and CHAPV for test case evaluation) based on the same approaches as previously published. RP1 also can easily also make versions of these VSVs that express the green fluorescent protein (GFP) reporter gene. We will obtain these all these VSV constructs from investigators in **PABVAX**. Existing VSV-CCHFV, VSV-NiV, and VSV-HeV vectors[45-47] will be provided by Dr. Geisbert overall PI and Lead of **Core E**. We already have Vanderbilt Institutional Biosafety Committee approval for use of these constructs in this format and assay. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression if used will identify mAbs with neutralizing activity against LASV. These mAbs will be moved forward for additional studies to identify an optimal anti-LASV mAb combination. Similar processes will be followed to determine neutralization capacity of MACV mAb candidates.

E.1.2. Identifying LASV and MACV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind LASV or MACV and validate their epitopes. We will perform competition-binding studies with GP or subunit proteins (as above) or virus-like particles (VLPs)[48] available from **Core E** at UTMB. **Core E** will also inactivate LASV and MACV virus particles that they have at UTMB by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from previously reported antibodies for the identification of mAbs that can concurrently bind LASV or MACV with a goal of finding more potent clones at those sites or clones that complement these inhibitory mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-LASV or anti-MACV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations for each virus, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key residues binding residues of human anti-LASV and anti-MACV mAbs.*** To determine the key residues for binding of down-selected mAbs, we will perform deep mutational scanning[49]. Briefly, all amino acids of the GP proteins will be mutated to the other 19 amino acids with a unique barcode using PCR-based mutagenesis. The LASV or MACV GP protein library will be incubated with the appropriate mAbs to identify residues with loss-of-binding phenotypes. A sequence 'logo' showing the relative contributions of each amino acid mutation will identify residues critical for binding. Multiple amino acid substitutions can then be assessed by reverse genetics at UTMB to confirm the epitopes recognized by the down-selected mAbs. This method also serves as a surrogate for *in vitro* neutralization escape studies. ***(d) Neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-LASV or VSV-MACV constructs at BSL2 in the presence of saturating concentrations of mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Confirmatory reverse genetics studies will be performed by introducing mutations into an infectious cDNA clone of LASV or MACV by **RP1** to test neutralization. We hypothesize that escape will be less likely in the presence of mAb combinations than single mAbs. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of escape variants in the presence of mAbs.

E.1.3. Structural basis of neutralization by mAb combinations. The studies above should identify optimal anti-LASV and anti-MACV mAb combinations that simultaneously bind, neutralize, and increase the barrier for viral escape under mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for arenavirus by identifying key epitope targets (**RP1**).

E.1.4. Protective efficacy of human anti-arenavirus mAbs. ***(a) Studies in guinea pigs***. MAbs that satisfy the criteria stated above and that display stage-appropriate manufacturability characteristics will advance to in vivo testing. We will use established models of LASV[22,50] and MACV[51,52] infection in guinea pigs. PABVAX **Core E**

has access to many strains from different LASV and MACV lineages, which will be tested. For prioritization, we will focus on LASV lineage II, III, and IV strains and MACV lineage I, II, and VIII strainsfor which uniformly lethal models are available. Groups of 6 outbred Hartley guinea pigs will be treated with low, medium, and high doses) of single or combinations of anti-LASV or anti-MACVs mAb, or isotype controls at day +1 after inoculation with the LASVs or MACVs This approach allows us to determine whether combinations provide advantage over the best monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-LASV mAbs and 10 single mAbs and 5 pairs of anti-MACV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements (blood) at day 5 or 6 after LASV or MACV challenge and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal LASV mAb pairs and two additional MACV mAb pairs that are comparable to or outperform previously reported mAbs for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-LASV and anti-MACV mAb combination against LASV or MACV in NHPs. As a final test of an optimal anti-LASV and anti-MACV mAb combinations, we will assess protective efficacy of the lead anti-LASV mAb pair in NHPs as well as the anti-MACV mAb pair in NHPs. These anti-LASV and anti-MACV mAb pairs will be selected based on the *in vitro* and guinea pig data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys will only be considered pending success in the guinea pig studies and consultation with the external Scientific Advisory Board (SAB) and NIAID program staff.

E.1.5. Applying lessons learned in LASV and MACV research with the selected test case pathogens for mAb generation: Lujo and Chapare mAbs. Once we have completed the LASV and MACV discovery campaigns, will turn to the test case pathogens to see if we can use the prototype pathogen approach to related viruses, Lujo (LUJV) and Chapare (CHAPV). We do not have access to human PBMCs from LUJV - or CHAPV-immune donors. As an alternative approach, we will use humanized mouse models as a source of B cells encoding fully human monoclonal antibodies. The Vanderbilt core investigators have an established fully executed agreement to use Alloy human antibody mice for this purpose (See Letter of Support). The Alloy ATX-Gx™ mouse is one of the most effective *in vivo* human antibody discovery platforms, with over 140 partners and counting. This mouse system was originally invented and validated inside a major pharma company and then further developed by Alloy. This foundational suite of highly immunocompetent transgenic mice is engineered to drive the greatest potential diversity of unique human antibodies binding to the viral target of interest, with broad epitopic coverage. The comprehensive functional human antibody repertoire in these mice is optimized for human Ab sequence developability and diversity. Alloy and Vanderbilt have already executed a simple licensing process for use of the ATX-Gx platform, and as an established partner the Vanderbilt core team will access the expanding portfolio of transgenic Alloy strains designed to address a range of discovery challenges (including diverse Ab isotypes). Mice will be immunized with conformationally correct recombinant protein antigens (**Table 2**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for the target of interest. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for LASV and MACV.

Table 2. Antigens available from RP1
LUJV NP NTD
LUJV GPe
LUJV NP NTD +GPe
CHAPV NP NTD
CHAPV GPe
CHAPV NP NTD +GPe

Anticipated results, potential problems, and alternative approaches. In this Aim, we will identify human anti-LASV and anti-MACV mAb combinations with optimal neutralizing and protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging LASV, MACV, or related arenaviruses. Given our previous ability to generate human antiviral mAbs, we do not anticipate problems in isolating anti-LASV or anti-MACV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently neutralizing anti-LASV or anti-MACV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody$^{\text{TM}}$) on enriched B cells (*i.e.*, memory B cells) isolated from human LASV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in LASV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-LASV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-LASV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-LASV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-LASV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the high-throughput Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E2. Specific Aim 2. Discover a protective Crimean-Congo hemorrhagic fever (CCHF) virus mAb combination therapy. Establishing the generality of protection approaches against nairoviruses, we will perform a mAb discovery proof-of-concept campaign for the Prototype CCHFV. Preliminary studies have identified human anti-CCHF mAbs with varying levels of protection in experimentally infected animals[25,53,54]. We will isolate a large panel of human anti-CCHFV mAbs and determine the rules governing optimal mAb combinations through competition-binding, epitope mapping, neutralization, and viral escape studies. Lead antibodies with acceptable manufacturability characteristics (**Core D**) and the highest capacity to overcome resistance while maintaining potency of protection will be selected. The structural basis of their activity will be established using structural biology techniques (electron microscopy and crystallography). Lead mAb cocktail therapies will be evaluated for *in vivo* protective efficacy in STAT1$^{-/-}$ mice (with **Core E).** As a final test of an optimal anti-CCHFV mAb combination, we will assess protective efficacy of the lead anti-CCHFV mAb pair in NHPs, to be selected based on the *in vitro* and mouse data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in cynomolgus monkeys studies would only be considered pending success in the mouse studies and consultation with the external Scientific Advisory Board and NIAID program staff. In years 4-5, we will apply the lessons learned to the plug and play test case Kasokero virus (KASV) in collaboration with **RP1**, **Core D**, and **Core E**.

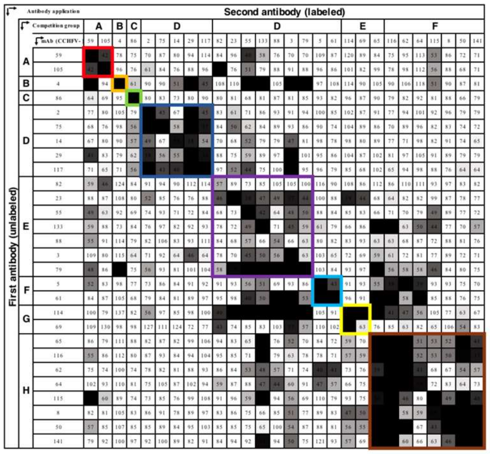


Figure 2. Competition assessment of human Abs to CCHFV using M-segment expressing cells. We tested 28 mAbs in competition assays. MAbs are displayed in 8 groups (A-H) based on their ability to compete for binding. Values shown are the % of binding that occurred during competition compared to non-competed binding of the mAb. This value was normalized to 100%. The values are also indicated by the box fill color; darker colors toward black indicate higher competition and lighter colors toward white indicate less competition, on a gradient scale. Unpublished data.

<u>Preliminary Data</u>. ***(a) Potently neutralizing and non-neutralizing human anti-CCHFV mAbs.*** In this Aim, we will rapidly discover human anti-CCHFV mAbs from CCHFV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them. Through these studies, we aim to identify an optimal anti-CCHFV mAb combination and further advance the discovery platform for rapid response against emerging nairoviruses. In preliminary experiments in collaboration with investigators in **RP1** and **Core E**, the Crowe laboratory has previously isolated and characterized panels of neutralizing human anti-CCHFV human mAbs from individuals who were convalescent after naturally-acquired laboratory-confirmed infection in Spain or Turkey. PBMCs were used to generate hybridomas and antibody variable genes sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against CCHFV that recognize the M-segment in transfected cells, and they exhibit a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 2**). This panel contains cross-binding and cross-neutralizing antibodies for diverse strains. We observed a human mAb designated CCHF-82 competing with competition group 1 and group 2 antibodies from the previously isolated GP38-reactive murine antibodies. CCHF-82 showed some protection in a STAT1$^{-/-}$ mouse model of infection conducted by investigators in **RP1 (Fig 3)**. Further investigation with this antibody in the IbAr10200 prophylaxis and therapeutic models is warranted. In contrast, the CCHFV-neutralizing mAbs that we isolated and tested *in vivo* did not perform well in the Turkish strain STAT1$^{-/-}$ mouse model. We now know protection in this model is possible, given the work from Fels et al., 2021 who showed that potent antibodies against various sites on the Gc surface can afford protection in this model[53]. However, in their study they did not test

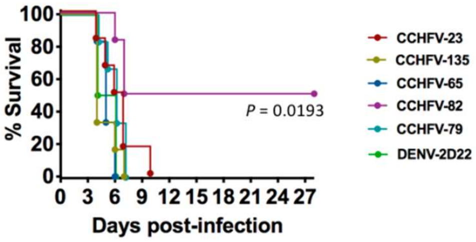


Figure 3. Human mAb CCHF-82 provides partial protection as a post-exposure prophylaxis against the Turkish strain of CCHFV in the STAT1$^{-/-}$ mouse model. A single dose of mAb was administered by the IP route to mice (n=6 per group (3 male, 3 female)) at 30 min post infections. A lethal dose of Turkish strain of CCHFVvirus was administered. CCHF-23, CCHF-135, CCHF-65, CCHF-82, CCHF-79, or DENV-2D22 (an isotype-matched negative control mAb) were tested in a single dose of 250 µg per mouse except for CCHFV-135 at 125 µg per mouse. Kaplan-Meier survival curves were statistically analyzed using a log-rank (Mantel-Cox) test where mAb-treated animals (*P* value shown in figure) were compared to animals treated with the DENV-2D22 negative control mAb. Unpublished data.

GP38-reactive antibodies. To our knowledge, our preliminary data is the first indication that a human GP38-targeting antibody can provide some protection in this mouse model of infection.

Non-neutralizing murine mAbs protect in mouse models of infection[25], but it has not been shown if the human antibody response could provide similar levels of protection. Murine mAb 13G8 binds to GP38 and does not neutralize CCHFV. This mAb provides protection against lethal challenge as a prophylaxis and a therapeutic against CCHFV. In preliminary experiments we sought to understand if human survivors of CCHFV infection possess B cells encoding mAbs that bind a similar epitope as 13G8 and provide protection. CCHF-82 competes for binding with 13G8 to preGn-transfected cells containing GP38. This antibody offered some protection as a post-exposure prophylaxis against the Turkish strain of CCHFV (**Fig 3**). Human antibodies like this should be further studied and the frequency of these clones should be elucidated to understand if this is a feasible strategy for future vaccine efforts for humans. The epitope that 13G8 and CCHF-82 identifies could inform vaccine design if these antibodies offer protection in larger animal models.

Understanding how non-neutralizing antibodies function is critical in efforts to validate the GP38-binding class of antibodies. Golden *et al.* undertook a study to understand the mechanisms 13G8 functioned and observed that complement activation contributes to the protective capacity of this antibody[25]. Here, in studies of CCHF-82, we also will assess complement activation in mouse models of infection to fully understand the function of this mAb *in vivo*. Furthermore, if this mAb protects in the CCHFV IbAr10200 strain challenge model, assessing its mechanism is warranted. Together with **Core D**, we will assess IgG molecules with complement knockout (“KA”) and Fc gamma receptor knockout (LALA-PG) mutations in the Fc domain to study this matter. Human mAb CCHF-82 displayed limited cross-protection against experimental infection with the Turkish strain of CCHFV. Our antibody isolation here will aim to identify cross-protective non-neutralizing antibodies reactive with GP38. Recently, vaccinating with NP was shown to be another way to elicit non- neutralizing antibodies[55]. The NP of CCHFV is more conserved than the glycoproteins and might also be a non-canonical target for the identification of cross-protective non-neutralizing antibodies[55].

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant viral surface protein antigens, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer GP-38-specific B cells.

E.2.1. Rapid isolation of human anti-CCHFV mAbs. ***(a) Immune cells.*** We have obtained human PBMCs from a number of previously CCHFV-infected individuals. One was a health care survivor who was infected while caring for a patient in Spain[56]. We also collected a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Turkey, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples were only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies were approved by the Vanderbilt University Medical Center IRB. PBMCs isolated from the blood samples were deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their used for mAb generation in this **RP4,** if funded.

(b) Ab screening and sequence analysis. Our goal is to identify human anti-CCHFV mAbs rapidly, as if we had to respond to a nairovirus outbreak in real time. Our ability to respond rapidly in the setting of outbreaks is well-documented in the lay press (60 Minutes features and others) and the scientific literature, for ex., 78 days from sample to protection of NHPs for Zika virus[43], and 25 days from PBMC sample receipt to delivery of the antibody genes for the approved COVID-19 drug Evusheld to AstraZeneca[1]. Here, we will single-cell sort antigen-specific B cells using recombinant CCHFV proteins (GP38, Gc, Gn, Gc/Gn) or non-infectious viral particles as bait. These cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF for downstream applications, including VSV-CCHFV neutralization assays. Alternatively, we will use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe CCHFV protein or virus-particle binding to single B cells. These cells will be exported for sequencing of the variable gene regions and analysis using the PyIR software. We will select a panel of up to 500 neutralizing anti-CCHFV mAbs to express as recombinant mAbs and advance for further characterization. We have demonstrated the capacity to execute multiple approaches simultaneously in parallel[1,43], which enhances the diversity of clones obtained. We will generate recombinant GP antigens for selecting GP-specific B cells, based on prior validated designs in the field, and use the antigens produced by **Core D**, listed in Table 3 or by **RP1** (Table 4).

Table 3. CCHFV antigens from Core D

Antigen	Manufacturer (expression system)
CCHF Turkey GnGc	Mapp (Drosophila)
CCHF Turkey GP38	Mapp (Drosophila)
CCHF NP	Genovac (mammalian)

Table 4. Antigens available from RP1
CCHF Turkey GnGc
CCHF Turkey GP38
CCHF Turkey NP
CCHF Turkey GnGc+GP38+NP
CCHF Turkey GnGc+GP38
CCHF Turkey GP38+NP
CCHF Turkey GnGc+NP

(c) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using the custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors. We will design human anti-LASV mAbs as IgG1 with the Fc mutation, LALA-PG, to abrogate Fc effector functions to assess role of Fc-mediated effector functions to protection in animal models. We also will engineer IgG1 molecules with extended-half-life Fc mutations based on enhanced binding to FcRn including LS (M428L +N434S)[26] and YTE (M252Y +S254T +T256E)[27] mutations. These Fc engineering approaches will generate mAbs with vaccine-like properties. The LS and YTE mutations were developed in commercial settings (and have been used safely in the clinic), but the patent life of these mutations will expire prior to approval of antibodies developed here.

(d) Micro-scale mAb expression. The Crowe laboratory will perform large-throughput micro-scale mAb expression and purification that, with our integrated approach allows, screening assays to identify candidate mAbs This approach allows early-stage assays to identify candidate mAbs that will then be produced at larger scale by **Core D** with diverse Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(e) A CPE-based CCHFV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of CCHFV. We will use a replication-competent vesicular stomatitis virus (VSV) CCHFV surrogate that expresses CCHFV surface proteins and causes CPE[46]. Investigators in **RP1** have the VSV-CCHFV-GFP versions of clades 1 through 7 based on this publication. We will obtain these all these VSV constructs from investigators in **PABVAX**. Existing VSV vectors will be provided by investigators in **RP1 and Core E**. We already have Vanderbilt Institutional Biosafety Committee approval for use of these constructs in this format and assay. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against CCHFV. These mAbs will be moved forward for additional studies to identify an optimal anti-CCHFV mAb combination. GP38-reactive clones will be advanced based on avidity ranking since they are not likely to be neutralizing but may be protective *in vivo*.

E.2.2. Identifying CCHFV mAb combinations. *(a) Epitope mapping through competition-binding studies.* Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 2**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind CCHFV surface proteins and validate their epitopes. We will perform competition-binding studies with CCHFV Gc/Gn or subunit proteins (as above) or CCHFV virus-like particles (VLPs) made from a replicon system in hand. **Core E** will inactivate CCHFV virus particles that they have in the UTMB center by gamma irradiation and safety test and send those to our Vanderbilt site (we have previously accomplished this transfer with Ebola and Marburg particles). Inactivated virus particles will be used in ELISA. We will include positive controls made recombinantly from some of the mAbs shown in the unpublished preliminary data above for the identification of mAbs that can concurrently bind CCHFV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti-CCHFV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. GP-38-reactive clones will be screened for synergistic binding as they are not expected to be neutralizing. ***(c) Identification of key binding residues of human anti-CCHFV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-CCHFV constructs at BSL2 in the presence of saturating concentrations of CCHFV mAbs (single or combination) using the RTCA platform to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test this concept *in vivo* by determining the level of infection of VSV-CCHFV escape variants in the presence of mAbs.

E.2.3. Structural basis of neutralization by anti-CCHFV mAb combinations. The studies above should identify optimal anti-CCHFV mAb combinations that simultaneously bind, neutralize, and increase the barrier for CCHFV to escape mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for nairoviruses by identifying key epitope targets (**RP1**).

E.2.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies in STAT1$^{-/-}$ mouse models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (Core D), will advance to *in vivo* testing. We will use an established STAT1$^{-/-}$ mouse model of CCHFV infection that the **RP1** and **Core E** investigators have deployed[46]. To benchmark our mAbs, a separate set of STAT1$^{-/-}$ mice will be treated with CCHF-82, which we showed in unpublished preliminary data above confers substantial but incomplete protection (**Fig 3**). The **Core E** laboratory has access to strains representing all 6 clades that can be tested. Groups of 10 STAT1$^{-/-}$ mice will be treated with low, medium, and high doses) of single or combinations of anti-CCHFV mAb, or isotype controls at day +1 after inoculation with CCHFVs. This approach allows us to determine whether combinations provide advantage over the best CCHFV monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after CCHFV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal CCHFV mAb pairs that are comparable to or outperform the mAbs we reported above. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-CCHFV mAb combination against CCHFV in NHPs. As a final test of an optimal anti-CCHFV mAb combination, we will assess efficacy of the lead anti-CCHFV mAb pair in NHPs (**Core E**), to be selected based on the *in vitro,* STAT1$^{-/-}$ mice, and manufacturability data (**Core D**). Down-selection for testing in cynomolgus monkeys would only be considered with success in the STAT1$^{-/-}$ mouse studies and in consultation with the external SAB and NIAID program staff.

E.2.5. Applying lessons learned in CCHFV research to Kasokero mAbs. Once we have completed the CCHFV discovery campaigns, will turn to the test case pathogen Kasokero virus to see if we can use the prototype pathogen approach for this virus. We do not have access to human PBMCs from Kasokero-immune donors. We will use Alloy humanized mouse models as a source of B cells encoding fully human monoclonal antibodies, as described above. Mice will be immunized with conformationally correct recombinant protein antigens (**Table 5**), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Kasokero. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for CCHFV.

Table 5. Antigens available from RP1
Kasokero GnGC
Kasokero GP38
Kasokero NP
Kasokero GnGc +GP38 +NP
Kasokero GnGc +GP38
Kasokero GnGc +NP
Kasokero GP38 +NP

__Anticipated results, potential problems, and alternative approaches__**.** In this Aim, we will identify human anti-CCHFV mAb combinations with optimal protective activity. These mAb combinations can be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging CCHFV or related nairoviruses. Given our previous ability to generate human anti-CCHFV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-CCHFV mAbs. We may observe that mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-CCHFV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (*i.e.*, memory B cells) isolated from human CCHFV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in CCHFV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-CCHFV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-CCHFV mAb combinations that can inform vaccine design (**RP1**) for related viruses being used as test cases in years 4 and 5. In the case that VSV-CCHFV does not cause sufficient CPE via the RTCA platform (highly unlikely), established focus reduction neutralization assays that detect VSV-CCHFV-infected foci will be used to identify mAbs with synergistic properties. Furthermore, for competitive binding assays, biolayer interferometry using the Octet HTX instrumentation also can be used to assess binding of mAbs in solution.

E3. Specific Aim 3. Establish optimal mAb combination therapies against henipaviruses Hendra (HeV) and Nipah (NiV) viruses. We will use a large panel of potent anti-henipavirus mAbs that we previously made from the memory B cells of immune individuals and generate new antibodies targeting the fusion (F) protein. **RP2** will provide new antigens for mAb selection and BSL-2-compatible viruses for neutralization screens including Cedar virus constructs displaying HeV and NiV F and G proteins. We will identify optimal mAb combinations to enhance henipavirus therapy, minimize viral escape, and improve treatment efficacy through synergy. *In vitro* competition-binding, neutralization, and mechanistic studies will be conducted, including authentic virus neutralization with **PABVAX Core E**. We will refine the recognized epitopes and investigate the structural basis for optimal combination neutralization of henipaviruses using HeV and NiV as prototypes. *In vivo* assessment of mAb combinations will determine the minimal effective dose for protection and the ability to prevent *in vivo* escape. Protective efficacy of lead mAb combinations will be assessed in hamster models of NiV and HeV infection with **Core E**. Leads will be transferred to **Core D**. As a final test of optimal henipavirus mAb combinations, we will assess protective efficacy of the lead henipavirus mAb pairs in NHPs, to be selected based on the *in vitro* and hamster data from **Core E** and manufacturability data from **Core D**. Down-selection for testing in African green monkey (AGM) henipavirus model studies would only be considered pending success in the hamster studies and consultation with the external SAB and NIAID program staff. The HeV+NiV studies will be conducted in years 1-3 and then applied to the plug-and-play test case Langya virus in years 2-5. If **RP2** and **Core E** cannot rescue recombinant Langya virus, the lessons learned in Years 1-3 will be applied to the alternate plug-and-play test viruses Angavokely or Sosuga (SOSV) virus. Preliminary data on SOSV mAbs are provided.

Preliminary Data. ***(a) Potently neutralizing and non-neutralizing human anti-NIV/HeV mAbs.*** In this Aim, we will further adapt approaches we have developed in preliminary experiments with henipavirus mAb studies by identifying a potently protective combination of anti-NiV/HeV human mAbs. We already have in hand some outstanding potency anti-G antibodies with extensive characterization, including synergistic neutralization. In the work proposed here, we will rapidly discover human NiV/HeV anti-F mAbs from NiV-immune individuals and perform synergy studies to identify optimal mAb combinations by pairing them with each other and with anti-G antibodies. Through these studies, we aim to identify an optimal anti-NiV+HeV mAb combination and further advance the discovery platform for rapid response against emerging paramyxoviruses, especially henipaviruses. In previous experiments in collaboration with investigators in **RP2** and **Core E**, the Crowe laboratory has isolated characterized panels of neutralizing human anti-G NiV/HeV human mAbs from an individual with G-based immunity following occupational exposure to an equine HeV G subunit vaccine. Isolated peripheral blood mononuclear cells (PBMCs) were used to generate human B cell hybridomas secreting human mAbs to G, and antibody variable genes were sequenced to generate mAbs recombinantly. We isolated a panel of antibodies against the G protein that recognize the HeV G, NiV Bangladesh G, and NiV Malaysia G proteins. The mAbs exhibited a diversity of recognition patterns in that they fall into multiple competition-binding groups (**Fig 4**). This panel contains cross-binding and cross-neutralizing antibodies for diverse NiV and HeV strains.

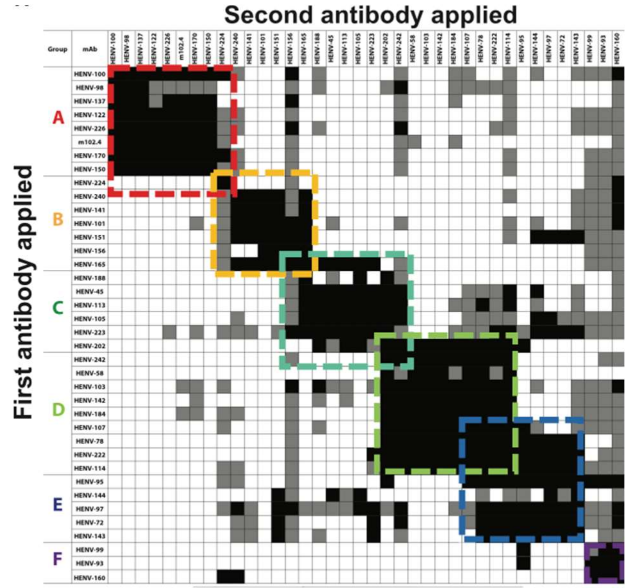


Figure 4. Identification of major antigenic sites for recognition of HeV RBP by human mAbs. SPR competition binding of human Abs against HeV-RBP. A first Ab was applied to a gold-coated sensor chip, and recombinant HeV-RBP head domain was associated to the coupled Ab. A second Ab was applied to the sensor chip to determine binding to RBP. Black boxes - a pairwise interaction in which the binding of the second Ab is blocked by the first. White - both Abs could bind simultaneously. Gray - intermediate competition phenotype.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific cells with recombinant henipavirus F protein antigens, perform cloning of Ab variable genes, and then express mAbs as recombinant IgGs. Alternatively, F-specific B cells will be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through 2 different single-cell RNA-seq approaches (10X Genomics & Rhapsody).

E.3.1. Rapid isolation of human anti-HeV/NiV mAbs. ***(a) Immune cells.*** We are obtaining human PBMCs from a number of previously NiV-infected individuals who acquired immunity following natural infection in collaboration with the **icddr,b**, an international health research institute based in Dhaka, Bangladesh. We are collecting a panel of 10 fully de-identified PBMC samples from individuals naturally infected in Bangladesh, in sample acquisition work supervised by the Vanderbilt Vaccine Center Biorepository with separate institutional funding. Blood samples are only obtained after informed consent, de-identified, and assigned random specimen numbers. The studies are approved by the icddr,b and Vanderbilt University Medical Center IRBs. PBMCs isolated from the blood samples are deposited into the Vanderbilt Vaccine Center Biorepository, but we can designate their use in this **RP4,** if funded.

(b) Applying mAb technologies for new mAb isolation. We will use sorting of antigen-specific B cells, cloning of Ab variable genes, and then expression of mAbs as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. As above, we can use high-throughput systems to capture Ab genes directly from viral protein-specific Ab-secreting B cells on the Berkeley Lights Beacon® optofluidic platform or through single-cell RNA-seq approaches (10X Genomics and BD Rhapsody™), and this may be helpful for rarer antigen-specific B cells. We will sort soluble fusion (sF) or attachment (G) protein-specific cells with recombinant viral surface protein antigens from **Core D** (Table 6).

Table 6. Henipavirus antigens from Core D

Antigen	Manufacturer (expression system)
Nipah sF	Genovac (mammalian)
Hendra sF	Genovac (mammalian)
NiV G	Geneovac (mammalian)

(c) Ab screening and sequence analysis. Our goal is to identify human anti-NiV/HeV mAbs rapidly, as if we had to respond to a paramyxovirus outbreak in real time. We will single-cell sort antigen-specific B cells using recombinant HeV and NiV proteins (sF or G) as bait. These cells will be expanded on feeder layers expressing CD40L, IL-21, and BAFF for downstream applications, including VSV-CCHFV neutralization assays. We also can use the Berkeley Lights Beacon® optofluidic platform, which can functionally ascribe CCHFV protein or virus-particle binding to single B cells. These cells will be exported for sequencing of the variable gene regions and analysis using the PyIR software. We will select a panel of up to 500 neutralizing anti-HeV/NiV mAbs to express as recombinant mAbs and advance for further characterization.

(d) Recombinant mAb cloning. We will synthesize heavy and light chain variable regions for cloning, expression, and downstream functional assays to validate the mAb sequence. We will perform high-throughput synthesis of mAb genes using the custom, commercial synthesis platform (Twist Bioscience). MAb genes are Gibson-assembly-cloned on-instrument into our custom full-length Ig expression vectors. We will design human anti-LASV mAbs as IgG1 with the Fc mutation, LALA-PG, to abrogate Fc effector functions to assess role of Fc-mediated effector functions to protection in animal models. We also will engineer IgG1 molecules with extended-half-life Fc mutations based on enhanced binding to FcRn including LS (M428L +N434S)[26] and YTE (M252Y +S254T +T256E)[27] mutations. These approaches will generate mAbs with vaccine-like properties.

(e) Micro-scale mAb expression. The Crowe laboratory will perform large-throughput micro-scale mAb expression and purification that, with our integrated approach allows, screening assays to identify candidate mAbs. Early-stage assays will identify candidate mAbs that will then be produced at larger scale by **Core D** with a variety of Fc mutants to assess the role of Fc-mediated effector functions *in vivo* (**Core E**).

(f) CPE-based HeV/NiV neutralization assay using RTCA. As above, we have established a high-throughput assay to assess mAb-mediated neutralization of viruses through the detection of CPE. In this aim, we will apply this method to assess neutralization of HeV and NiV. We will use replication-competent vesicular stomatitis virus (VSV) HeV and NiV surrogates that expresses henipaviurs surface proteins and cause CPE. Investigators in **RP2 and RP3** have VSV-NiV F and VSV-NiV G with GFP[45], VSV-NiV G without GFP[47] and VSV-HeVG (Geisbert and Cross, unpublished). We will obtain these all these VSV constructs from investigators in **PABVAX**. We already have Vanderbilt Institutional Biosafety Committee approval for use of these constructs in this format and assay. A decrease in cell impedance due to CPE and a corresponding decrease in GFP expression will identify mAbs with neutralizing activity against HeV and NiV. We also can use Cedar virus constructs for neutralization assays as the have reduced pathogenicity[57] and can be used in high-throughput screening assays for henipavirus antiviral discovery[58]. Cedar viruses will be provided by Dr. Broder of **RP3**. The mAbs with most potent activity will be moved forward for additional studies to identify an optimal anti-HeV/NiV mAb combination.

E.3.2. Identifying HeV/NiV mAb combinations. ***(a) Epitope mapping through competition-binding studies.*** Using previously established methods for competition-binding assays in Octet HTX biosensor (biolayer interferometry) (as above, **Fig 4**) or high-throughput analytical flow cytometry (using an iQue Plus screener), we will identify mAb pairs that can simultaneously bind HeV/NiV surface proteins and validate their epitopes. We will perform competition-binding studies with HeV/NiV sF or G proteins (as above). We will include positive controls made recombinantly from some of the mAbs shown in the preliminary data above for the identification of mAbs that can concurrently bind HeV/NiV proteins with a goal of finding more potent clones at those sites or clones that complement these mAbs. ***(b) Synergy studies using RTCA.*** To assess functional compatibility of the anti- HeV/NiV mAb pairs, we will assess neutralization potency using the RTCA platform. We anticipate testing up to 15 mAb combinations, using rational selection based on preliminary epitope mapping data. We can assess for synergy, additivity, or antagonism of neutralization. Based on these results, we will down-select neutralizing mAb pairs, with our priority on combinations with synergistic effects. ***(c) Identification of key binding residues of human anti-HeV/NiV mAbs by neutralization escape analysis***. The generation of mAb combinations should increase the barrier to viral escape. To assess whether combination mAb pairs reduce the incidence of viral escape, we will perform *in vitro* neutralization escape mutant analyses. For these analyses, we will passage VSV-NiV F, VSV-NiV G and VSV-HeV G constructs at BSL2 in the presence of saturating concentrations of HeV/NiV mAbs (single or combination) using RTCA to detect virus-induced CPE. Resistant virus will induce CPE, and supernatants will be extracted for sequencing. Even if partial escape occurs, virus fitness may suffer. We can test the level of infection of VSV-HeV/NiV escape variants in the presence of mAbs.

E.3.3. Structural basis of neutralization by anti-HeV/NiV mAb combinations. The studies above should identify optimal anti-HeV/NiV mAb combinations that simultaneously bind, neutralize, and increase the barrier for CCHFV to escape mAb selective pressure. For a limited number of mAb pairs (n = 3), we will determine the structural basis of this synergy with cryo-EM and crystallography, for which we have all capabilities and equipment in our laboratory in the Vanderbilt Vaccine Center, Vanderbilt Structural Biology Core, and external synchrotron sources. An understanding of the structural basis and rules governing an optimal neutralizing mAb combination can be applied to mAb therapy development against arenavirus pandemic threats and inform vaccine design for henipaviruses by identifying key epitope targets.

E.3.4. Protective efficacy of human anti-CCHF mAbs. ***(a) Studies hamster models***. MAbs that meet the above criteria and demonstrate acceptable manufacturability (**Core D**), will advance to *in vivo* testing. We will use an established hamster model of NiV or HeV infection that the **Core E** investigators have deployed. To benchmark our mAbs, a separate set of hamsters will be treated with G mAbs HENV-103+HENV-117, which we showed in previous data above confers substantial protection[18]. Groups of 10 hamsters will be treated with low, medium, or high IgG doses) of single or combinations of anti-HeV/NiV mAbs, or isotype controls at day +1 after inoculation with HeV or NiV. This approach allows us to determine whether combinations provide advantage over the best HeV or NiV mAb monotherapy treatments. These experiments will be repeated at least two independent times. We expect to test 10 single mAbs and 5 pairs of anti-CCHFV mAbs in these studies. Protection will be assessed using the following metrics: (i) survival; (ii) weight loss; (iii) clinical scores; (iv) viral burden measurements at day 3 after HeV or NiV exposure and the day 35 study endpoint by plaque and qRT-PCR assays. For any animals that show virological breakthrough of infection, deep sequencing will be performed from recovered viruses to assess for possible emergence of *in vivo* resistance. From these studies, we expect to down-select further to 2 optimal HeV/NiV mAb pairs that are comparable to or outperform the mAbs we reported previously. The lead mAbs will be advanced for further testing in **Core E** for additional dose-down studies.

(b) Validation of the protective efficacy of a down-selected human anti-HeV/NiV mAb combination against HeV and NiV in NHPs. As a final test of an optimal anti-HeV/NiV mAb combination, we may assess efficacy of the lead anti-HeV/NiV mAb pair in NHPs (**Core E**), selected based on the *in vitro,* hamster data, and manufacturability data (**Core D**). Down-selection for testing in African green monkeys would only be considered with success in the hamster studies and in consultation with the external SAB and NIAID program staff.

E.3.5. Applying lessons learned in HeV/NiV research to Langya, Angavokely and Sosuga viruses. Once we have completed the HeV/NiV discovery campaigns, will turn to the test case pathogen Langya, Angavokely and Sosuga (SOSV) viruses to see if we can use the prototype pathogen approach for this virus. We have plenty of human PBMCs from the sole identified SOSV-immune individual and have already shown that it is possible to obtain HN- and F-specific neutralizing antibodies from this individual's B cells[59]. We also have already prepared recombinant SOSV prefusion F and HN proteins[59]. We do not have access to human PBMCs from Langya- or Angavokely-immune donors. For those mAb discovery campaigns, we will use Alloy humanized mouse models as a source of B cells encoding human mAbs, as above. Mice will be immunized with recombinant protein antigens (Table 7), cDNAs encoding protective antigens, or replication-competent VSV strains encoding protective antigens for Langya or Angavokely. After the immunization protocol is completed, animals will be sacrificed humanely, spleens collected, and suspensions of splenocytes (enriched in B cells) will be purified by density gradient selection. Downstream, the generation of human mAbs follows our well-established methods as above for making human mAbs from B cells using single-cell RNAseq or human hybridoma methods. The development will follow as for Hev/NiV described above.

Table 7. Paramyxovirus antigens from Core D

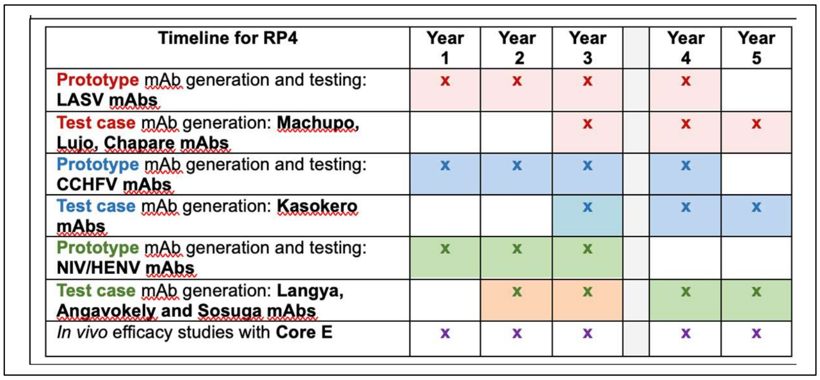
Antigen	Manufacturer (expression system)
Mojang sF	Genovac (mammalian)
Angavokely sF	Genovac (mammalian)
Langya sF	Genovac (mammalian)

<u>Anticipated results, potential problems, and alternative approaches</u>. In this Aim, we will identify human anti-HeV/NiV mAb combinations with optimal protective activity. These mAb combinations will be endowed genetically with half-life-extended Fc modifications to produce vaccine-like therapy options (for humans) for rapid response to emerging HeV/NiV or related paramyxoviruses. Given our previous ability to generate human anti-HeV/NiV mAbs, as shown in preliminary data, we do not anticipate problems in isolating anti-HeV/NiV mAbs. We may observe that new mAb combinations do not add any benefit over individual mAbs. If we do not identify potently protective anti-HeV/NiV mAbs via the planned approaches, we can also take a target-agnostic approach and perform single-cell RNA-seq (10X Genomics and BD Rhapsody^{TM}) on enriched B cells (*i.e.*, memory B cells) isolated from human NiV-immune PBMC samples. In addition, we can deep sequence the B cell repertoires present in NiV-immune PBMC samples and identify clonal lineages, including siblings. Often, additional Abs in the lineages may be more potently neutralizing than the ones obtained directly from human B cells. Together, through these complementary approaches (antigen-specific mAb isolation + single-cell RNA-seq + bulk NGS), we likely can select a diverse panel of human anti-HeV/NiV mAbs. We also anticipate defining key epitopes targeted by potently neutralizing anti-HeV/NiV mAb combinations that can inform vaccine design (**RP2**) for related viruses being used as test cases in years 2 through 5.

F. Scientific rigor. ***Experimental design.*** Experiments will include biological and technical replicates, and results will be analyzed by parametric or non-parametric analyses (depending on data distribution) and corrections for multiple comparisons. EC_{50} (binding) and IC_{50} (neutralization) values will be reported only following at least 3 identically conducted experiments. All mAb screening will incorporate appropriate + and - controls. Positive signals in binding assays will be defined as >3 SD above background. ***Protein quality.*** We have a high capacity to purify proteins using a Protein Maker workstation that allows purification chromatography on 24 parallel columns. We perform QC on protein reagents using a Maurice instrument with capillary electrophoresis technology to automate our protein profiling either by size or charge, providing cIEF and CE-SDS data. Animal ***studies*** will be performed blinded with independent replicates and include negative (*e.g.*, placebo), and positive (when available) controls. We will use power calculations (**Data Management Core B**) to determine sample sizes. For virological studies, power analysis was performed using accepted values for type I error (0.05) and power (80%). The values for population variance (15×) are based on existing data sets and our experience with rodent challenge studies. We will perform experiments at least 2-3 independent times (n = 4-5 each) to attain n = 12-15 per arm. Smaller group sizes can be used if differences are larger than expected.

G. Relevant biological variables. The human mAbs are derived from immune human donors or Alloy mice. Humans. Human Abs will be isolated for biological, structural, and functional studies at a clonal level. The sample size is not powered for large scale epidemiologic or clinical study conclusions. There is no evidence that sex, age, body mass index, or underlying health conditions other than immunodeficiency or immunosuppressive treatments alters the activity of cloned Abs in structure/function studies. The samples are de-identified without patient specific identifiers to protect the health information of the donors; thus, research with these samples is considered *Not Human Subjects Research* category activity. Mice, hamsters, guinea pigs, NHP, and viruses are also biological systems used in this application. Conventional animal housing conditions will be used because there is no evidence that altered room temperatures and light/dark cycles influence paramyxovirus, nairovirus, or arenavirus pathogenesis. Mice, hamsters, guinea pigs, and NHPs of both sexes will be used, and phenotypes will be monitored for sex-based differences. Virus stocks will be either low passage seed stocks from natural isolates or generated from existing cDNA clones and sequence-confirmed prior to use for infection studies.

H. Milestones and quantitative Go/No-Go criteria. Through **Aims 1-3**, we have proposed experiments that will advance our knowledge of the mAb-mediated mechanisms of action against paramyxoviruses, nairoviruses, and arenaviruses. In each Aim, we will use down-selection criteria (as below) to advance mAb combinations or variants for additional studies. By year 3, we will have defined the principles that govern optimal mAb combinations for paramyxoviruses, nairoviruses, and arenaviruses and the rational approaches needed to select mAbs for potency and breadth. We will use the following metrics to advance mAbs: ***(a) Neutralizing activity*.** All mAbs will have neutralizing potency < 10 ng/mL, EC_{50} value; ***(b) Binding affinity***. MAbs will be of high affinity to surface proteins or virions (K_D < 5 nM). ***(c) Protein stability and production quality*.** MAbs must produce at high levels without optimization (> 100 mg/L) and show biochemical/functional stability at 4°C or -80°C (>90% retention of binding and neutralization activity at 30 d). ***(d) Synergy***. Combinations of 2 mAbs against a given virus ideally will show synergy of neutralization *in vitro* or at a minimum, additive inhibition (no antagonism); ***(e) Epitopes***. To avoid resistance, mAbs in combination will bind 2 epitopes (determined by competition binding (<20% reduction in binding), mapping, and structural studies. ***(f) Resistance*** against mAbs will be assessed for virus escape. Deep sequencing of viruses that emerge from cells or animals treated with mAbs will inform prioritization. Combinations of mAbs with high genetic barriers to resistance (<10× loss in EC_{50}) will be advanced. ***(g) Protection in mice, hamsters, guinea pigs, or NHPs.*** The following metrics can be used: (i) Protection against lethal challenge by homologous virus (>90% survival; (ii) Protection against clinical



Timeline for RP4	**Year 1**	**Year 2**	**Year 3**		**Year 4**	**Year 5**
Prototype mAb generation and testing: **LASV mAbs**	x	x	x		x	
Test case mAb generation: **Machupo, Lujo, Chapare mAbs**			x		x	x
Prototype mAb generation and testing: **CCHFV mAbs**	x	x	x		x	
Test case mAb generation: **Kasokero mAbs**			x		x	x
Prototype mAb generation and testing: **NIV/HENV mAbs**	x	x	x			
Test case mAb generation: **Langya, Angavokely and Sosuga mAbs**		x	x		x	x
In vivo efficacy studies with **Core E**	x	x	x		x	x

disease by homologous virus (>80%); (iii) Protection against infection by homologous virus (>100-fold reduction in viral titers in multiple organs). ***(h) pK measurements***. For lead protective mAbs with mutations that should confer extended half-life, we will perform pK studies in NHPs (with **Core D** and **E**) to assess levels over time with a goal of a human predicted serum half-life of >6 months.

LITERATURE CITED – RP4

1. Zost, S.J.*, et al.* Rapid isolation and profiling of a diverse panel of human monoclonal antibodies targeting the SARS-CoV-2 spike protein. *Nat Med* **26**, 1422-1427 (2020).
2. Fischer, W.A., II & Wohl, D.A. Moving Lassa Fever Research and Care Into the 21st Century. *The Journal of Infectious Diseases* **215**, 1779-1781 (2017).
3. Ezeomah, C.*, et al.* Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. *Open Forum Infect Dis* **6**, ofz512 (2019).
4. Li, A.L.*, et al.* Ophthalmic manifestations and vision impairment in Lassa fever survivors. *PLoS One* **15**, e0243766 (2020).
5. Whitmer, S.L.M.*, et al.* New Lineage of Lassa Virus, Togo, 2016. *Emerg Infect Dis* **24**, 599-602 (2018).
6. Ibukun, F.I. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. *Viruses* **12**(2020).
7. Webb, P.A., Johnson, K.M., Mackenzie, R.B. & Kuns, M.L. Some characteristics of Machupo virus, causative agent of Bolivian hemorrhagic fever. *The American journal of tropical medicine and hygiene* **16**, 531-538 (1967).
8. Aguilar, P.V.*, et al.* Reemergence of Bolivian hemorrhagic fever, 2007-2008. *Emerg Infect Dis* **15**, 1526-1528 (2009).
9. Enria, D.A., Ambrosio, A.M., Briggiler, A.M., Feuillade, M.R. & Crivelli, E. [Candid#1 vaccine against Argentine hemorrhagic fever produced in Argentina. Immunogenicity and safety]. *Medicina (B Aires)* **70**, 215-222 (2010).
10. Enria, D.A. & Maiztegui, J.I. Antiviral treatment of Argentine hemorrhagic fever. *Antiviral research* **23**, 23-31 (1994).
11. Hawman, D.W. & Feldmann, H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology* (2023).
12. Chinikar, S.*, et al.* Genetic Diversity of Crimean Congo Hemorrhagic Fever Virus Strains from Iran. *J Arthropod Borne Dis* **10**, 127-140 (2016).
13. Shepherd, A.J., Swanepoel, R. & Leman, P.A. Antibody response in Crimean-Congo hemorrhagic fever. *Rev Infect Dis* **11 Suppl 4**, S801-806 (1989).
14. Lombe, B.P.*, et al.* Mapping of Antibody Epitopes on the Crimean-Congo Hemorrhagic Fever Virus Nucleoprotein. *Viruses* **14**(2022).
15. Mackenzie, J., Wang, L. & Broder, C.C. Henipaviruses. in *Fields Virology*, Vol. 2 1070-1085 (2013).
16. Whitmer, S.L.M.*, et al.* Inference of Nipah virus evolution, 1999–2015. *Virus Evolution* **7**(2020).
17. Dong, J.*, et al.* Potent Henipavirus Neutralization by Antibodies Recognizing Diverse Sites on Hendra and Nipah Virus Receptor Binding Protein. *Cell* **183**, 1536-1550 e1517 (2020).
18. Doyle, M.P.*, et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021).
19. Mire, C.E.*, et al.* A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis* **221**, S471-S479 (2020).
20. Dang, H.V.*, et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021).
21. Cross, R.W.*, et al.* Antibody therapy for Lassa fever. *Curr Opin Virol* **37**, 97-104 (2019).
22. Cross, R.W.*, et al.* Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. *Antiviral research* **133**, 218-222 (2016).
23. Cartwright, H.N., Barbeau, D.J. & McElroy, A.K. Isotype-Specific Fc Effector Functions Enhance Antibody-Mediated Rift Valley Fever Virus Protection In Vivo. *mSphere* **6**, e0055621 (2021).

24. Gutjahr, B., *et al.* Two monoclonal antibodies against glycoprotein Gn protect mice from Rift Valley Fever challenge by cooperative effects. *PLoS Negl Trop Dis* **14**, e0008143 (2020).
25. Golden, J.W., *et al.* GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection. *Sci Adv* **5**, eaaw9535 (2019).
26. Zalevsky, J., *et al.* Enhanced antibody half-life improves in vivo activity. *Nat Biotechnol* **28**, 157-159 (2010).
27. Dall'Acqua, W.F., *et al.* Increasing the affinity of a human IgG1 for the neonatal Fc receptor: biological consequences. *J Immunol* **169**, 5171-5180 (2002).
28. Enria, D.A., Briggiler, A.M., Fernandez, N.J., Levis, S.C. & Maiztegui, J.I. Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma. *Lancet* **2**, 255-256 (1984).
29. Jahrling, P.B. & Peters, C.J. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infect Immun* **44**, 528-533 (1984).
30. Chen, E.C., *et al.* Systematic analysis of human antibody response to ebolavirus glycoprotein shows high prevalence of neutralizing public clonotypes. *Cell Rep* **42**, 112370 (2023).
31. Buck, T.K., *et al.* Neutralizing Antibodies against Lassa Virus Lineage I. *mBio* **13**, e0127822 (2022).
32. Enriquez, A.S., *et al.* Delineating the mechanism of anti-Lassa virus GPC-A neutralizing antibodies. *Cell Rep* **39**, 110841 (2022).
33. Hastie, K.M., *et al.* Convergent Structures Illuminate Features for Germline Antibody Binding and Pan-Lassa Virus Neutralization. *Cell* **178**, 1004-1015 e1014 (2019).
34. Hastie, K.M., *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928 (2017).
35. Li, H., *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991 (2022).
36. Gilchuk, P., *et al.* Pan-ebolavirus protective therapy by two multifunctional human antibodies. *Cell* **184**, 5593-5607 e5518 (2021).
37. Chen, E.C., *et al.* Convergent antibody responses to the SARS-CoV-2 spike protein in convalescent and vaccinated individuals. *Cell Rep* **36**, 109604 (2021).
38. Dong, J., *et al.* Genetic and structural basis for SARS-CoV-2 variant neutralization by a two-antibody cocktail. *Nat Microbiol* **6**, 1233-1244 (2021).
39. Greaney, A.J., *et al.* Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host Microbe* **29**, 44-57 e49 (2021).
40. Kramer, K.J., *et al.* Potent neutralization of SARS-CoV-2 variants of concern by an antibody with an uncommon genetic signature and structural mode of spike recognition. *Cell Rep* **37**, 109784 (2021).
41. Suryadevara, N., *et al.* Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. *Cell* **184**, 2316-2331 e2315 (2021).
42. Zost, S.J., *et al.* Potently neutralizing and protective human antibodies against SARS-CoV-2. *Nature* **584**, 443-449 (2020).
43. Gilchuk, P., *et al.* Integrated pipeline for the accelerated discovery of antiviral antibody therapeutics. *Nat Biomed Eng* **4**, 1030-1043 (2020).
44. Cross, R.W., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep* **40**, 111094 (2022).
45. Mire, C.E., *et al.* Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J* **10**, 353 (2013).
46. Rodriguez, S.E., *et al.* Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep* **9**, 7755 (2019).
47. Foster, S.L., *et al.* A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against lethal Nipah virus disease. *Proc Natl Acad Sci U S A* **119**, e2200065119 (2022).

48. Branco, L.M., *et al.* Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. *Virol J* **7**, 279 (2010).
49. Starr, T.N., *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310 e1220 (2020).
50. Deschambault, Y., *et al.* An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. *Viruses* **15**(2023).
51. Bell, T.M., *et al.* Pathogenesis of Bolivian Hemorrhagic Fever in Guinea Pigs. *Vet Pathol* **53**, 190-199 (2016).
52. Golden, J.W., *et al.* An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. *Sci Rep* **7**, 4679 (2017).
53. Fels, J.M., *et al.* Protective neutralizing antibodies from human survivors of Crimean-Congo hemorrhagic fever. *Cell* **184**, 3486-3501 e3421 (2021).
54. Mishra, A.K., *et al.* Structural basis of synergistic neutralization of Crimean-Congo hemorrhagic fever virus by human antibodies. *Science* **375**, 104-109 (2022).
55. Leventhal, S.S., *et al.* Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. *EBioMedicine* **82**, 104188 (2022).
56. Negredo, A., *et al.* Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *N Engl J Med* **377**, 154-161 (2017).
57. Laing, E.D., *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018).
58. Amaya, M., *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084 (2021).
59. Parrington, H.M., *et al.* Potently neutralizing human mAbs against the zoonotic pararubulavirus Sosuga virus. *JCI Insight* **8**(2023).

From: Geisbert, Thomas W. on behalf of Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
To: Crowe, James; Larry Zeitlin; CHRISTOPHER BRODER
Cc: Cross, Robert W.
Subject: Re: RP4
Date: Wednesday, June 7, 2023 9:26:54 AM
Attachments: RP4 Research Strategy-Final-7June23.docx

OK thanks all! I made a few final minor edits and just fired this off to Sponsored Programs.

From: Crowe, James <james.crowe@vumc.org>
Sent: Wednesday, June 7, 2023 8:14 AM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Cc: Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: RP4

Ok, I think this is final from me
Tom, I added a Fig 4 with sosv data to E3.5, since there was space

---

From: Crowe, James <james.crowe@vumc.org>
Date: Wednesday, June 7, 2023 at 8:02 AM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, Geisbert, Thomas W. <twgeisbe@utmb.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Cc: Cross, Robert W. <rwcross@utmb.edu>
Subject: Re: RP4

Ok, I am just touching it one last time. Give me 10 min

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Date: Wednesday, June 7, 2023 at 7:57 AM
To: Geisbert, Thomas W. <twgeisbe@utmb.edu>, Crowe, James <james.crowe@vumc.org>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Cc: Cross, Robert W. <rwcross@utmb.edu>
Subject: Re: RP4

With a revised final timeline figure….

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Date: Wednesday, June 7, 2023 at 8:36 AM

To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Crowe, James <james.crowe@vumc.org>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Cc: Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: RP4

References are all set. Jim, in the timeline figure (I couldn't edit it), please move Machupo to Prototype with Lassa…

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Date: Wednesday, June 7, 2023 at 8:30 AM
To: Crowe, James <james.crowe@vumc.org>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Larry Zeitlin <larry.zeitlin@mappbio.com>
Cc: Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: RP4

OK I accepted all of Larry's changes and then some of my own and we were still about half a page over. The only way I could really make this work without ripping the guts out of the approach was to cut back the Background section. This made it work. Larry, you will need to fix the references. If anyone is inclined to add some text back to the Background we do have a little space left now. We have about a hour or so before I have to send this to Sponsored Programs. Many thanks!

From: Crowe, James <james.crowe@vumc.org>
Sent: Wednesday, June 7, 2023 7:12 AM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Larry Zeitlin <larry.zeitlin@mappbio.com>
Cc: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: RP4

I'm happy to touch it again now. As needed.
Jim

From: Broder, Christopher <christopher.broder@usuhs.edu>
Date: Wednesday, June 7, 2023 at 6:33 AM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>
Cc: Geisbert, Thomas W. <twgeisbe@utmb.edu>, Crowe, James <james.crowe@vumc.org>, Cross, Robert W. <rwcross@utmb.edu>

Subject: Re: RP4

Its nearly there
let me know if you need help this AM
now Tom

On Wed, Jun 7, 2023 at 7:24 AM Larry Zeitlin <larry.zeitlin@mappbio.com> wrote:

> Tom is active on it now…
>
> ---
>
> **From:** Larry Zeitlin <larry.zeitlin@mappbio.com>
> **Date:** Wednesday, June 7, 2023 at 7:17 AM
> **To:** Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Crowe, James <james.crowe@vumc.org>, Cross, Robert W. <rwcross@utmb.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
> **Subject:** Re: RP4
>
> I used my bag of tricks and cut it down 1 ½ pages…still ½ page to go…
>
> ---
>
> **From:** Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
> **Date:** Wednesday, June 7, 2023 at 6:21 AM
> **To:** Larry Zeitlin <larry.zeitlin@mappbio.com>, Crowe, James <james.crowe@vumc.org>, Cross, Robert W. <rwcross@utmb.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
> **Subject:** Re: RP4
>
> Thanks Jim! Larry is on it now. I will take it when he is done. Get some rest.
>
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>
> **From:** Larry Zeitlin <larry.zeitlin@mappbio.com>
> **Sent:** Wednesday, June 7, 2023 5:13 AM
> **To:** Crowe, James <james.crowe@vumc.org>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Cross, Robert W. <rwcross@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
> **Subject:** Re: RP4
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>
> I'm trimming it now…
>
> ---
>
> **From:** Crowe, James <james.crowe@vumc.org>
> **Date:** Wednesday, June 7, 2023 at 3:02 AM
> **To:** Thomas Geisbert (twgeisbe@UTMB.EDU) <twgeisbe@utmb.edu>,

Larry Zeitlin <larry.zeitlin@mappbio.com>, Robert Cross <rwcross@utmb.edu>, Chris Broder (christopher.broder@usuhs.edu) <christopher.broder@usuhs.edu>
Subject: RP4

Ok, I got all of the important pieces into this draft and cleaned it up

I need to give it a little rest.

Its 2 pages too long now

Tom, if you really reengage at ~5 am (3 hrs from now) I wont likely be up yet. So you could review it, and if you wanted to start cutting the length, that is good. There is methodologic redundancy in SA2 and SA3 that can be cut and converted to “as in SA1 above”

I can do that in the morning if no one gets to it before I get up. But is someone is in process of cutting it, let me know!

Cheers

Jim

RESEARCH STRATEGY – RP1 (Antigen Design and Testing of Arenavirus and Nairovirus Vaccines)

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) – are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness[3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocompromised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success[4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability[5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other arenaviruses and nairoviruses.

The Arenaviridae: All mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge[7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible.[9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

The *Nairoviridae*: Unique from other Bunyaviruses, orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever

which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC[20]. Like arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 7 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the virion), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine[9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation[20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface[20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge[33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control arenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g.*, immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE[34, 35] and LA[36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral

families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

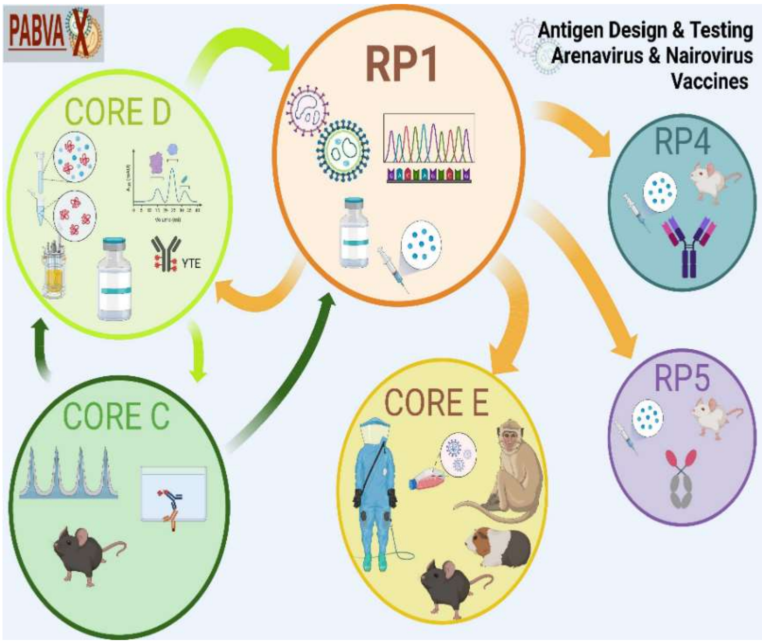


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

Preliminary Results

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant,

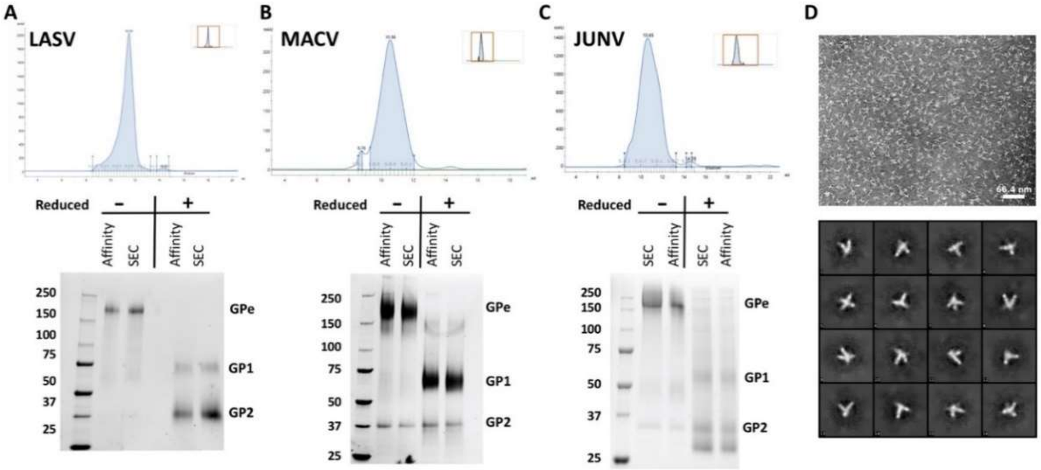


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

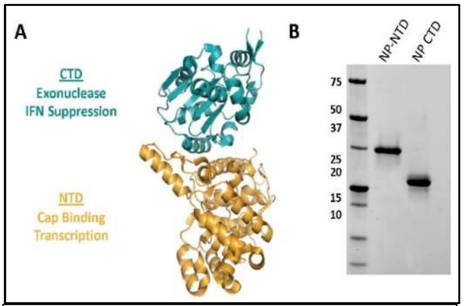
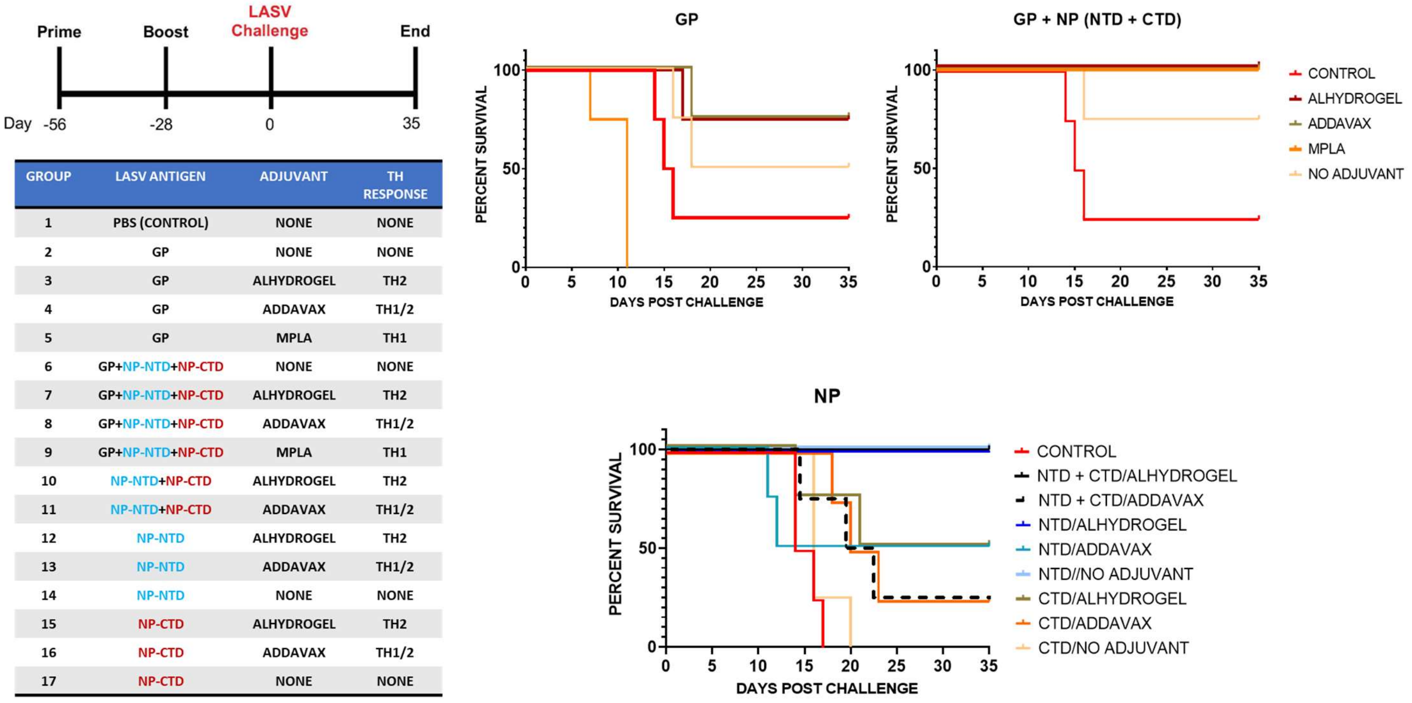


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival whereas Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

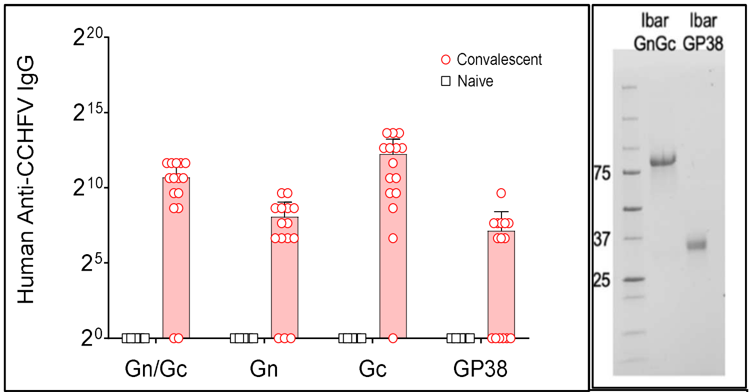


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

<u>*Engineering glycoproteins antigens for CCHFV:*</u> The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

<u>***Scientific Approach:***</u> The choice of Bunyavirales Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- <u>*Old Word Arenaviruses (OWAV):*</u> In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- <u>*New World Arenaviruses (NWAV):*</u> A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- <u>*Nairoviruses:*</u> In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A

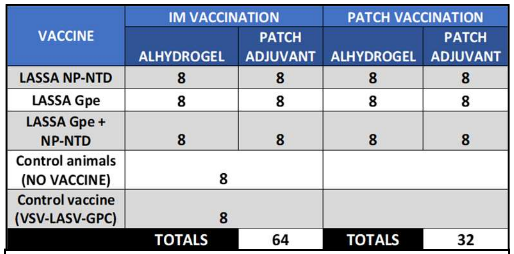
suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model[53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38. Once a candidate subunit-patch vaccine approach is selected, **RP1** will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative "test case" pathogen from each prototype virus group for which no vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV in the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat associated orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats[58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant

viruses for research and vaccine design for several different virus families[46, 61, 62] . An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.

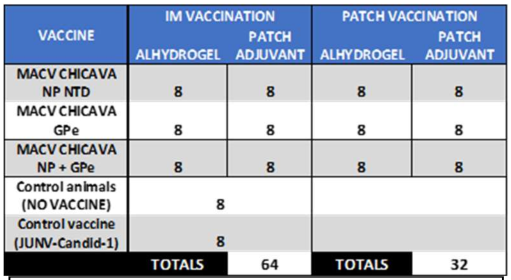


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

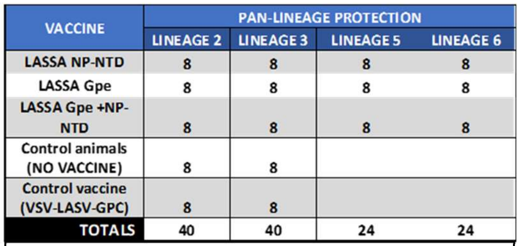


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].

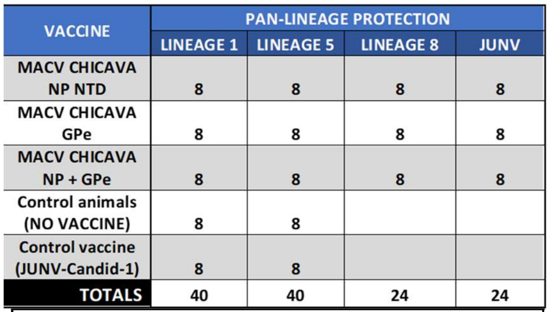
Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.C

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]**.** If Candid-1 confers protection in guinea pigs outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs[46, 47]

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of efficacy studies for MACV Milestone 1.1.2.A

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV. We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
	TOTALS	128	TOTALS	112

Table 8: Outline of efficacy studies for CCHF Milestone 1.1.2.B

Milestone 1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing STAT-1KO mice for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

Milestone 1.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. PBMCs will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for CCHF Milestone 1.1.1.C

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of efficacy studies for LUJV, CHAPV, & KASV Milestone 1.1.1.D

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10).**

***Milestone* 1.1.3: Determination of cellular and humoral immune correlates associated with protection.**

Milestone 1.1.3.A: Define the phenotype and function of arenavirus or nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols[67, 68]. PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.B: Systems serology characterization of arenavirus and nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups*:* We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument[70, 72]. Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

Milestone 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates: We will use existing mammarenaviruses[62, 73] and orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog[74] but is still pathogenic in mice and humans. There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Specific Aim 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

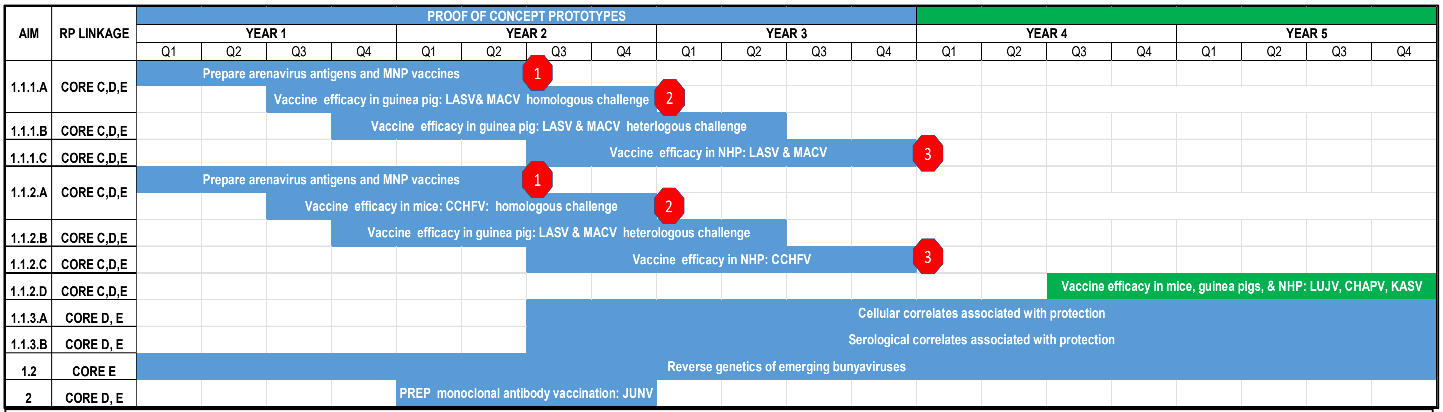
Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months[34-37].

PREP	JUNV NHP CHALLENGE
5 mg/kg	**5**
15mg/kg	**5**
Control animals (NO PREP)	**5**
TOTALS	**15**

Table 11: PREP schedule outline in Milestone 2

***Scientific Approach**: A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine:* In conjunction with **Cores D** and **E**, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11) Further** details are described in **Core E**. These data will be reviewed by Scientific Advisory Board (SAB) to aid decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the "go" criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and **SAB**. *Please note: Discussion of expected results, other alternative approaches, and criteria for "go" or "no go" decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

RESEARCH STRATEGY – RP1 (Antigen Design and Testing of Arenavirus and Nairovirus Vaccines)

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) – are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness[3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocompromised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success[4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability[5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other arenaviruses and nairoviruses.

<u>The Arenaviridae</u>**:** All mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge[7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible.[9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

<u>The *Nairoviridae*</u>: Unique from other Bunyaviruses, orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever

which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC[20]. Like arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 7 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the virion), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine[9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation[20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface[20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge[33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control arenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g.*, immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE[34, 35] and LA[36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral

families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

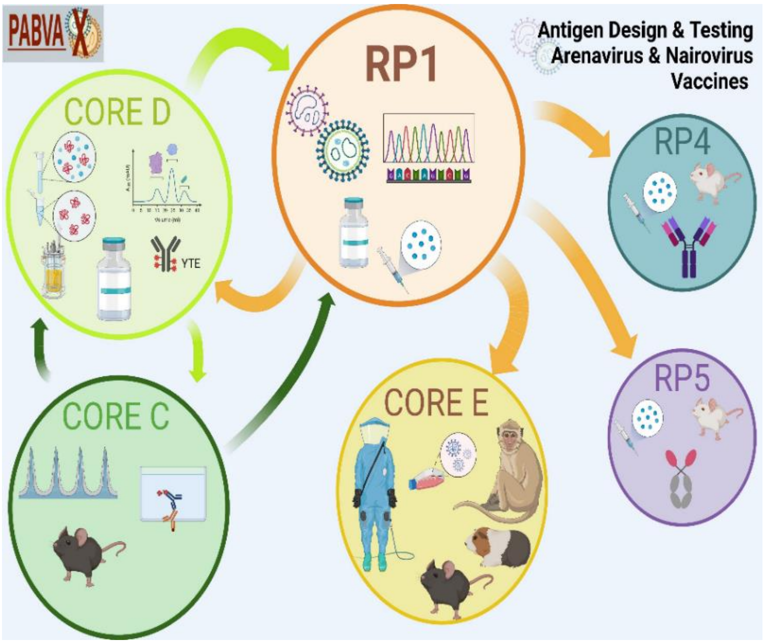


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

Preliminary Results

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant,

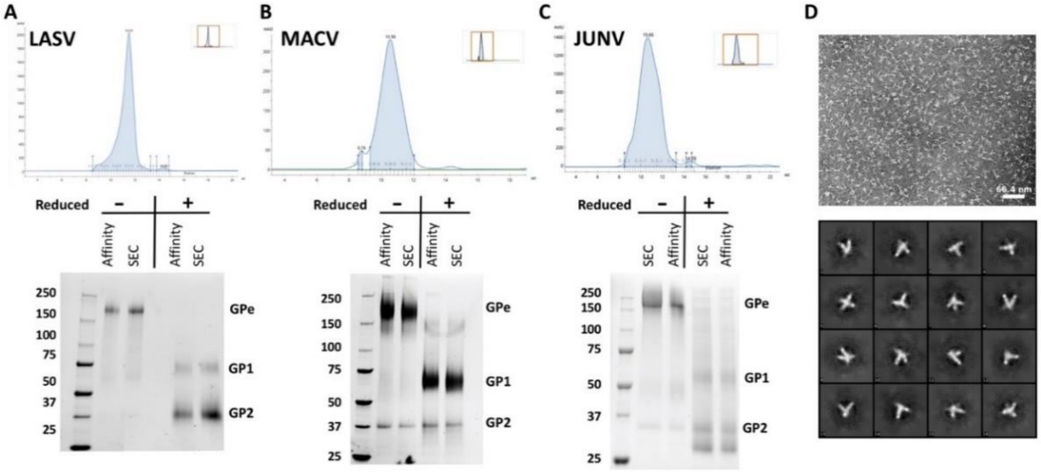


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

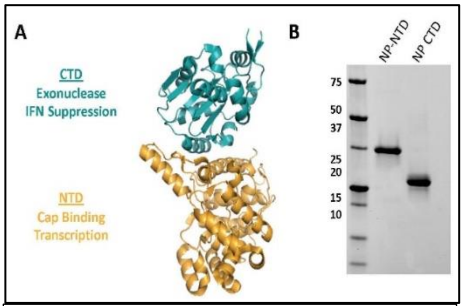
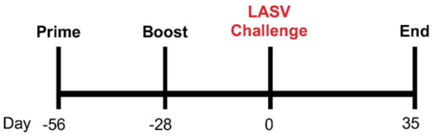


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

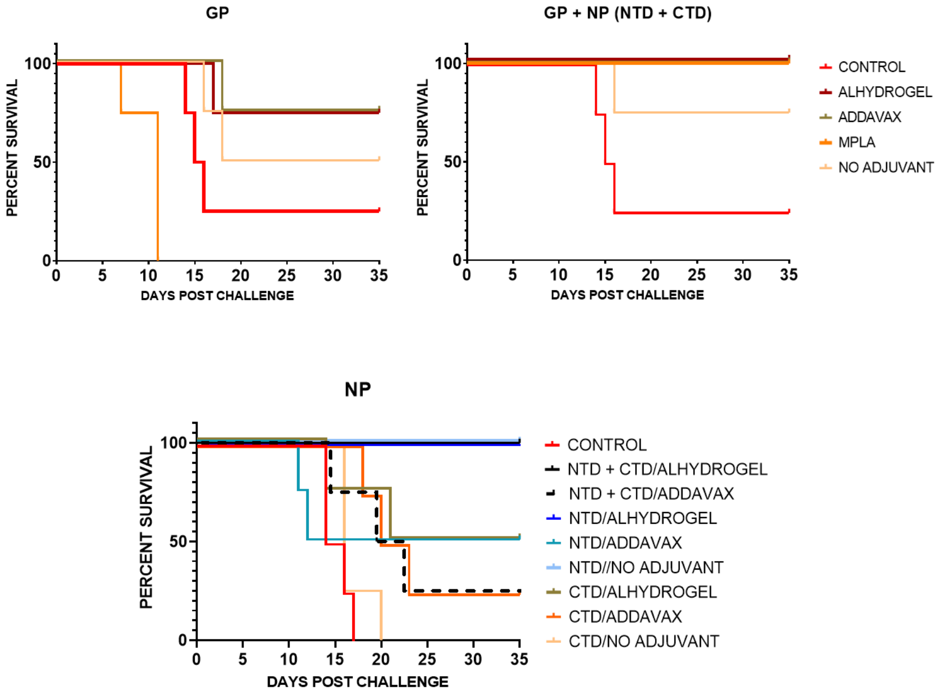


Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival whereas Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

<u>*Engineering glycoproteins antigens for CCHFV:*</u> The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

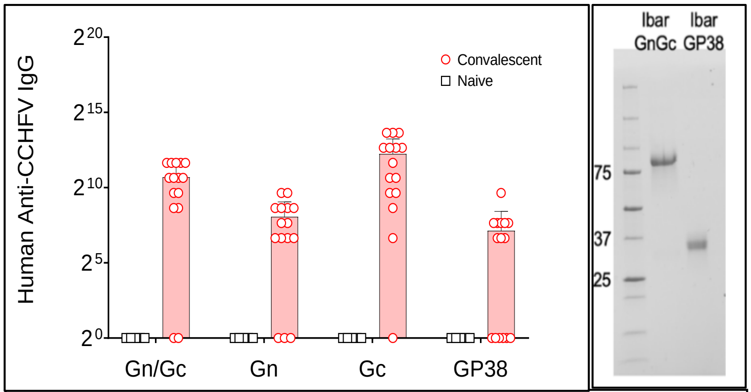


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

<u>***Scientific Approach:***</u> The choice of Bunyavirales Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- <u>*Old Word Arenaviruses (OWAV):*</u> In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- <u>*New World Arenaviruses (NWAV):*</u> A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- <u>*Nairoviruses:*</u> In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A

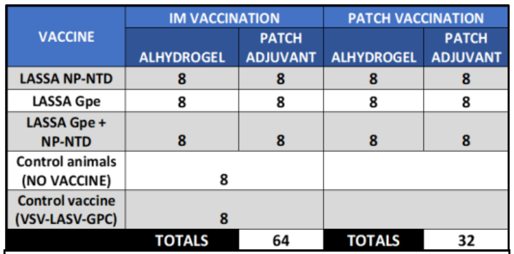
suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model[53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38. Once a candidate subunit-patch vaccine approach is selected, **RP1** will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative "test case" pathogen from each prototype virus group for which no vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV in the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat associated orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats[58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant

viruses for research and vaccine design for several different virus families[46, 61, 62]. An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.



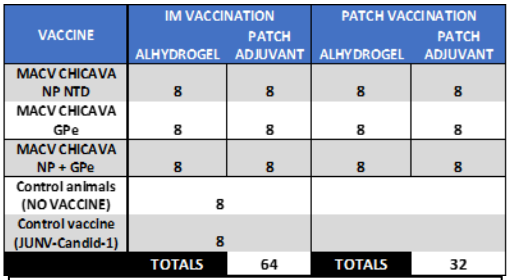
VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
TOTALS	64		TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

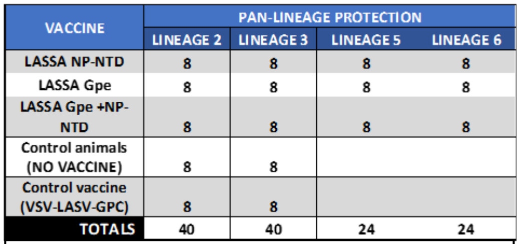
Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].



VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
TOTALS	64		TOTALS	32

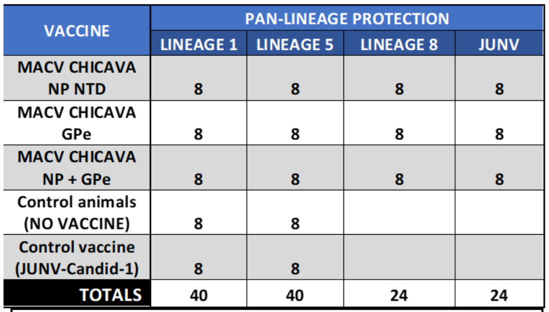
Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but

distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.C

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]**.** If Candid-1 confers protection in guinea pigs outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs[46, 47]

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV. We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of efficacy studies for MACV Milestone 1.1.2.A

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
	TOTALS	128	TOTALS	112

Table 8: Outline of efficacy studies for CCHF Milestone 1.1.2.B

Milestone 1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing STAT-1KO mice for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

Milestone 1.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. PBMCs will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for CCHF Milestone 1.1.1.C

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of efficacy studies for LUJV, CHAPV, & KASV Milestone 1.1.1.D

***Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP:* RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10).**

***Milestone* 1.1.3: Determination of cellular and humoral immune correlates associated with protection.**

Milestone 1.1.3.A: Define the phenotype and function of arenavirus or nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols[67, 68]. PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.B: Systems serology characterization of arenavirus and nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument[70, 72]. Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

Milestone 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates: We will use existing mammarenaviruses[62, 73] and orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog[74] but is still pathogenic in mice and humans. There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Specific Aim 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

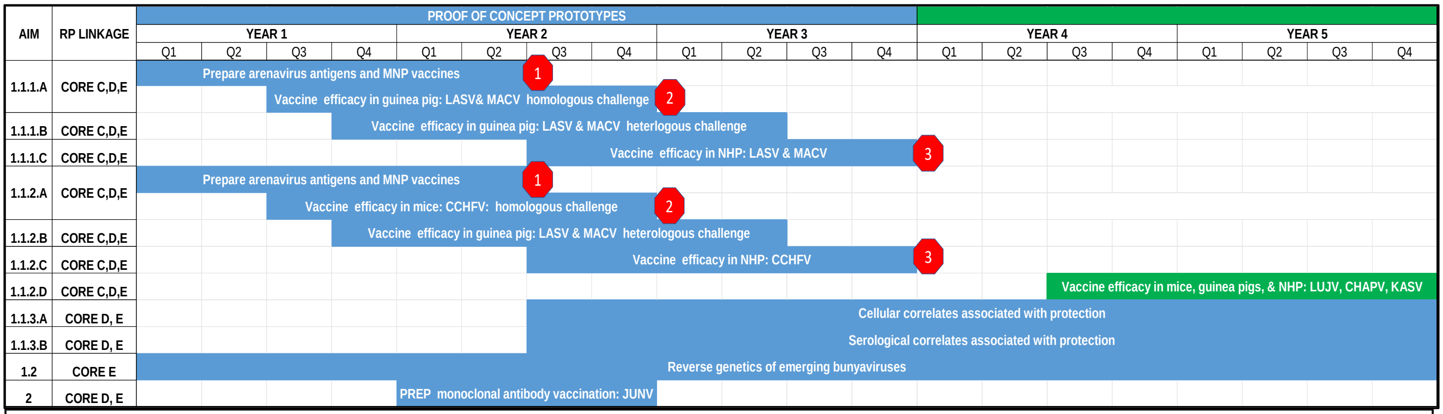
Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months[34-37].

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

Scientific Approach: *A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine:* In conjunction with **Cores D** and **E**, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11) Further** details are described in **Core E**. These data will be reviewed by Scientific Advisory Board (SAB) to aid decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the "go" criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and **SAB**. *Please note: Discussion of expected results, other alternative approaches, and criteria for "go" or "no go" decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

RESEARCH STRATEGY – RP1 (Antigen Design and Testing o~~O~~f Arenavirus ~~A~~and Nairovirus Vaccines)

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) – are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness[3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocompromised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success[4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability[5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other arenaviruses and nairoviruses.

<u>The Arenaviridae</u>**:** All mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge[7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible.[9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

<u>The *Nairoviridae*</u><u>:</u> Unique from other Bunyaviruses, orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever

which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC[20]. Like arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the virion), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine[9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation[20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface[20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge[33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP “vaccination” as a measure to control arenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g*., immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE[34, 35] and LA[36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral

families within **Bunyavirales** **(Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

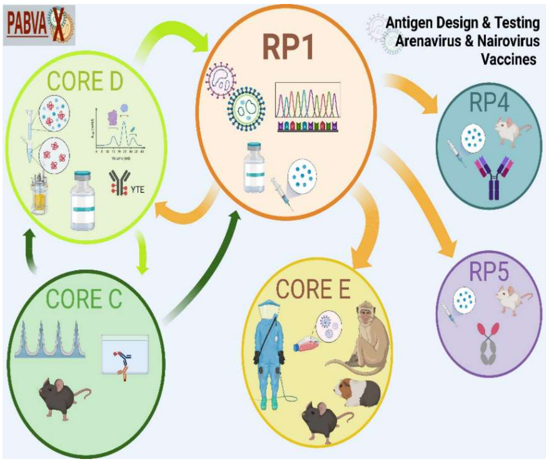


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

Preliminary Results

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the

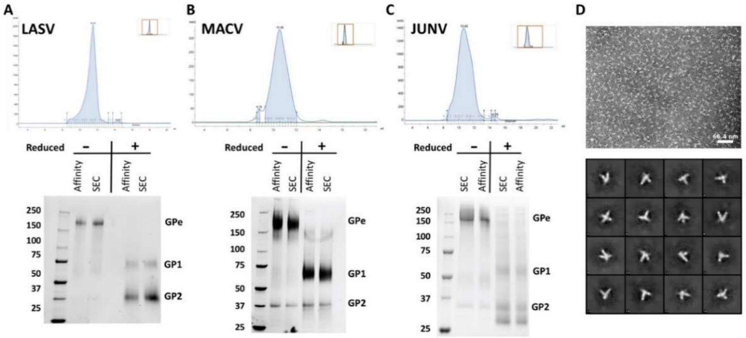


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

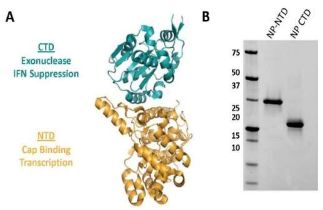
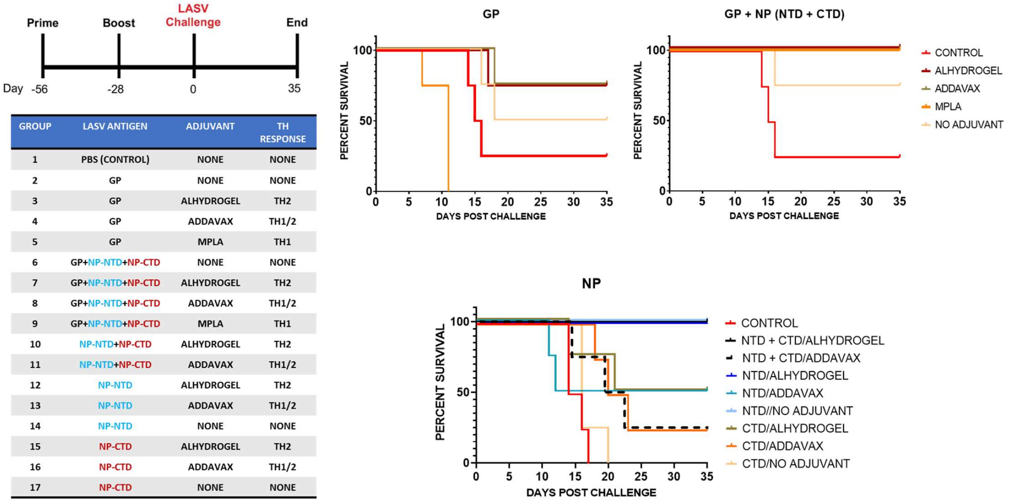


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

<u>LASV subunit vaccine protects guinea pigs against lethal challenge</u>

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival whereas Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

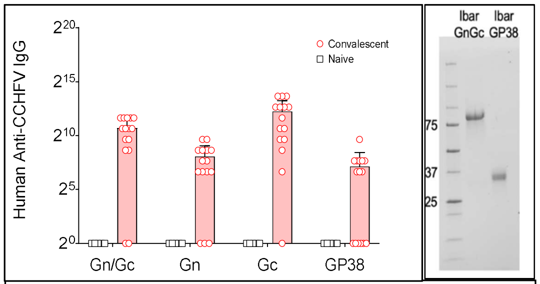


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

Engineering glycoproteins antigens for CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

Scientific Approach: The choice of Bunyavirales Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Arenaviruses (OWAV):* In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Arenaviruses (NWAV):* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Nairoviruses:* In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A

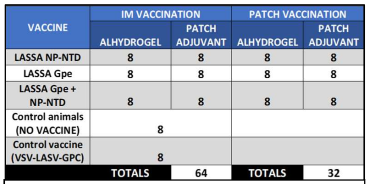
suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model[53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38. Once a candidate subunit-patch vaccine approach is selected, **RP1** will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative "test case" pathogen from each prototype virus group for which no vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV in the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat associated orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats[58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant

viruses for research and vaccine design for several different virus families[46, 61, 62] . An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.

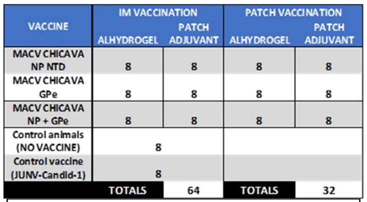


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

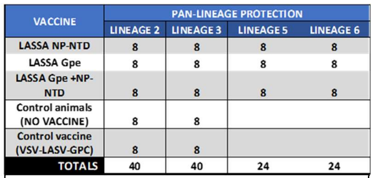


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

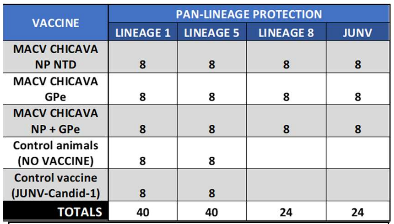
Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but

distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.CB

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]. If Candid-1 confers protection in guinea pigs outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs[46, 47]

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of ~~NHP~~ efficacy studies for MACV Milestone

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV. We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
	TOTALS	128	TOTALS	112

Table 8: Outline of ~~NHP~~ efficacy studies for ~~MACV~~ CCHF Milestone 1.1.2~~1~~.B

Milestone 1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-

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Commented [SSJ2]: Table 7 for mice milestone 1.1.2.A-legend for NHP 1.1.1B

1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing ~~optimized strain 13 or outbred, Hartley guinea pig models~~STAT-1KO mice for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

Milestone ~~2~~1.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. PBMCs will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for CCHF~~MACV~~ Milestone 1.1.1.~~B~~C

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10).** **RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10).**

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of ~~NHP~~ efficacy studies for ~~MACV~~ LUJV, CHAPV, & KASV

Milestone 1.1.3: Determination of cellular and humoral immune correlates associated with protection.

Milestone 1.1.3.A: Define the phenotype and function of arenavirus or nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

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Commented [SSJ6]: Table 10 for mice/GP/NHP; Milestone 1.1.2.D-legend for NHP 1.1.1.B

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols[67, 68]. PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37ºC for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37ºC. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37ºC. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4ºC or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37ºC), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.~~A~~B:~~:~~ Systems serology characterization of arenavirus and nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups*:* We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument[70, 72]. Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

Milestone 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates: We will use existing mammarenaviruses[62, 73] and orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog[74] but is still pathogenic in mice and humans. There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

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~~Milestone~~ Specific Aim 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

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Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months[34-37].

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

Scientific Approach:

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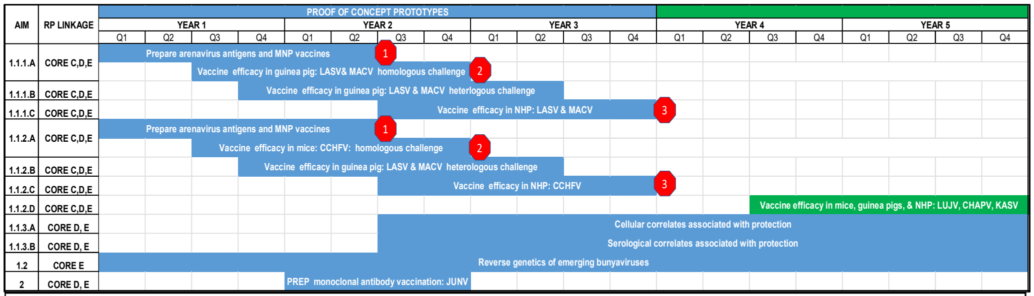
A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine: In conjunction with **Cores D** and **E**, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11) Further** details are described in **Core E**. These data will be reviewed by Scientific Advisory Board (SAB) to aid decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three “test case” viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the “go” criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and **SAB**. *Please note: Discussion of expected results, other alternative approaches, and criteria for “go” or “no go” decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future “plug and play” pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

RESEARCH STRATEGY:

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) – are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness[3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocompromised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success[4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability[5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other arenaviruses and nairoviruses.

<u>The Arenaviridae</u>: All mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge[7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible.[9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

<u>The *Nairoviridae*</u>: Unique from other Bunyaviruses, orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited

efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC[20]. Like arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the virion), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine[9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation[20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface[20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge[33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control arenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g.*, immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE[34, 35] and LA[36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families.

These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

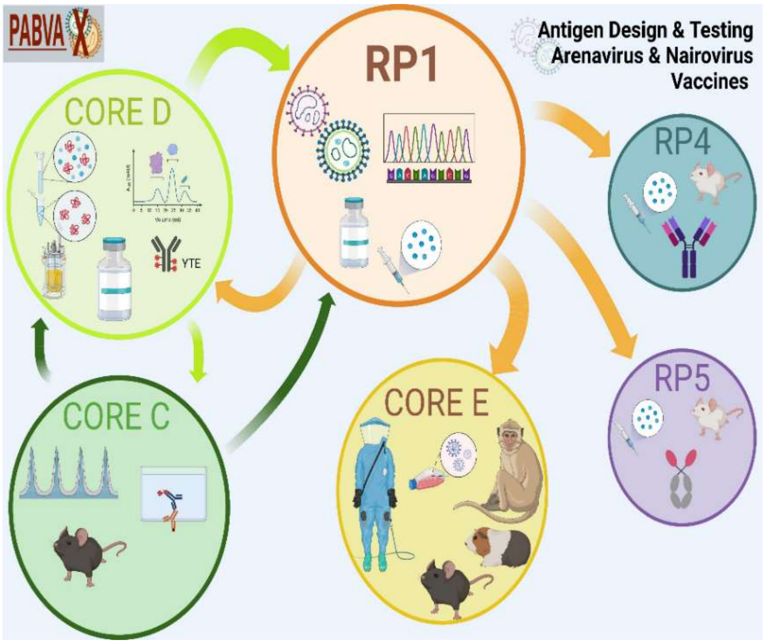


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

<u>Preliminary Results</u>

<u>Engineering trimeric glycoprotein antigens for the arenaviruses</u>

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE

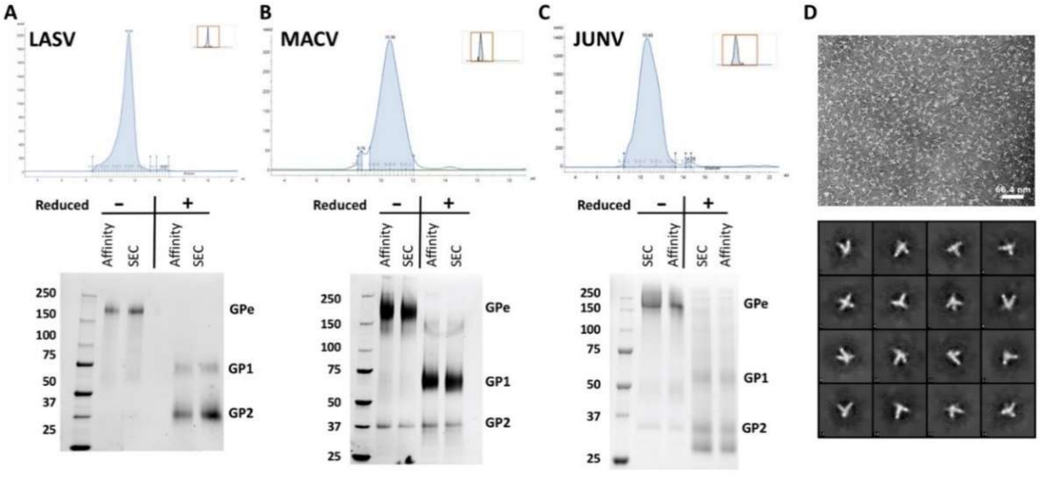


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

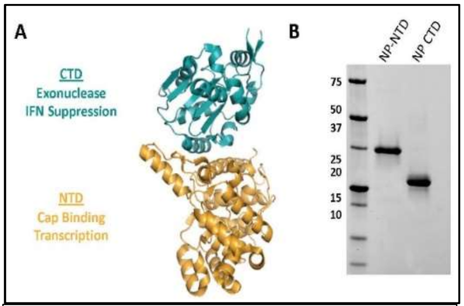
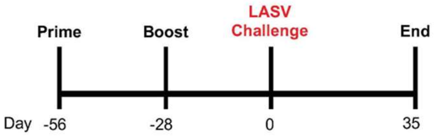


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were**



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

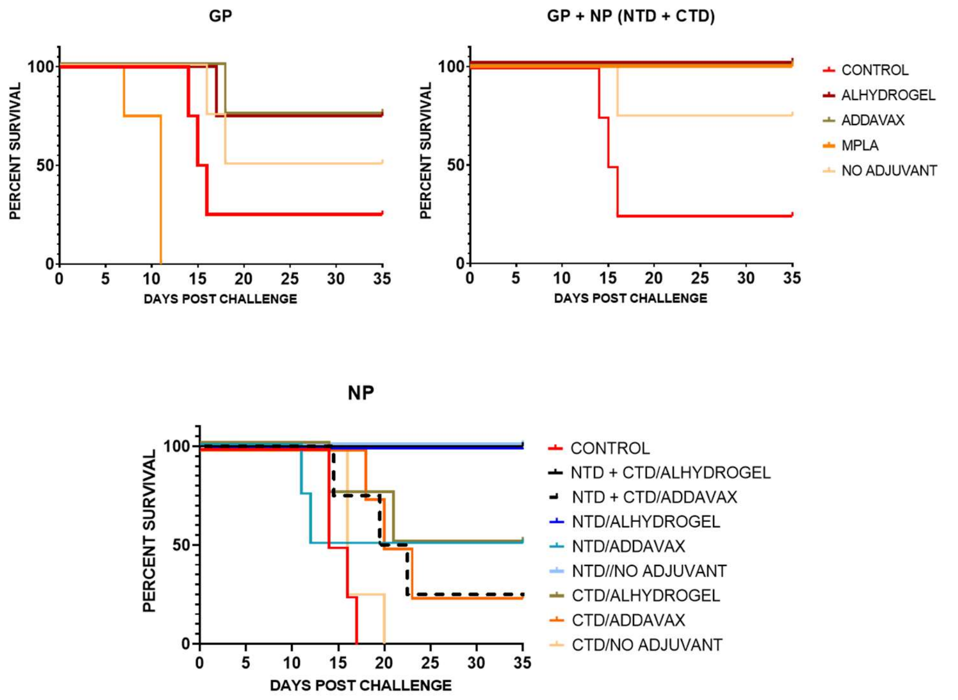


Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV. Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival whereas Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

Engineering glycoproteins antigens for CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

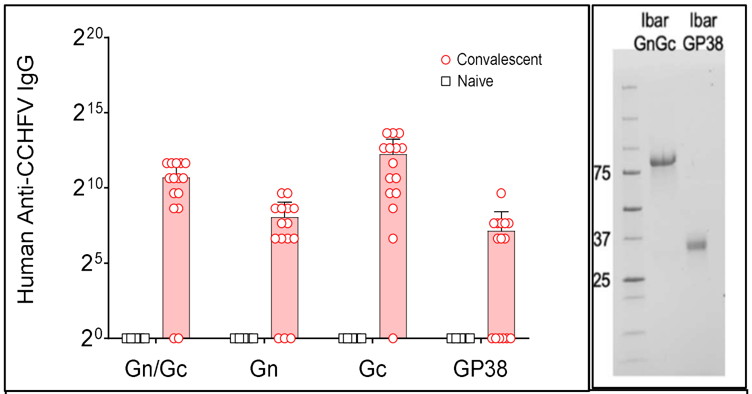


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

Scientific Approach: The choice of Bunyavirales Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Arenaviruses (OWAV):* In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Arenaviruses (NWAV):* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Nairoviruses:* In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying

efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model[53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38. Once a candidate subunit-patch vaccine approach is selected, **RP1** will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative "test case" pathogen from each prototype virus group for which no vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV in the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat associated orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats[58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families[46, 61, 62] . An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared

with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve. Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.B

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]**.** If Candid-1 confers protection in guinea pigs outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs[46, 47]

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
	TOTALS	128	TOTALS	112

Table 8: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

Milestone 2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. PBMCs will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10).**

Milestone **1.1.3: Determination of cellular and humoral immune correlates associated with protection.**

Milestone 1.1.3.A: Define the phenotype and function of arenavirus or nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

<u>*Immune subset phenotyping*</u>**:** We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell

phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols[67, 68]. PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.A:: Systems serology characterization of arenavirus and nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups*:* We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the

GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument[70, 72]. Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

Milestone **1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing mammarenaviruses[62, 73] and orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog[74] but is still pathogenic in mice and humans. There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months[34-37].

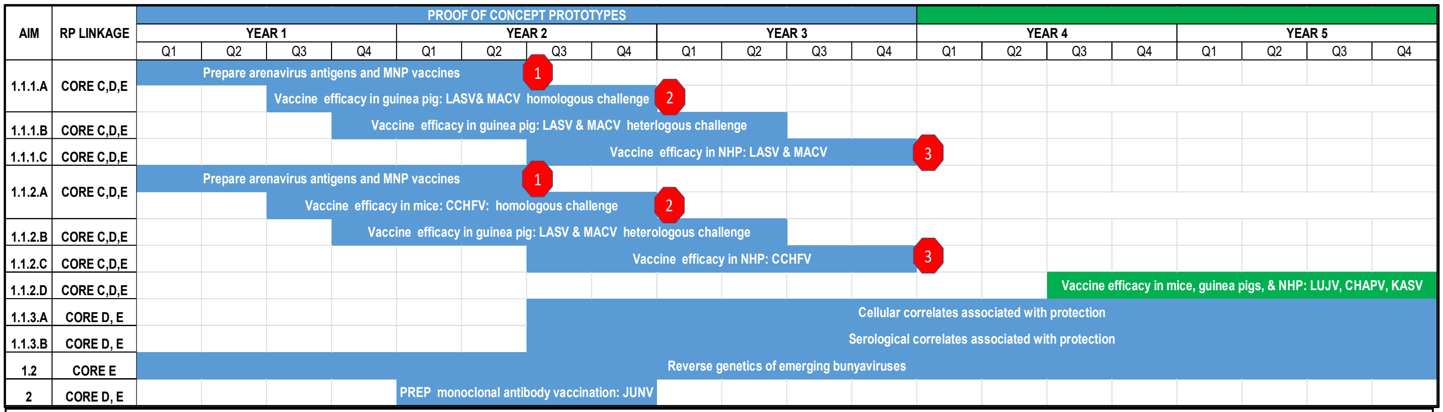
PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

Scientific Approach:

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine: In conjunction with **Cores D** and **E**, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11) Further** details are described in **Core E**. These data will be reviewed by Scientific Advisory Board (SAB) to aid decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the "go" criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and **SAB**. *Please note: Discussion of expected results, other alternative approaches, and criteria for "go" or "no go" decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*



AIM	RP LINKAGE	PROOF OF CONCEPT PROTOTYPES																			
		YEAR 1				YEAR 2				YEAR 3				YEAR 4				YEAR 5			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1.1.1.A	CORE C,D,E	Prepare arenavirus antigens and MNP vaccines						1													
				Vaccine efficacy in guinea pig: LASV& MACV homologous challenge						2											
1.1.1.B	CORE C,D,E				Vaccine efficacy in guinea pig: LASV & MACV heterlogous challenge																
1.1.1.C	CORE C,D,E							Vaccine efficacy in NHP: LASV & MACV						3							
1.1.2.A	CORE C,D,E	Prepare arenavirus antigens and MNP vaccines						1													
				Vaccine efficacy in mice: CCHFV: homologous challenge						2											
1.1.2.B	CORE C,D,E				Vaccine efficacy in guinea pig: LASV & MACV heterologous challenge																
1.1.2.C	CORE C,D,E							Vaccine efficacy in NHP: CCHFV						3							
1.1.2.D	CORE C,D,E															Vaccine efficacy in mice, guinea pigs, & NHP: LUJV, CHAPV, KASV					
1.1.3.A	CORE D, E							Cellular correlates associated with protection													
1.1.3.B	CORE D, E							Serological correlates associated with protection													
1.2	CORE E	Reverse genetics of emerging bunyaviruses																			
2	CORE D, E					PREP monoclonal antibody vaccination: JUNV															

RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

References:

1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.
2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.
5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.
6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.
8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.
9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.
10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia I, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.
11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.
12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.
13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.

16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.

17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.

18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.

19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.

20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.

21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.

22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.

23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 2019/05/28. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.

24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.

25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.

26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.

27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.

28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.

29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.

30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF. Lassa fever in post-conflict sierra leone. PLoS Negl Trop Dis. 2014;8(3):e2748. Epub 20140320. doi: 10.1371/journal.pntd.0002748. PubMed PMID: 24651047; PMCID: PMC3961205.

43. Li AL, Grant D, Gbakie M, Kanneh L, Mustafa I, Bond N, Engel E, Schieffelin J, Vandy MJ, Yeh S, Shantha JG. Ophthalmic manifestations and vision impairment in Lassa fever survivors. PLoS One. 2020;15(12):e0243766. doi: 10.1371/journal.pone.0243766.

44. Ezeomah C, Adoga A, Ihekweazu C, Paessler S, Cisneros I, Tomori O, Walker D. Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. Open Forum Infect Dis. 2019;6(12):ofz512. Epub 20191203. doi: 10.1093/ofid/ofz512. PubMed PMID: 31879673; PMCID: PMC6923636.

45. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

46. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.

47. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.

48. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

49. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.

50. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.

51. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.

52. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.

53. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.

54. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.

55. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.

56. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.

57. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.

58. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.

59. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.

60. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.

61. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

62. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

63. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

64. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

65. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

66. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

67. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods. 2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

68. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

69. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

70. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

71. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

72. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

73. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

74. Walker PJ, Widen SG, Firth C, Blasdell KR, Wood TG, Travassos da Rosa AP, Guzman H, Tesh RB, Vasilakis N. Genomic Characterization of Yogue, Kasokero, Issyk-Kul, Keterah, Gossas, and Thiafora Viruses: Nairoviruses Naturally Infecting Bats, Shrews, and Ticks. Am J Trop Med Hyg. 2015;93(5):1041-51. Epub 20150831. doi: 10.4269/ajtmh.15-0344. PubMed PMID: 26324724; PMCID: PMC4703270.

75. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

76. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D,

Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

RESEARCH STRATEGY:

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) – are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness[3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocompromised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success[4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability[5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other arenaviruses and nairoviruses.

***The Arenaviridae*:** All mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge[7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible.[9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

The *Nairoviridae*: Unique from other Bunyaviruses, orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited

efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC[20]. Like arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the virion), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine[9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation[20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface[20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge[33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control arenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g.*, immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE[34, 35] and LA[36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families.

These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

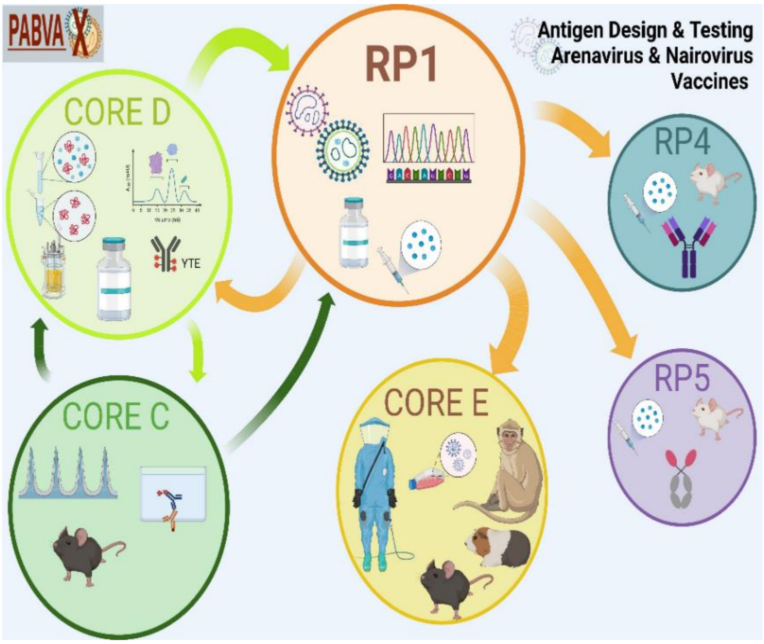


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

Preliminary Results

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE

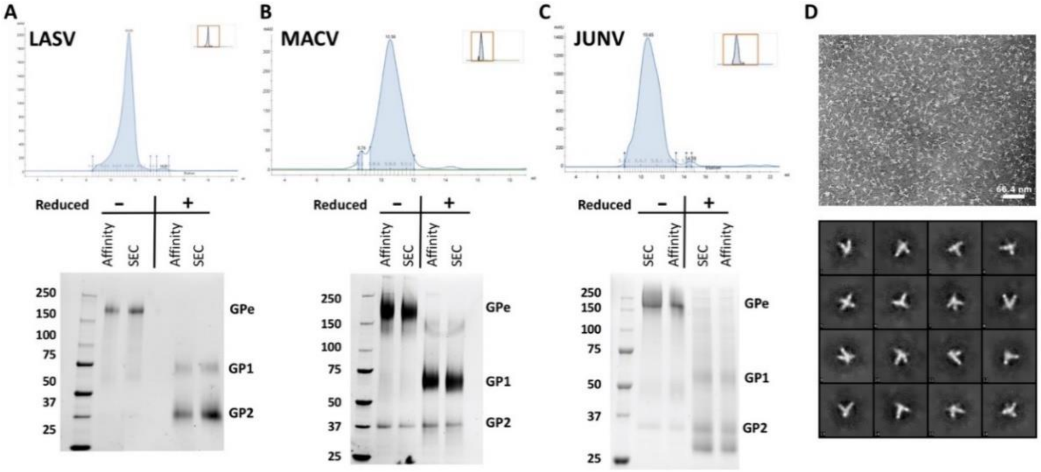


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

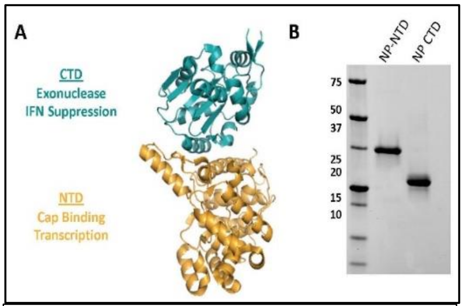
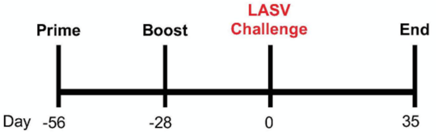


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were**



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

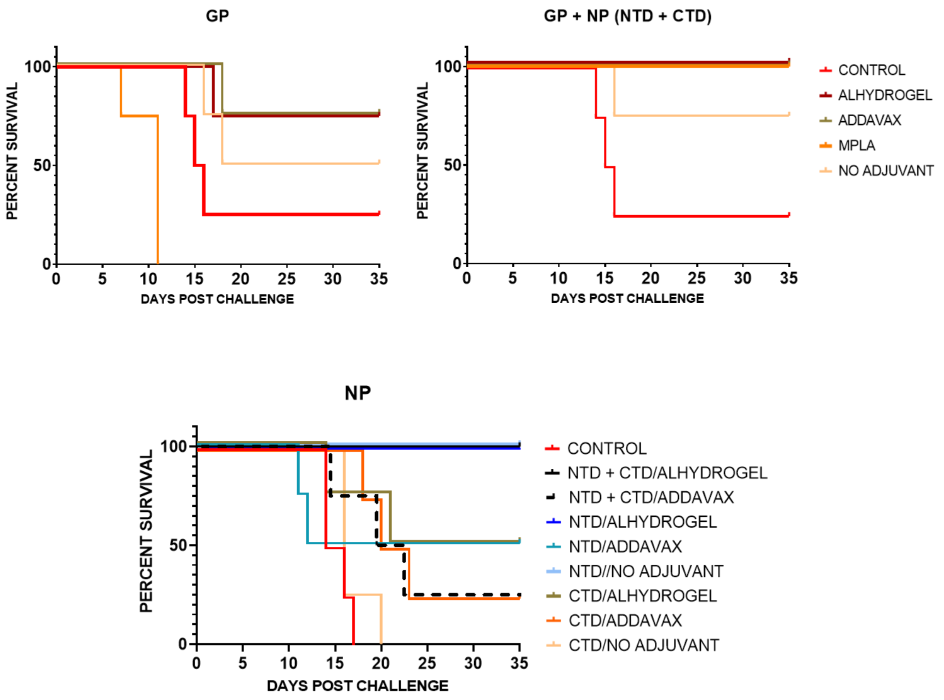


Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 μg of GP, or 2x50 μg doses of GP + 2x25μg of NP-NTD + 2x25 μg of NP-CTD, or either 2x25 μg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV. Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival whereas Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

Engineering glycoproteins antigens for CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

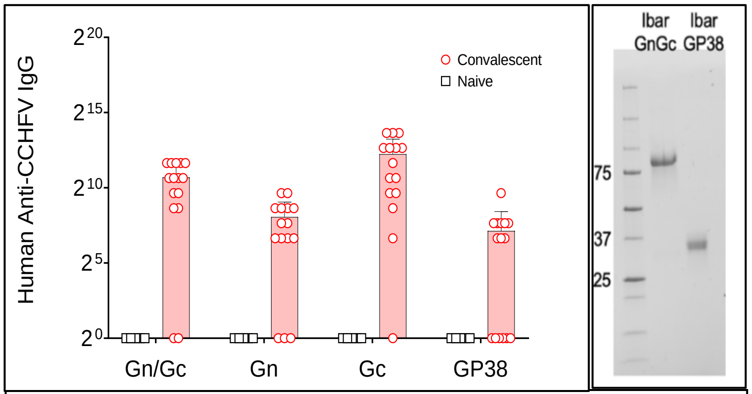


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

Scientific Approach: The choice of Bunyavirales Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Arenaviruses (OWAV):* In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Arenaviruses (NWAV):* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Nairoviruses:* In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying

efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model[53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38. Once a candidate subunit-patch vaccine approach is selected, **RP1** will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative "test case" pathogen from each prototype virus group for which no vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV in the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat associated orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats[58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

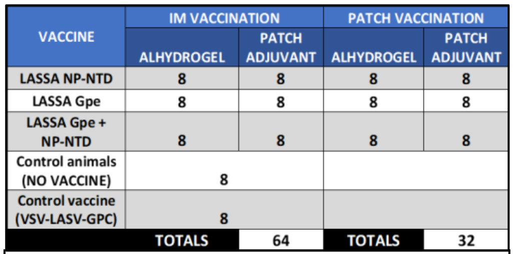
Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families[46, 61, 62] . An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared

with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.

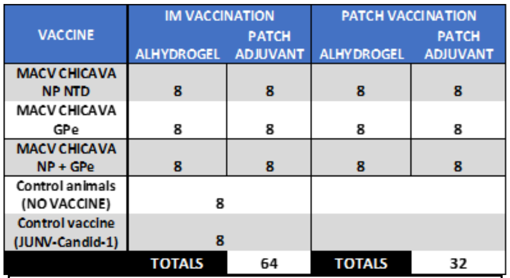
Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve. Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].



VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
TOTALS	64		TOTALS	32

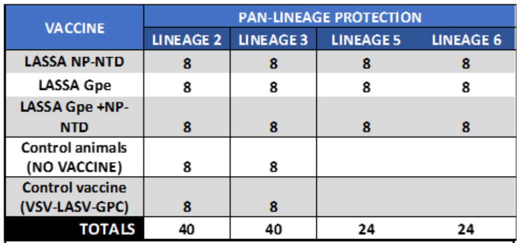
Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A



VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
TOTALS	64		TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

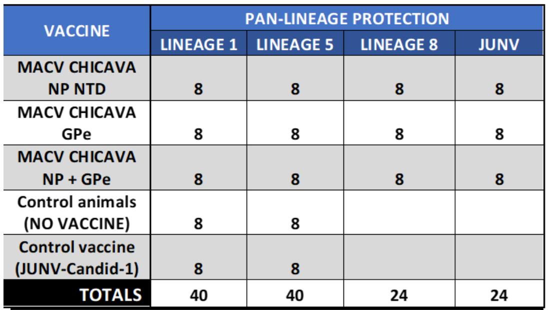
Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]**.** If Candid-1 confers protection in guinea pigs outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs[46, 47]

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.B

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
	TOTALS	128	TOTALS	112

Table 8: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

Milestone 2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. PBMCs will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10).**

***Milestone* 1.1.3: Determination of cellular and humoral immune correlates associated with protection.**

Milestone 1.1.3.A: Define the phenotype and function of arenavirus or nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols[67, 68]. PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.A:: Systems serology characterization of arenavirus and nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups*:* We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the

GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument[70, 72]. Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

***Milestone* 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing mammarenaviruses[62, 73] and orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog[74] but is still pathogenic in mice and humans. There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months[34-37].

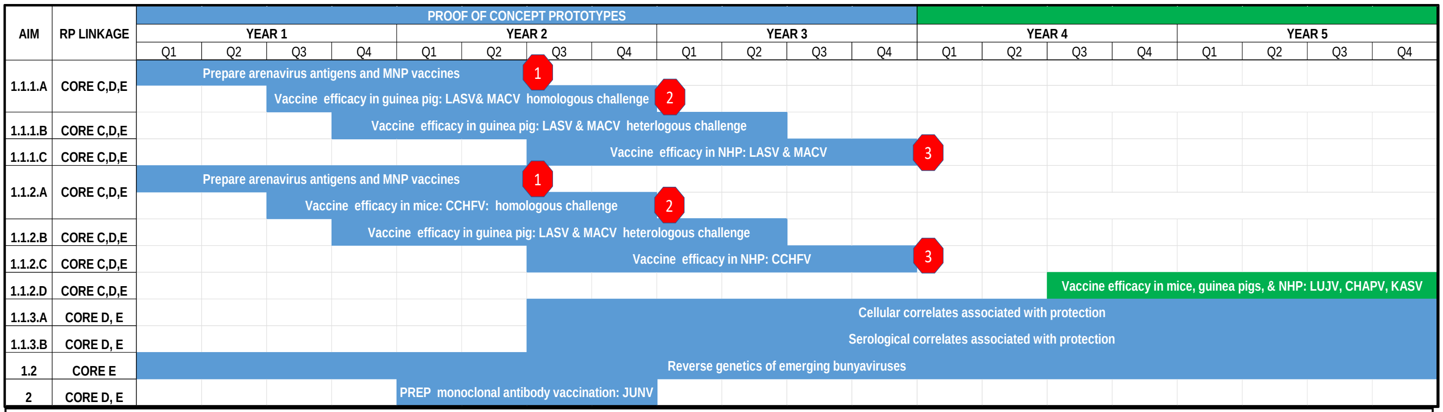
PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

Scientific Approach:

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine: In conjunction with **Cores D** and **E**, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11) Further** details are described in **Core E**. These data will be reviewed by Scientific Advisory Board (SAB) to aid decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the "go" criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and **SAB**. *Please note: Discussion of expected results, other alternative approaches, and criteria for "go" or "no go" decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*



AIM	RP LINKAGE	PROOF OF CONCEPT PROTOTYPES
1.1.1.A	CORE C,D,E	Prepare arenavirus antigens and MNP vaccines 1 Vaccine efficacy in guinea pig: LASV& MACV homologous challenge 2
1.1.1.B	CORE C,D,E	Vaccine efficacy in guinea pig: LASV & MACV heterlogous challenge
1.1.1.C	CORE C,D,E	Vaccine efficacy in NHP: LASV & MACV 3
1.1.2.A	CORE C,D,E	Prepare arenavirus antigens and MNP vaccines 1 Vaccine efficacy in mice: CCHFV: homologous challenge 2
1.1.2.B	CORE C,D,E	Vaccine efficacy in guinea pig: LASV & MACV heterologous challenge
1.1.2.C	CORE C,D,E	Vaccine efficacy in NHP: CCHFV 3
1.1.2.D	CORE C,D,E	Vaccine efficacy in mice, guinea pigs, & NHP: LUJV, CHAPV, KASV
1.1.3.A	CORE D, E	Cellular correlates associated with protection
1.1.3.B	CORE D, E	Serological correlates associated with protection
1.2	CORE E	Reverse genetics of emerging bunyaviruses
2	CORE D, E	PREP monoclonal antibody vaccination: JUNV

RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

References:

1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.
2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.
5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.
6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.
8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.
9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.
10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia l, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.
11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.
12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.
13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.

16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.

17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.

18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.

19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.

20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.

21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.

22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.

23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 2019/05/28. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.

24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.

25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.

26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.

27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.

28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.

29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.

30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF. Lassa fever in post-conflict sierra leone. PLoS Negl Trop Dis. 2014;8(3):e2748. Epub 20140320. doi: 10.1371/journal.pntd.0002748. PubMed PMID: 24651047; PMCID: PMC3961205.

43. Li AL, Grant D, Gbakie M, Kanneh L, Mustafa I, Bond N, Engel E, Schieffelin J, Vandy MJ, Yeh S, Shantha JG. Ophthalmic manifestations and vision impairment in Lassa fever survivors. PLoS One. 2020;15(12):e0243766. doi: 10.1371/journal.pone.0243766.

44. Ezeomah C, Adoga A, Ihekweazu C, Paessler S, Cisneros I, Tomori O, Walker D. Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. Open Forum Infect Dis. 2019;6(12):ofz512. Epub 20191203. doi: 10.1093/ofid/ofz512. PubMed PMID: 31879673; PMCID: PMC6923636.

45. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

46. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.
47. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.
48. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.
49. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.
50. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.
51. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.
52. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.
53. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.
54. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.
55. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.
56. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.
57. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.
58. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.
59. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.
60. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.
61. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

62. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

63. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

64. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

65. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

66. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

67. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods. 2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

68. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

69. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

70. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

71. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

72. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

73. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

74. Walker PJ, Widen SG, Firth C, Blasdell KR, Wood TG, Travassos da Rosa AP, Guzman H, Tesh RB, Vasilakis N. Genomic Characterization of Yogue, Kasokero, Issyk-Kul, Keterah, Gossas, and Thiafora Viruses: Nairoviruses Naturally Infecting Bats, Shrews, and Ticks. Am J Trop Med Hyg. 2015;93(5):1041-51. Epub 20150831. doi: 10.4269/ajtmh.15-0344. PubMed PMID: 26324724; PMCID: PMC4703270.

75. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

76. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D,

Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

RESEARCH STRATEGY:

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) – are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness[3]. Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success[4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability[5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other Arenaviruses and Nairoviruses.

<u>The Arenaviridae</u>**:** All Mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge[7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible.[9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

<u>The *Nairoviridae*</u><u>:</u> Unique from other Bunyaviruses, Orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic Orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited

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efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC[20]. Like Arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine ~~in active development by Inovio Pharmaceuticals~~. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the ~~surface of arenavirus~~virion), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine[9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

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Unique Nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation[20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface[20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge[33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control Arenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g.*, immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE[34, 35] and LA[36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will

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be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select Mammarenaviruses and Orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

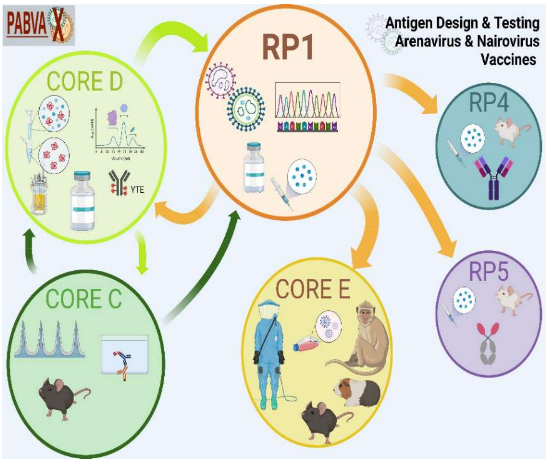

Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

~~PRELIMINARY RESULTS~~

Preliminary Results

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to

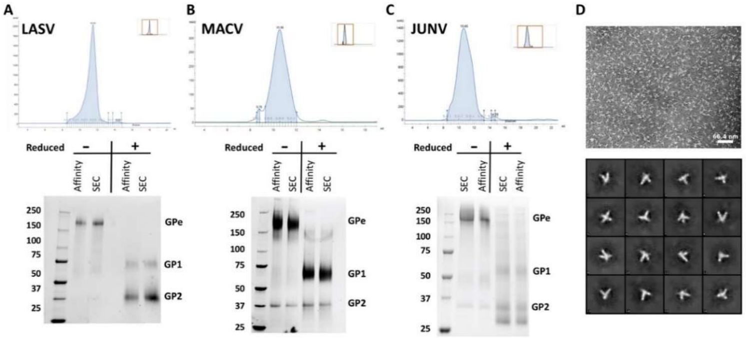

Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

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stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

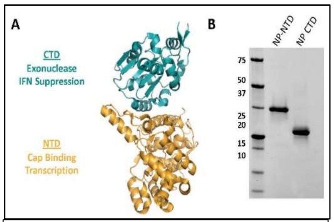
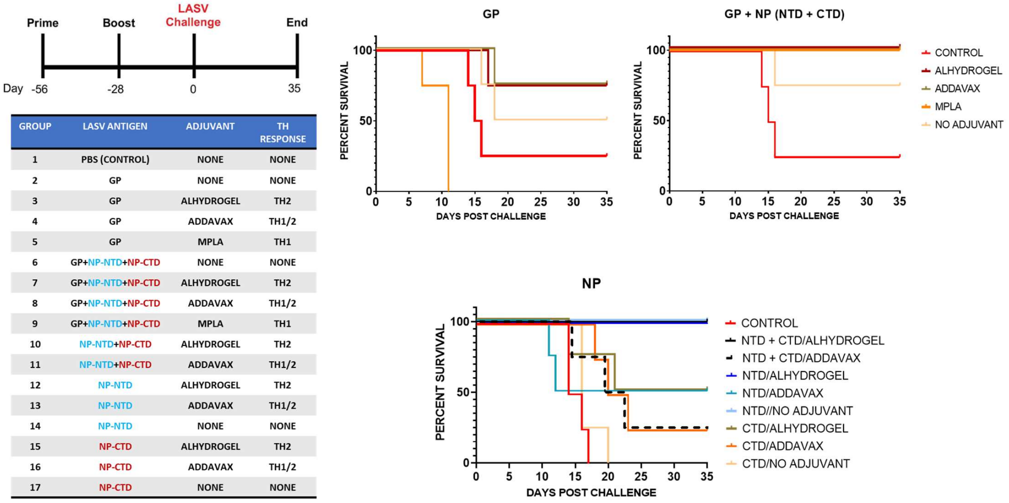


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

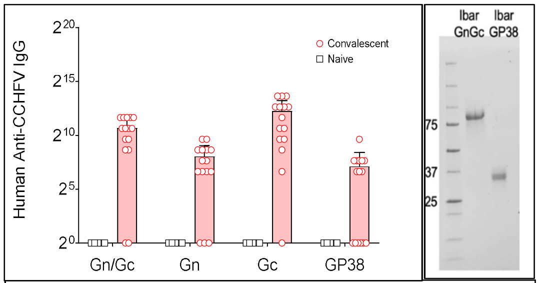


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

__Engineering glycoproteins antigens for CCHFV:__ The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

__Scientific Approach:__ The choice of Arenavirus Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *__Old Word Arenaviruses (OWAV):__* In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *__New World Arenaviruses (NWAV):__* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *__Nairoviruses:__* In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in

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Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model[53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38.

- Once a candidate subunit-patch vaccine approach is selected, ~~this~~ **RP1** will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative~~,~~ "test case" ~~human~~ pathogen from each prototype virus group for which no ~~research grade~~ vaccines have been created and tested in animal models.

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- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne Mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV ~~as part of~~in the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats[58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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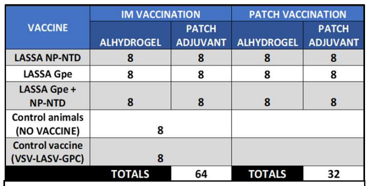
Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families-[46, 61, 62] . An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.

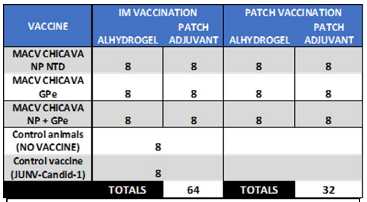


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

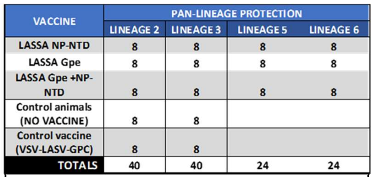


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), ~~all~~ animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after ~~LASV~~ challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]**.** If Candid-1 confers protection in guinea pigs ~~study~~ outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs[46, 47]

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.B

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
TOTALS		128	TOTALS	112

Table 8: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. ~~Peripheral blood mononuclear cells (~~PBMCs~~)~~ will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10)**

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***Milestone* 1.1.3: Determination of cellular and humoral immune correlates associated with protection.**

Milestone 1.1.3.A: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

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Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences~~, Franklin Lakes, NJ~~) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols~~.~~[67, 68]. PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative ~~(BD Biosciences, Franklin Lakes, NJ~~) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.A:: Systems serology Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups*:* We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

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Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument~~.~~[70, 72]. Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

***Milestone* 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing Mammarenaviruses[62, 73] and Orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog[74] but is still pathogenic in mice and humans. There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

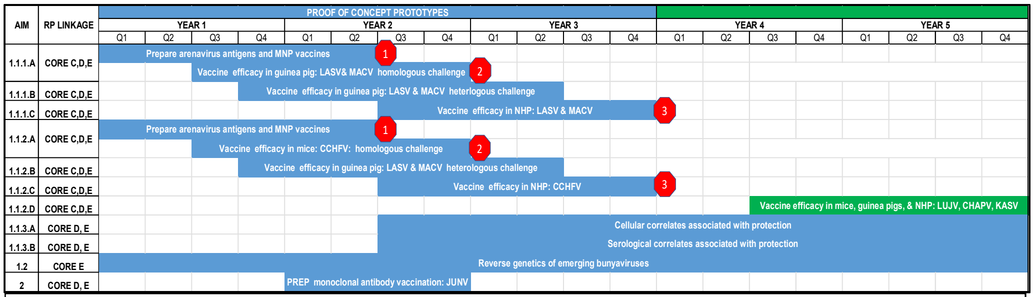
Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months~~(~~[34-37]~~)~~.

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

Scientific Approach: *A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine:* In conjunction with **Cores D** and **E**, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11)**~~.~~ ~~For f~~Further details ~~of the NHP testing, please~~are described in~~ see~~ **Core E**. These data will be ~~used by the~~reviewed by Scientific Advisory Board (SAB) to ~~inform~~ aid decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the "go" criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and SAC. *Please note: Discussion of expected results, other alternative approaches, and criteria for "go" or "no go" decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*



AIM	RP LINKAGE	PROOF OF CONCEPT PROTOTYPES
1.1.1.A	CORE C,D,E	Prepare arenavirus antigens and MNP vaccines (Year 1 Q1 – Year 2 Q2) ① Vaccine efficacy in guinea pig: LASV& MACV homologous challenge (Year 1 Q3 – Year 2 Q4) ②
1.1.1.B	CORE C,D,E	Vaccine efficacy in guinea pig: LASV & MACV heterlogous challenge (Year 1 Q4 – Year 3 Q2)
1.1.1.C	CORE C,D,E	Vaccine efficacy in NHP: LASV & MACV (Year 2 Q3 – Year 3 Q4) ③
1.1.2.A	CORE C,D,E	Prepare arenavirus antigens and MNP vaccines (Year 1 Q1 – Year 2 Q2) ① Vaccine efficacy in mice: CCHFV: homologous challenge (Year 1 Q3 – Year 2 Q4) ②
1.1.2.B	CORE C,D,E	Vaccine efficacy in guinea pig: LASV & MACV heterologous challenge (Year 1 Q4 – Year 3 Q2)
1.1.2.C	CORE C,D,E	Vaccine efficacy in NHP: CCHFV (Year 2 Q3 – Year 3 Q4) ③
1.1.2.D	CORE C,D,E	Vaccine efficacy in mice, guinea pigs, & NHP: LUJV, CHAPV, KASV (Year 4 Q3 – Year 5 Q4)
1.1.3.A	CORE D, E	Cellular correlates associated with protection (Year 2 Q3 – Year 5 Q4)
1.1.3.B	CORE D, E	Serological correlates associated with protection (Year 2 Q3 – Year 5 Q4)
1.2	CORE E	Reverse genetics of emerging bunyaviruses (Year 1 Q1 – Year 5 Q4)
2	CORE D, E	PREP monoclonal antibody vaccination: JUNV (Year 2 Q1 – Year 2 Q4)

RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and

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Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future “plug and play” pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

References:

1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.
2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.
5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.
6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.
8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.
9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.
10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia I, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.

11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.

12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.

13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.

16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.

17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.

18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.

19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.

20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.

21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.

22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.

23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 2019/05/28. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.

24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.

25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.

26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.

27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.

28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.

29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.

30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF. Lassa fever in post-conflict

sierra leone. PLoS Negl Trop Dis. 2014;8(3):e2748. Epub 20140320. doi: 10.1371/journal.pntd.0002748. PubMed PMID: 24651047; PMCID: PMC3961205.
43. Li AL, Grant D, Gbakie M, Kanneh L, Mustafa I, Bond N, Engel E, Schieffelin J, Vandy MJ, Yeh S, Shantha JG. Ophthalmic manifestations and vision impairment in Lassa fever survivors. PLoS One. 2020;15(12):e0243766. doi: 10.1371/journal.pone.0243766.
44. Ezeomah C, Adoga A, Ihekweazu C, Paessler S, Cisneros I, Tomori O, Walker D. Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. Open Forum Infect Dis. 2019;6(12):ofz512. Epub 20191203. doi: 10.1093/ofid/ofz512. PubMed PMID: 31879673; PMCID: PMC6923636.
45. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.
46. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.
47. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.
48. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.
49. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.
50. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.
51. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.
52. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.
53. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.
54. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.
55. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.
56. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.
57. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.
58. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.

59. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.

60. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.

61. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

62. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

63. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

64. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

65. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

66. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

67. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods. 2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

68. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

69. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

70. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

71. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

72. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

73. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

74. Walker PJ, Widen SG, Firth C, Blasdell KR, Wood TG, Travassos da Rosa AP, Guzman H, Tesh RB, Vasilakis N. Genomic Characterization of Yogue, Kasokero, Issyk-Kul, Keterah, Gossas, and Thiafora Viruses: Nairoviruses Naturally Infecting Bats, Shrews, and Ticks. Am J Trop Med Hyg. 2015;93(5):1041-51. Epub 20150831. doi: 10.4269/ajtmh.15-0344. PubMed PMID: 26324724; PMCID: PMC4703270.

75. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

76. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

From: Cross, Robert W. on behalf of Cross, Robert W. <rwcross@UTMB.EDU>
To: Stutz, Sonja J.; CHRISTOPHER BRODER; Geisbert, Thomas W.
Cc: Haller, Sherry
Subject: RE: RP1
Date: Tuesday, June 6, 2023 4:25:15 PM
Attachments: 06JUN23 RP1-BUNYAVIRALES VACCINES--RESEARCH STRATEGY RWC 1033-sjs RWC1520.docx
06JUN23 RP1-BUNYAVIRALES VACCINES--RESEARCH STRATEGY RWC 1033-sjs RWC1520.pdf

Accepted your changes and fixed a small blip I found. Added PDF version of same word document for context incase anything moves.

From: Stutz, Sonja J. <sjstutz@utmb.edu>
Sent: Tuesday, June 6, 2023 3:08 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Cc: Haller, Sherry <shhaller@UTMB.EDU>
Subject: RE: RP1

Hello Dr. Cross,

I have a few comments/edits in the attached research strategy. Primarily, there are a few discrepancies between the table legends and text for specific aim 1. I tried to correct as I saw appropriate, but please review and edit as needed.

Thanks,
Sonja

From: Cross, Robert W. <rwcross@UTMB.EDU>
Sent: Tuesday, June 6, 2023 10:42 AM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Cc: Stutz, Sonja J. <sjstutz@utmb.edu>; Haller, Sherry <shhaller@UTMB.EDU>
Subject: RE: RP1

Here is the final clean version after Chris' comments. Fixed a few other glitches as well. Added a PDF here for reference in case anything grows legs.

Let me know if anything else needed.

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Tuesday, June 6, 2023 8:48 AM
To: Cross, Robert W. <rwcross@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: RP1

hey Bob

here are some ideas to tighten up,
you are ok on space. but i would use 3pt breaks between major sections (less dense)
just have to remove text here and there to delete hanging sentences.

page 11 toi 12 has some weird anchor
might have to cut and move fig or text

there some weird stuff, like forced font capitals on preliminary results header.
i didnt want to keep going but i can, because i figured you have already started another version
reads great
cb

A. Significance

Henipaviruses (HNV) and arenaviruses (ARV) are two families of viruses that pose significant global public health concerns[1-5]. HNV, which includes Nipah virus (NiV) and Hendra virus (HeV), has caused outbreaks in Asia and Australia, resulting in severe respiratory and central nervous system (CNS) illnesses in humans[6-8]. These infections have been associated with high mortality rates ranging from 40% to 90%[4,9-13]. Similarly, ARV, such as Lassa virus (LASV) and Machupo virus (MACV), can cause severe illness and death in humans, with LASV being endemic in West Africa and MACV responsible for Bolivian hemorrhagic fever[14-19]. The geographical distribution and diversity of these viruses make them a global public health threat. While there are some vaccines and antiviral drugs available[4,13,20,21], there are no licensed treatments specifically targeting these viruses. Thus, there is a pressing need to develop new therapies that can effectively neutralize and prevent the spread of these viruses.

Significance of Developing Nanobody Therapy

The urgent need for effective treatments against HNV and ARV infections, highlighted by the ongoing COVID-19 pandemic, emphasizes the importance of rapid drug development and innovative antiviral therapies. Nanobodies, also known as VHH or single-domain antibodies, derived from camelid animals, offer unique advantages that make them promising candidates for antiviral therapy[22-25].

One significant advantage of nanobodies is their inherent stability[26-28]. Their small size and single-domain structure make them highly resistant to denaturation and degradation, allowing them to maintain their binding activity under various conditions. Nanobodies also offer ease of engineering. Their single-domain format simplifies the modification and optimization of their binding properties. Through techniques such as affinity maturation and structure-guided design, nanobodies can be readily engineered to enhance their affinity, specificity, and neutralization potency against specific viral targets. This flexibility in engineering enables the development of tailored nanobodies with improved therapeutic properties[29,30].

Also, nanobodies exhibit modular characteristics[26]. Their small size and independent folding domains allow for the construction of multi-specific or bi-specific nanobodies, where different specificities can be combined into a single molecule[31,32]. This modularity opens up possibilities for targeting multiple epitopes on viral proteins or simultaneously targeting different viral strains, providing enhanced breadth and efficacy in combating HNV and ARV infections.

Combined with their specificity, small size, stability, ease of engineering, and modular nature, nanobodies represent a promising approach for developing effective antiviral therapies against HNV and ARV infections.

Three nanobody development platforms to enhance diversity and coverage

The use of multiple nanobody development platforms, including camelid animals, nanomice, and low-immunogenic human nanobody libraries (**Fig.1**), enhances the diversity and coverage of nanobody libraries, increasing the likelihood of identifying potent nanobody candidates. Notably, **Dr. Jianliang Xu**, a nanobody research expert, is **the inventor of the "nanomice" platform**[29], which was commented by *Nature News and Views* that "*Just as the development of mice with antibodies containing human variable domains (such as Regeneron's VelocImmune mouse) has helped to deliver the 100th FDA-approved monoclonal antibody, perhaps the nanomouse will give nanobody-based therapeutics a push in the same direction*"[33]. In addition, we have recently cloned the CDR regions of human antibodies (IgM, IgD, IgG1-4, IgE, IgA1-2) heavy chain from 60 healthy donors of various demographic backgrounds, assembled them on Ighv3 family framework, and generated a human nanobody library that is close to natural human antibody heavy chain variable domain. The potentially low immunogenicity of such a library will enable us to quickly identify nanobody leads that need minimum optimization for clinical use.

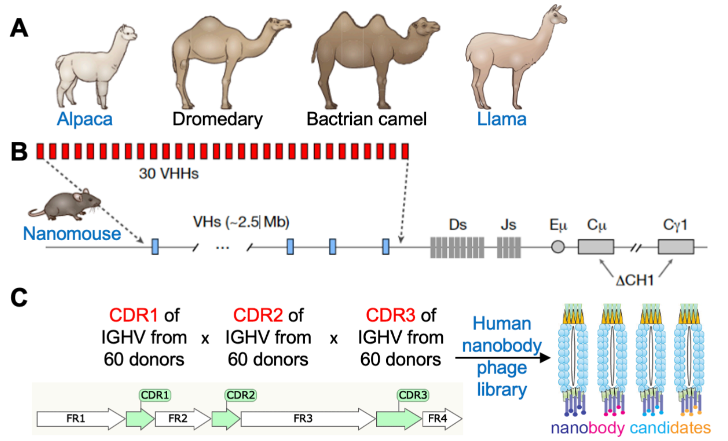


Figure 1: Three nanobody development platforms. (A) Camelid animals; (B) Nanomouse; (C) Human nanobody phage library.

The deep sequencing of antigen-specific enriched nanobody libraries ensures a comprehensive exploration of the nanobody repertoire[29], allowing for the rapid identification of leads with minimal optimization required for clinical use. This expedited development process is critical in responding to emerging viral threats and addressing the urgent need for effective antiviral therapies.

Targeting CNS diseases caused by viral infection

Nanobodies hold great promise for targeted therapy of CNS diseases as they can efficiently cross the blood-brain barrier (BBB) due to their small size[34-37]. To further enhance brain delivery, we have developed a bi-specific nanobody strategy by coupling another nanobody that targets TfR1[38,39], a receptor expressed at the BBB. This bi-specific nanobody can cross-react with both human and mouse TfR1, facilitating enhanced cargo delivery to the brain. In our upcoming study, we will compare the brain delivery and therapeutic efficacy of un-engineered nanobodies with the bi-specific nanobodies using animal models in **RP3 and Core E**. This study aims to evaluate the abilities of these two approaches to effectively penetrate the BBB and deliver therapeutic nanobodies to the central nervous system. Through this evaluation, we hope to understand the benefits of utilizing bi-specific nanobodies for targeted therapy of CNS diseases caused by HNV and ARV infections.

Generalizable research strategy

The significance of developing nanobody therapy extends beyond the four selected prototypic viruses in HNV and ARV. By applying and testing the research strategy developed in this project during **phase 2** of the project, the scope can be expanded to include other members of these viral groups, such as Langya virus (LayV)[40], Lujo virus (LUJV)[41], and Chapare virus (CHAV)[42]. This approach expands the scope of nanobody-based therapies, tackling global health risks from emerging viral diseases.

B. Innovation

The study incorporates several innovative aspects in the development of nanobody-based therapies for HNV and ARV infections:

- Utilization of diverse nanobody sources: The study leverages nanobodies derived from camelid animals, nanomice, and low-immunogenic human nanobody libraries. This approach expands the repertoire of nanobodies available for antiviral therapy development.
- Targeting viral entry machinery: Nanobodies are employed to target and neutralize viral proteins involved in viral attachment and entry. This innovative approach provides a novel avenue to inhibit viral infection at the early stages.
- Integration of structural characterization techniques: The study integrates advanced structural characterization techniques such as X-ray crystallography and Electron Microscopy Single Particle Analysis. This enables a detailed understanding of nanobody epitopes and viral neutralization mechanisms, facilitating the rational design of nanobody-based therapies.
- Development of BBB-penetrating nanobodies: The study focuses on designing nanobodies with the ability to penetrate the BBB, enabling effective targeting of the central nervous system. This is particularly relevant for combating the severe neurological manifestations associated with HNV and ARV infections.
- Generalizable research strategy: The research strategy developed in this study can be applied to other members of the HNV and ARV viral groups, such as Langya virus, Lujo virus, and Chapare virus. This expands the potential impact of nanobody therapies to address a broader range of emerging viral diseases.
- Deep sequencing for comprehensive coverage: The study utilizes deep sequencing to ensure comprehensive coverage of antigen-specific enriched nanobody libraries. This approach facilitates the rapid identification of potent nanobody leads with minimal optimization required for clinical use.
- Rapid and efficient therapy development: By leveraging the advantages of nanobodies and employing a well-designed research strategy, the study aims to rapidly and efficiently develop nanobody-based therapies. This contributes to the field of antiviral research and addresses the urgent need for effective treatments against HNV and ARV infections, thereby enhancing public health protection.

C. Approach

Scientific Premise: Our hypothesis is that nanobodies targeting viruses' key entry machinery can effectively inhibit virus infection. By utilizing structure-based analysis and rational design, we can enhance the efficacy of nanobody therapy by increasing its potency and breadth. Through the design of bi-specific nanobodies coupled with brain receptor-mediated transcytosis, we aim to enhance their ability to penetrate the blood-brain barrier, thus effectively combating central nervous system diseases caused by HNVs and ARVs.

Aim 1: To identify cross-reactive nanobodies to HNVs.

Rationale and Preliminary Data:

Envelope fusion glycoprotein F and the attachment glycoprotein G of Hendra virus (HeV) and Nipah virus (NiV) play crucial roles in host cell receptor engagement and membrane fusion, making them the main targets of neutralizing antibodies[43-46]. The high degree of similarity, approximately 80-90%, between the glycoproteins of HeV and NiV presents an attractive opportunity to identify cross-reactive neutralizing nanobodies capable of providing broad protection against both viruses[1,47-49]. To achieve this goal, we conducted immunization experiments using four groups of nanomice, with each group consisting of four mice. The mice were immunized with soluble F and G proteins of HeV and NiV, namely HeV_sF, HeV_sG, NiV_sF, and NiV_sG. These proteins, designed by **Dr. Kai Xu** and **Dr. Christopher Broder's** group, served as the immunogens[43,50,51] (**Fig. 2A**).

Following four injections in the nanomice, we observed various levels of serum antibody responses against all four immunogens, as depicted in **Figure 2B** for F proteins (data for G proteins not shown). To validate our approach, we isolated RNA from the bone marrow and spleen of mice immunized with NiV_sF and constructed nanobody phage libraries. Through a series of three rounds of phage panning using NiV_sF, subsequent individual colony picking, ELISA screening, and Octet affinity validation, we successfully identified several nanobody binders specific to NiV_sF. Of particular significance, one of the identified nanobodies, NiV_sF_Nb9, exhibited the ability to bind not only to NiV-sF protein but also to HeV-sF protein (**Fig. 2C**). This finding suggests that NiV_sF_Nb9 recognizes an epitope shared by the F proteins of both NiV and HeV. The results obtained from these experiments clearly demonstrate the potential of nanomice as a convenient and efficient model for the development of nanobodies. Consequently, we are confident in our ability to identify nanobodies against other F/G proteins of HeV and NiV using the same approach applied to the immunized mice.

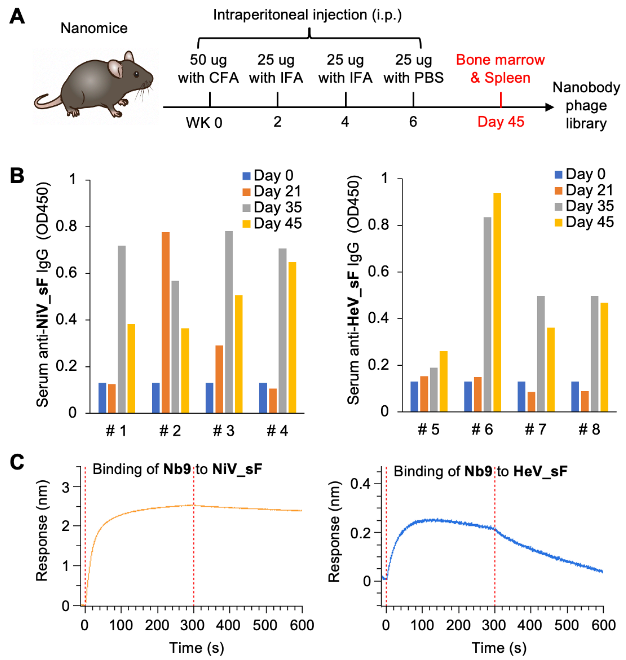


Figure 2: Nanomouse immunization and NiV_sF_Nb9 characterization. (A) Nanomouse immunization scheme. (B) HNV F proteins immunized nanomice serum response to the respective immunogen determined by ELISA; (C) Binding of NiV_sF_Nb9 to NiV_sF and HeV_sF determined by Octet.

In addition to the nanomice immunization strategy, we have also made significant progress using a **human library version 1.0 (Fig. 1C)** for screening purposes. Notably, we sequentially screened this human nanobody library with spikes of omicron variants (BA.4/5, BA.1) and WA-1, leading to the enrichment of numerous nanobody candidates. Among them, nine nanobodies exhibited binding affinity to the receptor-binding domain (RBD) of BA.4/5, BA.1, WA-1, SARS, and WIV16, indicating their ability to recognize a range of sarbecoviruses (**Fig. 3**). Importantly, all nine of these human nanobodies demonstrated potent neutralization against WA-1, and five of them exhibited cross-neutralization of Omicron subvariants (BA.4/5, BQ.1.1, XBB.1), SARS1, and Bat-WIV1. These results underscore the power and versatility of the human nanobody library platform, which holds significant potential for targeting various other antigens.

human nanobodies	WA1	Omicron (BA.4/5)	SARS1	Bat-WIV1	BQ.1.1	XBB.1
CoV2.hNb3	0.056	9.1	0.2	30.0	16.4	17.9
CoV2.hNb6	0.027	4.5	0.3	33.1	17.9	19.8
CoV2.hNb17	0.228	16.3	0.8	20.9	44.7	21.2
CoV2.hNb18	0.158	13.9	0.4	27.5	18.4	45.6
CoV2.hNb31	0.089	9.7	0.2	15.2	15.0	28.0
CoV2.hNb35	0.028	10.9	0.4	>50		
CoV2.hNb9	0.011	>50	4.4	>50		
CoV2.hNb28	0.123	>50	>50	>50		
CoV2.hNb30	0.122	>50	>50	>50		

Figure 3: SARS-CoV-2 neutralizing nanobodies identified by human nanobody library. Neutralization IC50 (ug/ml) of 9 human nanobodies against various pseudotyped sarbecoviruses.

Experimental Design:

To expand our search for cross-reactive nanobodies against HeV and NiV glycoproteins, we will immunize additional groups of nanomice sequentially with either HeV or NiV F or G proteins. Each group will serve as a source for constructing *in vivo* matured nanobody phage libraries, using bone marrow, spleen, and/or peripheral blood mononuclear cells (PBMCs). These libraries will undergo sequential screening with HeV and NiV F or G proteins to identify nanobodies that exhibit cross-recognition of the F or G proteins of both viruses. To ensure comprehensive coverage of potential binders, we will employ a two-pronged approach. Firstly, we will randomly pick colonies from the libraries, and secondly, we will subject the enriched libraries to deep sequencing using the MiSeq platform. This approach will enable us to identify nanobody candidates from all complementarity-determining region 3 (CDR3) families. Subsequently, we will assess the target specificity of individual nanobodies through enzyme-linked immunosorbent assay (ELISA). Moreover, the neutralization activity of the identified nanobody candidates will be evaluated using RP2 assays.

While nanomice have proven to be valuable for generating in vivo matured nanobodies, it is important to consider the potential immunogenicity associated with animal-derived nanobodies. Therefore, in parallel with the nanomice immunization strategy, we will also screen for F or G protein binders using a human nanobody library. By utilizing the human nanobody library, we can significantly minimize the risk of immunogenicity and obtain nanobody candidates that are better suited for therapeutic testing in human subjects.

1.1: Nanomice immunization. Four groups of nanomice have already been immunized with soluble HNV F and G separately. Our preliminary data showed that almost all mice developed good immune response after 3 injections. We will next immunize 2 more groups, 8 mice each, sequentially with HNV F or G proteins (**Fig.4**). Injections (i.p.) will be performed at 3 weeks interval to allow more time for mouse immune system to react to each immunization. Specifically, for F proteins, mice will be immunized with HeV_sF at week 0, 3, 6, NiV_sF at week 9, 12, and a mixture of HeV_sF and NiV_sF at week 15, followed by a final boost (i.v.) with HeV_sF and NiV_sF mixture 3 days later. Bone marrow, spleen and PBMCs will be harvested 3 days after the final i.v. injection. HeV_sG and NiV_sG proteins immunization will follow the same scheme. Test blood/serum will be collected one week after each injection except for the first and last two injections, and serum antibody response to F or G proteins will be monitored by ELISA. The same serum will be used for HNV neutralization **test by RP2**.

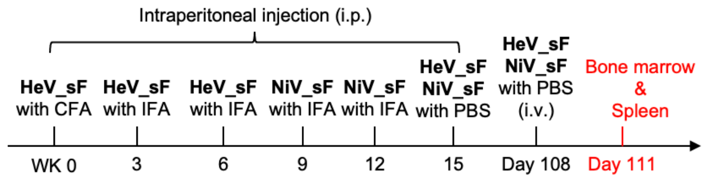


Figure 4: Nanomice sequential immunization scheme for HNV F proteins.

1.2: Nanobody phage library construction. We have extracted RNA from bone marrow and spleen from NiV_sF immunized mice, constructed nanobody phage library, screened for NiV_sF binders, and identified NiV_sF_Nb9 to be NiV_sF and HeV-sF cross binder (**Fig.2**). We will complete the nanobody phage library construction for all immunization groups and perform sequential screening to identify cross binders. Specifically, total RNA will be extracted from bone marrow, spleen and PBMCs from the best responding mice based on ELISA and neutralization test results. Two-step PCR will be performed to amply VHH-DJ (nanobody sequence) from the matured heavy chain-only IgG1 mRNA molecules (**Fig. 5**). First, we will amplify VHH-DJ-CH2 domain from IgG1 using 30 forward primers aligning to the leading exons of 30 VHH genes and one reverse primer aligning to CH2 sequence of IgG1. Next, a mixture of 30 forward primers aligning to 5’ end of individual VHH exon and 4 reverse primers aligning to JH1~4 will be used to amplify nanobody sequences, which will be cloned to pMES4 phagemid and subsequently transformed to TG1 E.coli cells.

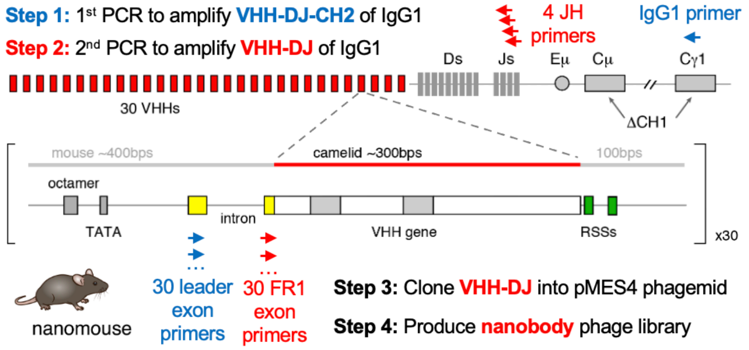


Figure 5: Nanobody phage library construction with nested PCR.

1.3: Screening nanobody libraries for HNV F/G binders. We will produce phages expressing individual nanobodies fused to their coat protein by infecting TG1 cells with VCSM13 helper phages. To identify nanobodies that recognize both HeV_sF and NiV_sF, libraries from F proteins immunized mice will be sequentially screened with HeV_sF and NiV_sF proteins in wells of 96-well Maxisorp plate; to identify nanobodies that recognize both HeV_sG and NiV_sG, libraries from G proteins immunized mice will be sequentially screened with HeV_sG and NiV_sG proteins. Multiple rounds of screening (phage panning) will be performed to ensure ideally over 100 times elution of phages from F or G protein coated wells than control wells (**Fig. 6A**). For each TG1 cell sub library from single or sequential F or G protein screening, 96 ~192 individual colonies will be picked and cultured in deep-well plates. Nanobodies (fused to phage coat protein pIII) induced by IPTG will be extracted from periplasmic region of TG1 cells and tested for binding to HNV F or G proteins by ELISA. Phagemid of ELISA positive clones will be extracted and Sanger sequenced, and then transformed into WK6 E.coli cells to produce individual nanobodies (monomer, 13 kDa). In addition, sub libraries will be deep sequenced (**Fig. 6B**) and nanobody sequences analyzed as described before[29]. Nanobodies will be clustered based on their CDR3 differences and cross checked with the sequences of ELISA-positive clones. Representative unique nanobodies from each CDR3 class will be codon optimized and synthesized, cloned into pMES4 phagemid, and nanobodies will be produced in WK6 cells. <u>We will perform step 1.3 with our newly designed and constructed human nanobody library to identify less immunogenic nanobodies against HNV F and G protein.</u>

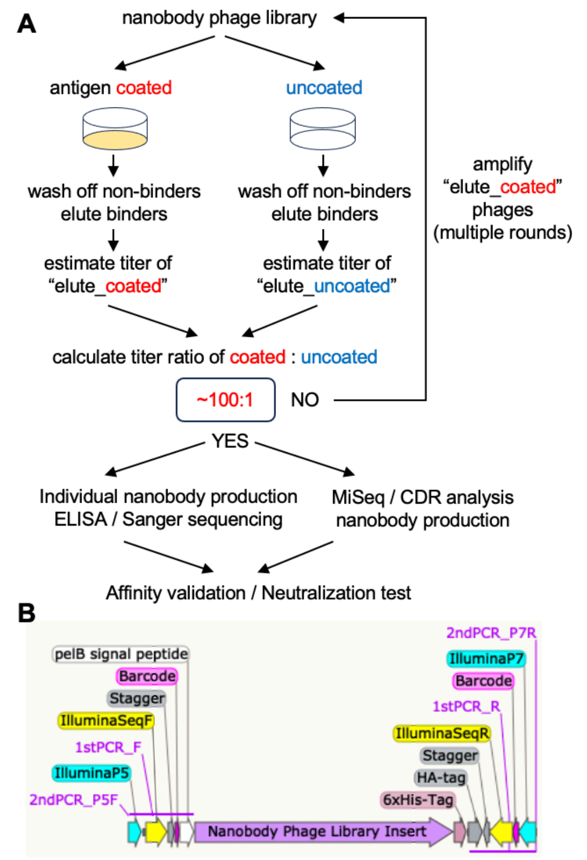


Figure 6: Phage screening, sanger sequencing, and MiSeq sequencing. (A) Nanobody phage screening strategy. (B) MiSeq sequencing library preparation. 1st round PCR will attach MiSeq F/R sequences to nanobodies, and 2nd round PCR will introduce P5/P7 adaptors to amplicon 5' and 3' ends.

1.4: Nanobody binding validation and neutralizing activity assessment. To validate the binding of nanobodies to HNV F/G proteins, we will employ two methods: ELISA and Octet Bio-Layer Interferometry (BLI) affinity analysis. ELISA will allow us to determine the binding of nanobodies to the target proteins, while Octet BLI will provide detailed information on affinity. Additionally, we will perform Octet epitope binning assays using available F/G antibodies to map the epitopes recognized by the nanobodies. Furthermore, we will assess the neutralizing activity of the identified nanobodies with the neutralization assay developed by **RP2**[52,53]. These comprehensive analyses will enable us to evaluate the binding specificity, affinity, and neutralizing capability of the nanobodies. We will further select representative neutralizing nanobodies for structural analysis (**Aim 3**) and consider various engineering approaches such as Fc conjugation and multimerization (**Aim 4**) to further optimize the properties and efficacy of the nanobodies.

<u>Expected results, potential pitfalls and alternatives:</u>

We expect to be able to produce 5-10 HNV F or G nanobodies that either specifically recognize and neutralize HeV or NiV, or cross-bind to HeV and NiV proteins and cross neutralize the two viruses. We also expect to identify nanobodies with novel epitopes compare to currently available human antibodies.

Based on our success in identifying HeV_sF and NiV_sF cross binder (NiV_sF_Nb9 from nanomouse immunization) and SARS-CoV-2/SARS/WIV1 cross neutralizers (hNb3, 6, 17, 18 and 31 from human nanobody library screening), we do not anticipate any major technical or methodological pitfalls. While we have high expectations for our study, there are potential pitfalls that we need to consider. One challenge could be the limited number of nanobody candidates that meet our criteria, particularly if their affinity or neutralizing activity is not optimal. In such cases, we have a strategy in place to perform in vitro PCR-based CDR maturation to generate nanobody variants with improved properties. Additionally, there is a possibility that our efforts to identify HNV F or G cross binders/neutralizers using nanomice or the human nanobody library may not yield satisfactory epitope diversity. To overcome this limitation, an alternative approach would involve immunizing camelid animals and applying the same selection and screening processes to identify target-specific nanobodies.

Aim 2: Identification of nanobodies to ARVs.

***<u>Rationale and Preliminary Data</u>*:**

The surface of LASV and MACV virions is covered by the heavily glycosylated trimeric type-1 fusion glycoprotein precursor (GPC)[54-57]. The GPC trimer consists of receptor-binding GP1, fusion machinery GP2, and the associated stable signal peptide (SSP)[57-62]. The extensive glycan shield on GP1 and GP2 poses a challenge for the immune system to generate a robust GPC-directed antibody response, resulting in weak and inconsistent neutralizing antibody development during natural infection[63-67]. Only a few LASV-neutralizing antibodies have been isolated from convalescent patients to date[68]. While LASV GPC has shown neutralizing response in rabbit and guinea pig studies, serum neutralization has not been achieved in mouse models[69-71]. In contrast, MACV neutralizing antibodies have been successfully isolated from GPC immunized mice[72]. However, there are currently no FDA-approved treatments or vaccines for LASV or MACV infections. In our study, we aim to leverage the small size of nanobodies to develop glycan shield-penetrating neutralizing nanobodies against LASV and MACV.

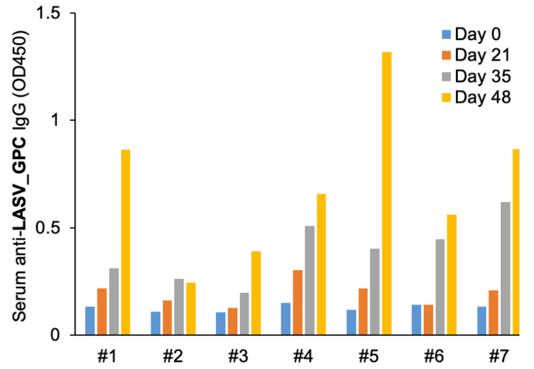


Figure 7: Antibody response aginst LASV GPC in immunized nanomice sera.

In our experiments, we immunized 8 nanomice with a stabilized LASV GPC trimer, following a modified scheme. We performed an intravenous boost injection on day 45 and harvested bone marrow and spleen on day 48. With one mouse accidentally died on day 46, 7 mice remain. Over time, 6 out of 7 mice developed an antibody response against GPC, reaching their highest titers on day 48 (**Fig.7**). Importantly, the serum from nanomouse #3 on day 48 showed weak but detectable neutralization activity against LASV Josiah strain. These findings demonstrate the potential of nanomice as a valuable model for developing neutralizing nanobodies against LASV and MACV.

Experimental Design:

We will construct nanobody phage library using the top responders from the LASV immunized nanomice, with a particular focus on nanomouse #3 due to its detectable neutralization activity. We will immunize nanomice with alternative versions of soluble stabilized trimeric LASV GPC and MACV GPC proteins **provided by RP1** and construct *in vivo* matured nanobody phage libraries. These libraries and the human nanobody library will be screened with GPC proteins to identify nanobody binders. Individual nanobodies will be assessed for target specificity by ELISA and Octet, and neutralization activity of nanobody candidates will be **evaluated by RP1**.

2.1: Nanomice immunization and nanobody phage library construction. In our previous LASV GPC immunization attempt, 1 out of 8 nanomice developed serum neutralization titer at day 48. We will immunize two groups of nanomice separately with LASV GPC or MACV GPC designed by RP1 with slightly modified immunization regimen. For both LASV and MACV, injections (i.p.) will be performed once every 3 weeks, and test blood/serum will be collected one week after each injection except for the first injection. Serum antibody response to GPC proteins will be monitored by ELISA and send to RP1 for neutralization test. Immunization (i.p.) will stop until serum neutralization titer is observed, or the 6th injection is performed on week 15. Three days after a final boost (i.v.), bone marrow, spleen and PBMCs will be harvested for nanobody phage library construction following the same strategy listed in Aim1.

2.2: Screening nanobody libraries for LASV GPC or MACV GPC binders. Nanobody phage library screening using LASV GPC or MACV GPC will be performed for multiple rounds until the ideally 100:1 ratio of eluted phages from coated vs uncoated wells is achieved. We will carry out random colony picking followed by ELISA and Sanger sequencing, as well as Miseq for comprehensive analysis of enrich sub libraries. Same screening will be applied to human nanobody library too. 13 kDa nanobody candidates will be produced in WK6 cells for further tests.

2.3: Nanobody binding analysis and neutralizing activity assessment. Individual nanobodies will be validated using ELISA and Octet assays to confirm their interaction with GPC proteins. Precise affinity will be measured by BLI method. Epitope competition mapping and binning will be performed using known human/mouse GPC antibodies. Nanobodies with positive binding will be evaluated for neutralization activity by **RP1**.

Expected results, potential pitfalls and alternatives: We expect to be able to produce 5-10 LASV or MACV neutralizing nanobodies targeting GPC proteins. The monoclonal antibodies that are currently known to have LASV neutralizing activity are categorized into four competition groups: GP1-A, GPC-A, GPC-B, and GPC-C[68]. We expect to identify LASV neutralizing nanobodies lie within these 4 groups. Given the small size of nanobodies and their potential to target hidden epitopes, we also expect to identify novel epitopes of vulnerability on LASV and MASC GPC surface. Even though the immunogenicity of LASV GPC seems to be very poor in mouse, nanomouse #3 developed serum neutralizing response to LASV Josiah strain. Furthermore, a nanobody neutralizer D5 was identified by screening the same LASV GPC with a naïve llama nanobody library[69]. Therefore, we do not anticipate any major technical or methodological pitfalls with nanomouse and human nanobody library platforms. However, if we are unsuccessful at isolating LASV/MACV binders and neutralizers using nanomice or human nanobody library, we will immunize camelid animals instead. We have a standing by llama from Capralogics Inc. ready for immunization. In the past a few years, we have immunized this animal with HIV envelop trimer, SARS-CoV-2 spike and HMPV F proteins, and all three studies led to the identification of potent nanobody neutralizers against these virus targets. We expect this llama will perform well against LASV and MACV. If our selected nanobody candidates are less-than-optimal, *in vitro* PCR-based CDR maturation will be performed and nanobody variants with improved activity will be screened.

Aim 3: Structural characterization.

Rationale and Preliminary Data.

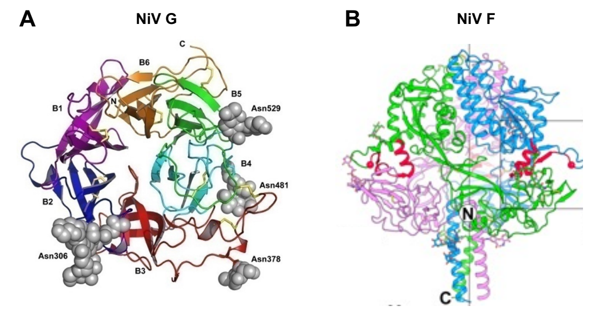


Figure 8: Structures of NiV G and F glycoproteins by X-ray crystallography. (A) NiV G head domain; (B) NiV F in the native prefusion conformation.

Dr. Kai Xu is a distinguished researcher with a proven track record in the structural determination of Henipavirus (HNV) glycoproteins and their complexes with receptors and monoclonal antibodies[50,73-75]. Notably, he achieved a significant milestone by determining the first crystal structures of the Nipah virus (NiV) G protein head domain and NiV F protein in the prefusion conformation (**Fig.8**). Moreover, Dr. Xu possesses exceptional expertise in designing HNV protein constructs that faithfully present their native conformation[43,74]. This capability enables him to create accurate representations of HNV glycoproteins, facilitating a deeper understanding of their functional properties and enabling investigations into their interactions with receptors and antibodies[51,76,77]. With his comprehensive skill set in protein design and structural determination, Dr. Xu stands as a leading authority in the field of HNV research.

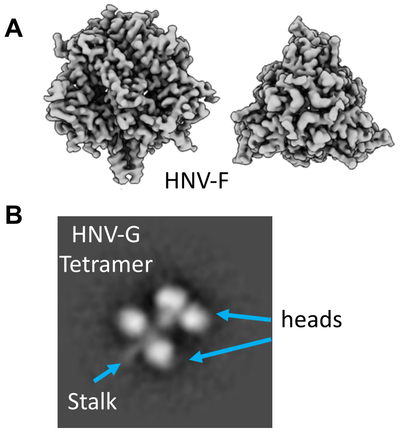


Figure 9: Validation of HNV F and G protein used in this study by EM single particle analysis. (A) Orthogonal views of HNV F protein cryo-EM 3D reconstruction at 2.5 A resolution; (B) 2D analysis of HNV G protein tetramer.

Our proposed research is driven by the need to present the native conformation of viral antigens, which is essential for antigenicity, immunogenicity, and the induction of protective immune responses. Glycoproteins of HNVs and ARVs are particularly prone to conformational flexibility, resulting in sample heterogeneity. To overcome this challenge, our preliminary data demonstrate the feasibility of creating viral antigens that display their native protein conformation. We have successfully stabilized viral antigens in their native states by designing prefusion conformation stabilizing mutations and incorporating specific stabilizing tags, such as GCNt and GCN4, to F and G proteins, respectively[50,73] (described in **RP1, 2 and Core D**). This stabilization approach has been validated through structural analysis (**Fig.9**), biochemical and biophysical characterization, and functional assessments, including receptor binding and the induction of neutralizing antibody responses.

Additionally, we have extensive experience in determining high-resolution structures of nanobodies in complex with viral antigens, such as SARS-CoV-2 spike proteins[29] (**Fig.10**). Leveraging advanced structural and functional characterization techniques such as X-ray crystallography, cryo-electron microscopy, and binding assays, we aim to unravel the detailed structural features and functional properties of the native-like viral antigens. This comprehensive understanding will provide critical insights into the interactions between antigens and antibodies, as well as the mechanisms of immune recognition. Ultimately, this knowledge will guide the rational design of effective vaccines and therapeutics targeting viral infections.

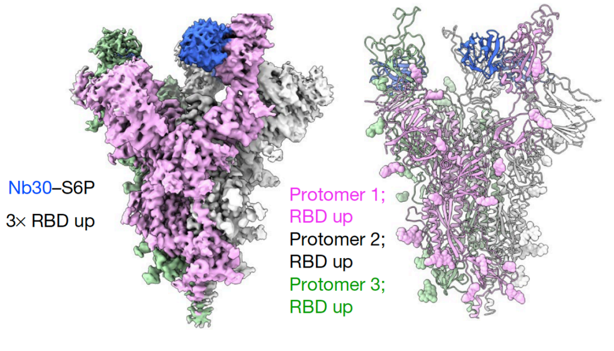


Figure 10: Cryo-EM single particle analysis of broadly protective nanobody against SARS-CoV2. 2.6 Angstrom high-resolution cryo-EM map (left) and structural model (right) of nanobody in complex with SARS-CoV2 spike protein. Nbs are in color blue.

Experimental Design:

Our experimental design aims to understand how nanobodies neutralize HNV and ARV viruses by analyzing their structure and interactions. Using X-ray crystallography and EM methods, we will determine the structures of nanobody-viral glycoprotein complexes, providing insights into their binding. Additionally, we will employ structure-based mutagenesis techniques to investigate the impact of specific mutations on viral entry and neutralization **in RP1 and 2**. By combining these approaches, we will gain detailed knowledge of nanobody epitopes and their interactions, contributing to the development of antiviral therapeutics for HNV and ARV.

3.1. Structural basis of nanobody cross-neutralization of both NiV and HeV. To determine the structure of the NiV or HeV F or G protein complexed with cross-neutralizing nanobodies, a series of steps will be undertaken. Initially, the NiV and HeV F and G proteins will be prepared in their native prefusion conformation by **Core D**. Subsequently, the purified nanobodies will be incubated with the F or G proteins to form stable complexes, optimizing the incubation conditions. High-resolution structures of the F or G protein complexes bound to the cross-neutralizing nanobodies will be determined using both X-ray crystallography and cryo-EM single particle analysis. Epitope analysis, including sequence analysis to identify conserved regions between the NiV and HeV F proteins, will provide insights into the potential neutralization mechanism. Additional experiments, such as site-directed mutagenesis, binding assays, and functional studies, will be conducted to validate the interaction between the nanobodies and the F proteins, confirming the importance of specific residues and regions identified in the structural analysis. Notably, a negative stain electron microscopy (ns-EM) analysis of a cross-neutralizing nanobody targeting both HeV and NiV G protein head domain has been performed, employing a compatible monoclonal antibody hAH1.3 to enhance particle 2D alignment (**Fig.11A**) and 3D reconstruction (**Fig. 11B**). This study demonstrates the feasibility of obtaining high-resolution cryo-EM structures. Ultimately, this study will yield crucial insights into the molecular mechanisms of neutralization and facilitate the development of effective antiviral therapies against HNVs.

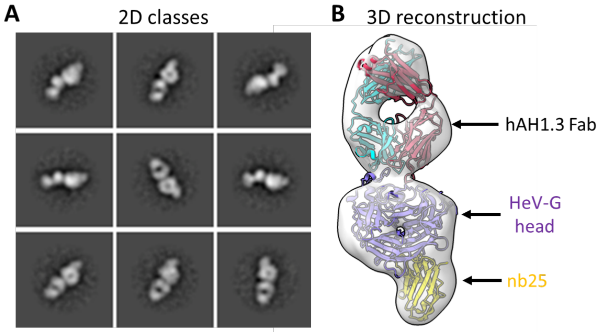


Figure 11: Anti-HeV-G nanobody epitope mapping by ns-EM analysis. (A) 2D classification; (B) 3D reconstruction showing a ternary complex of HeV-G head domain with a murine neutralizing antibody hAH1.3 and a nanobody nb25.

3.2. Structural analysis of LASV or MACV GPC proteins in complex with neutralizing nanobodies. The arenavirus glycoprotein precursor (GPC) proteins are key components of the viral envelope and play a crucial role in the entry and infection process of arenaviruses[78-80]. Arenaviruses are a family of RNA viruses that can cause severe diseases in humans, including hemorrhagic fevers[81,82]. The GPC proteins are synthesized as a single polypeptide chain, which undergoes post-translational processing to generate mature glycoproteins. The mature glycoproteins consist of two subunits, GP1 and GP2, that are responsible for receptor recognition and membrane fusion, respectively. The GP1 subunit is involved in receptor binding and determines the host tropism of the virus, while the GP2 subunit mediates membrane fusion between the viral envelope and host cell membrane. The GPC proteins are highly immunogenic and are targets for neutralizing antibodies. Understanding the structure and function of ARV GPC proteins is essential for developing effective antiviral strategies and vaccines against arenavirus infections. To investigate the structure of LASV and MACV GPC complexed with nanobodies using EM and X-ray crystallography, the experimental procedure involves preparation of stabilized GPC constructs, such as GPCysR4 and GPCysRRLL by **Core D**[58,83,84]. These constructs, developed with specific mutations, have been extensively used in structural studies of GPC-neutralizing antibodies. The purified GPC constructs are then mixed with the nanobodies of interest to form stable GPC-nanobody complexes. Cryo-EM analysis is performed by preparing cryo-EM grids, imaging them using a cryo-electron microscope, and generating a 3D reconstruction of the complex. X-ray crystallography is employed to crystallize the GPC-nanobody complex, collect diffraction data, and determine the crystallographic structure. The obtained structures are analyzed to identify nanobody-viral glycoprotein interactions and understand neutralization mechanisms. This combined approach provides valuable insights into the complex structures and guides the optimization of nanobody-based antiviral strategies.

Expected results, potential pitfalls and alternatives:

We anticipate that our research efforts will result in the generation of high-resolution structures of nanobody-antigen complexes, providing detailed insights into the precise epitope information and neutralization mechanisms. However, obtaining high-quality crystals suitable for X-ray analysis can be a challenging task. We are aware that extensive optimization of crystallization conditions may be necessary to promote crystal formation.

To overcome this potential pitfall, we will employ various strategies and screening methods to enhance crystal quality and obtain the desired structural information. The conformational flexibility and heterogeneity of viral antigens and their complexes pose another challenge. In such cases, cryo-EM SPA can serve as a valuable alternative to X-ray crystallography. Through the application of computational algorithms, we can classify different particle conformations and extract high-resolution structural information from cryo-EM data. This approach will provide valuable insights into the structural features and dynamics of the viral antigens, despite their inherent flexibility. While our GPC stabilization strategy is primarily derived from LASV antigens, it may not be equally effective for MACV antigens due to their distinct structural and stability characteristics. As an alternative approach, we propose co-expressing MACV GPC with specific antibodies or nanobodies that can assist in stabilizing the native conformation of the antigens. This co-expression strategy will enhance the structural integrity of MACV antigens, enabling their characterization using various structural biology techniques.

Aim 4: Enhancing efficacy and CNS bioavailability of nanobodies.

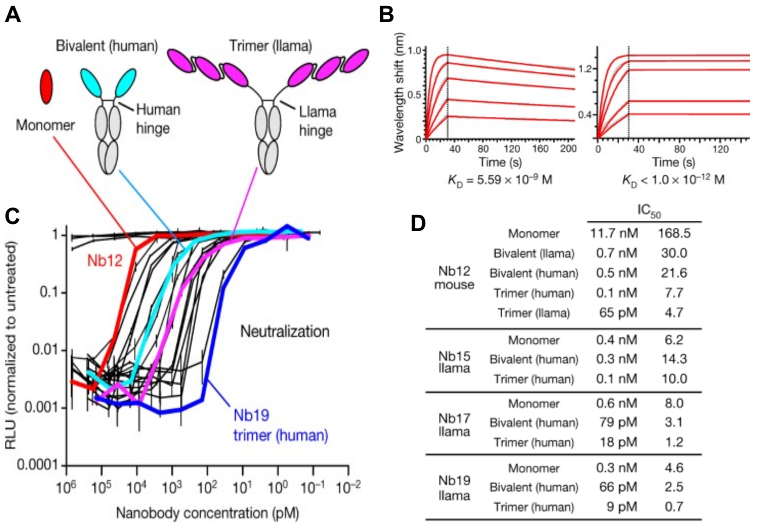
Rationale and Preliminary Data.

Our aim is to enhance the therapeutic efficacy of nanobodies through engineering strategies. We will achieve this by extending the half-life of nanobodies, enhancing their interaction with immune effector cells, and increasing their binding avidity and neutralization potency. In addition, we will focus on improving the therapeutic effectiveness and availability of nanobodies within the CNS. The BBB presents a challenge in delivering therapeutic substances to the brain and spinal cord[85,86]. This barrier, formed by specialized brain capillary endothelial cells, tightly regulates the passage of molecules into the CNS, limiting the transport of drugs and antibodies[87-89]. Nanobodies, with their small size, show promise for targeted therapy of CNS diseases as they have demonstrated the ability to cross the BBB in recent studies[34-37]. In Aim 4, our research focuses on enhancing the therapeutic effectiveness and availability of nanobodies within the CNS, specifically for addressing viral infections that affect the CNS. We propose utilizing receptor-mediated transcytosis (RMT) as a strategy to facilitate the transport of nanobodies across the BBB[90,91]. RMT has emerged as a promising approach for delivering biologics, including antibodies, to the CNS[92]. RMT involves targeting specific receptors or transporters on brain capillary endothelial cells to enhance the uptake of nanobodies into the CNS. By engineering bi-specific nanobodies to selectively bind to these receptors or transporters, we can improve their transport across the BBB.

Our goal is to optimize the targeting and transcytosis efficiency of nanobodies through RMT, thereby improving their delivery and distribution within the CNS. This optimization will ultimately enhance the therapeutic efficacy of nanobodies in combating viral infections and CNS-related diseases.

Experimental Design:

4.1. Generate nanobody Fc-fusion and tri-valent nanobodies*:* In our previous study focusing on SARS-CoV-2 nanobody therapy development, we successfully implemented two crucial engineering strategies: Human IgG1 Fc fusion and nanobody multimerization[29] (**Fig.12**). These strategies have shown significant advancements in improving the effectiveness of nanobody-based therapeutics.



		IC_{50}	
Nb12 mouse	Monomer	11.7 nM	168.5
	Bivalent (llama)	0.7 nM	30.0
	Bivalent (human)	0.5 nM	21.6
	Trimer (human)	0.1 nM	7.7
	Trimer (llama)	65 pM	4.7
Nb15 llama	Monomer	0.4 nM	6.2
	Bivalent (human)	0.3 nM	14.3
	Trimer (human)	0.1 nM	10.0
Nb17 llama	Monomer	0.6 nM	8.0
	Bivalent (human)	79 pM	3.1
	Trimer (human)	18 pM	1.2
Nb19 llama	Monomer	0.3 nM	4.6
	Bivalent (human)	66 pM	2.5
	Trimer (human)	9 pM	0.7

Figure 12: Fc fusion and multimerization improve potency of nbs against SARS-CoV2. (A) Diagrams show nanobody monomers, dimer, and trimers (fused to human IgG1 Fc) (B). Comparison BLI binding of monomer and trimer binding 1000x affinity improvement due to avidity. (C). Neutralization shows of SARS-CoV-2 pseudovirus by various nbs and multimers. (D). Table summarizes pseudovirus neutralization potency of selected nanobodies. Values given in molarity and ng/ml.

To extend the half-life of nanobodies, we incorporated Human IgG1 Fc fusion through a flexible hinge region (**Fig.12A**). This fusion strategy substantially increased the duration of nanobody activity within the body by improving their stability and protection against degradation. The Fc region of the antibody played a crucial role in enhancing the therapeutic effect by providing enhanced durability. Furthermore, we designed nanobody multimers, specifically tri-valent structures, to enhance the binding affinity (**Fig.12B**) and neutralization potency of the nanobodies (**Fig.12C and D**). The multimeric arrangement allowed for stronger and more effective binding interactions with the target viral proteins, leading to improved neutralization of the virus and ultimately enhancing the therapeutic efficacy of the nanobodies.

Our experimental strategy involves extending the half-life and enhancing the therapeutic efficacy of our top nanobody candidates through the generation of nanobody Fc-fusion proteins and tri-valent nanobodies. To extend the half-life, we will fuse the Fc region of IgG1 to the nanobodies using either the human or mouse IgG1 hinge region or the llama IgG2a hinge region, taking advantage of the FcRn-mediated recycling process and stability conferred by these hinge regions, respectively. For the generation of nanobody trimers, we will design rational linkers based on structural information of viral glycoproteins and the approaching angle of nanobody recognition. This will ensure proper orientation and spatial arrangement of the nanobodies within the trimeric structure, leading to increased avidity, binding affinity, and improved target engagement. By implementing these strategies, we aim to enhance the stability, therapeutic potential, and neutralization potency of our nanobodies, ultimately contributing to the development of more effective therapeutic interventions against viral infections.

4.2. Design multi-specific nanobodies by combining nanobodies with non-overlapping epitopes: In this aim, we will design nanobodies that target distinct and non-overlapping epitopes on the viral glycoproteins. These nanobodies will be linked together in a tandem fashion to create a synergistic effect, enhancing their overall binding affinity and neutralization potency against the target viruses. The design of these multi-specific nanobodies will involve careful consideration of the orientation and flexibility of the linker regions, ensuring optimal cooperative binding properties. Through this approach, we aim to create a robust and comprehensive neutralizing response against viral infections, ultimately advancing the development of more effective therapeutic interventions. (**Fig.13A**)

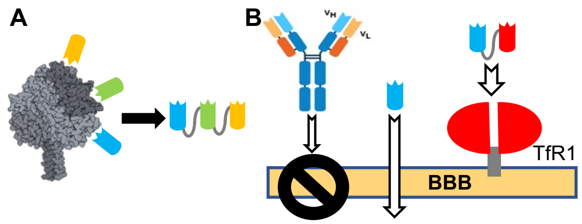


Figure 13: Bi-specific and multi-specific nanobody engineering. (A) Combination of epitope specificities to enable synergy, (B) Bi-specific nb with TfR1 specificity to improve brain targeted delivery.

4.3. Design Bi-specific nanobody by coupling with TfR1-targeting specificity to facilitate CNS delivery: In this aim, we will explore the incorporation of TfR1-specific nanobodies to enhance the delivery of nanobodies to the CNS (**Fig.13B**). The high expression of TfR1 on the blood-brain barrier makes it an attractive target for facilitating CNS delivery. To improve the penetration of nanobodies across the blood-brain barrier and enhance their bioavailability within the CNS, we will design bi-specific nanobodies by genetically fusing our candidate nanobodies against highly pathogenic neurotropic HNVs or ARVs with TfR1-specific nanobodies. By combining the targeting specificity of both nanobodies, we aim to achieve enhanced CNS delivery and therapeutic efficacy. We will evaluate several published TfR1-specific nanobodies, including FC5, a camelid single-domain antibody with known sequence[93]. FC5 has demonstrated the ability to transcytose across the blood-brain barrier and effectively deliver bioactive molecules to the CNS[36,94,95]. With its cross-reactivity across species and strong binding affinity to brain endothelial cells, FC5 holds promise for enhancing the CNS delivery of our nanobodies.

4.4. Efficacy test in both in vitro neutralization assay and animal studies. We will assess the efficacy of the original nanobodies identified in aims 1 and 2 and compare them with the engineered nanobodies in both *in vitro* neutralization assays and animal studies. Our goal is to evaluate the performance of the engineered nanobodies and determine their potential for therapeutic applications. The *in vitro* neutralization assays will assess the ability of the nanobodies to neutralize the target viruses, providing valuable insights into their antiviral potency. By measuring the reduction in viral replication or the inhibition of viral entry, we can determine the neutralization potency of the nanobodies. This will provide valuable insights into their antiviral activity and effectiveness. To further evaluate the efficacy of the nanobodies *in vivo*, we will utilize the rCedV-NiV-B chimera mouse model at the Uniformed Services University (USU) **in RP3** for HNVs, as well as animal models of ARVs developed at the University of Texas Medical Branch (UTMB) in **Core E**. These animal models will allow us to assess the therapeutic efficacy of the original and engineered nanobodies in terms of nanobody brain delivery efficiency, viral load reduction, disease progression, and overall survival rates. By comparing the outcomes between the two groups, we can determine the superiority of the engineered nanobodies in terms of therapeutic effectiveness.

Through these comprehensive in vitro and in vivo evaluations, we aim to demonstrate the improved efficacy of the engineered nanobodies compared to the original nanobodies. This assessment will provide crucial data for further development and optimization of nanobody-based therapeutics targeting HNVs and ARVs, with the ultimate goal of improving patient outcomes and combating viral infections.

Expected results, potential pitfalls and alternatives:

We anticipate that the design of tandemly linked nanobodies will result in enhanced neutralization potency and viral inhibition. The incorporation of a hinge and Fc region is expected to improve the half-life and stability of nanobodies, increasing their therapeutic potential. Coupling with TfR1-specific nanobodies is likely to improve brain delivery efficiency. However, potential pitfalls of using VHH FC5 as bi-specific nanobodies include short half-life due to susceptible to proteolytic degradation, thus limiting its development for clinical use. Alternative strategies, such as utilizing alternative receptor targeting or seeking to identify an antibody that binds to the same epitope, but that is derived from a fully human antibody library and has the requisite properties for clinical development, will be explored if necessary. Overall, these enhancements in efficacy and CNS bioavailability of nanobodies will significantly contribute to the development of potent antiviral therapies against CNS infections.

Aim 5: Expanding to other members of the viral groups.

Rationale

Building upon the valuable insights and progress achieved in Aims 1-4, Aim 5 aims to expand our nanobody-based therapeutic approach to target other members of the HNV and ARV in the **2nd phase** of this project. This expansion is based on the premise that the knowledge gained from studying NiV, HeV, LASV, and MACV can be extrapolated to other related viruses within these groups.

Experimental Design:

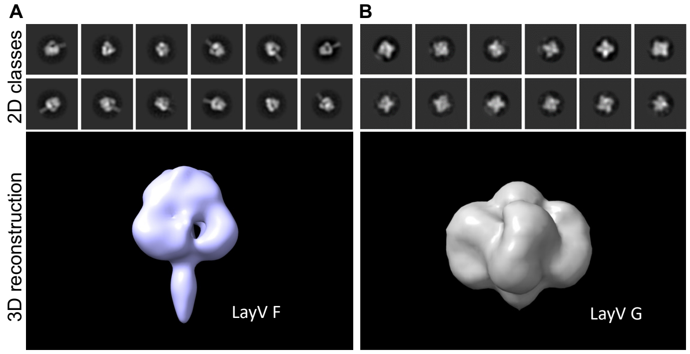


Figure 14: LayV F and G protein analysis. 2D classification and 3D reconstruction of LayV prefusion F (A), and G tetramer (B)

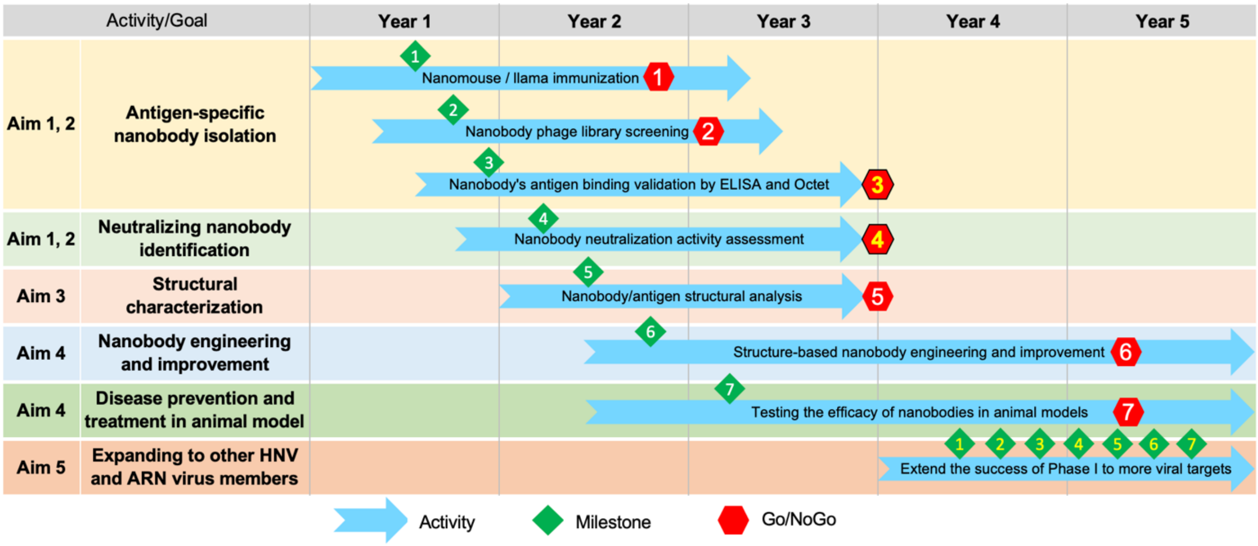
5.1. Langya virus: Langya virus (LayV) is a newly emerged member of the Henipavirus (HNV) family that has been linked to respiratory illness in humans[40,96]. LayV shares a close genetic relationship with Nipah virus and Hendra virus, both of which are known to cause severe disease in humans. To develop effective nanobody therapies against LayV infection, we have successfully generated native viral glycoprotein antigens by design, specifically the G and F glycoproteins (**Fig. 14**). By following our established research strategy, we will utilize nanobody development platforms, employ structural characterization techniques, and implement optimization approaches to identify nanobodies with protective properties against LayV. This process will involve evaluating the selected nanobodies with the neutralization assay and the animal disease model developed in collaboration with **RP2 and Core E**, and ready during the **phase 2** of this project. These efforts will not only contribute to development of nanobody-based therapies targeting LayV but also demonstrate the plug-and-play nature and rapid generalizability of our research strategy. By successfully applying our established approach to a newly emerged member of the Henipavirus family, we will showcase the versatility and effectiveness of our methodology in identifying and optimizing nanobodies against emerging viral pathogens. This will have broader implications for future studies on other viral infections, as our research strategy can be readily adapted and applied to different viral targets.

5.2. Lujo virus and Chapare virus: In our continued research, we will apply a similar strategy to the one used in Aim 2-4 for Lujo virus (LUJV)[41,97,98] and Chapare virus (CHAV)[42,99,100], which are closely related to the prototype LASV and MACV Arenaviruses. This strategy involves stabilizing the glycoprotein complex (GPC) using existing mutation designs transferred from LASV, isolating nanobodies specific to LUJV and CHAV, and performing structural characterization. By leveraging the knowledge and techniques gained from previous aims, we aim to identify nanobodies that can effectively neutralize LUJV and CHAV.

Expected results, potential pitfalls and alternatives:

We anticipate that our research strategy, combined with the knowledge gained from the phase 1 study, will enable us to identify potent neutralizing nanobodies against LayV, LUJV, and CHAV, providing promising therapeutic options for these viral infections. However, it is important to acknowledge potential challenges, such as limited cross-reactivity due to the genetic and antigenic differences between these additional viral targets and the prototypes studied in phase 1. In such cases, alternative approaches, including structure-guided rational design, may be explored to combine viral specificity and expand the protection breadth. Another potential limitation could be the difficulty to generate native prefusion GPC for the additional ARVs. In such instances, we will focus on utilizing the immune-dominant GP1 subunit, which is more stable and contains vulnerable sites for neutralizing antibody recognition. Rational design will be employed to shield non-native surfaces and minimize the generation of non-neutralizing nanobodies. Overall, the successful expansion of our research to other members of the HNV and ARV groups will make a significant contribution to broadening the effectiveness of nanobody-based therapies and addressing the global health risks associated with these viral pathogens.

D. Project Milestones and Timelines



<u>**Milestones**</u>: (<u>Same milestones are expected to be achieved during phase II with a faster pace).</u>

1. Serum neutralization activity observed (ID_{50}>10).
2. Nanobody phage library panning good enrichment achieved (enrichment>100:1).
3. High affinity viral protein-specific nanobodies isolated (affinity<1 nM).
4. Neutralizing nanobodies obtained (IC_{50}<1 ug/ml; neutralize multiple strains).
5. High resolution nanobody/antigen complex structure solved (resolution<3A).
6. Engineered nanobody molecules with improved activity obtained (IC_{50}<0.1 ug/ml; neutralize more strains).
7. Positive therapeutic effect of nanobodies in virus-induced diseases achieved (no symptoms or weight loss at 10 mg/kg or lower dose).

<u>**Criteria to be met for each activity before moving forward (Go/NoGo)**</u>:

1. ELISA signal of post-immunization sera is at least 3 times higher than pre-immunization sera.
2. Nanobody phage library panning reach to at least 5 times enrichment.
3. Viral protein-specific nanobody with at least 1 nM affinity <u>**(if not achieved, No Go for Phase II)**</u>.
4. Nanobodies neutralize at IC_{50}<10 ug/ml and/or multiple strains (<u>**if not achieved, NoGo for Phase II)**</u>.
5. Resolve structure with high enough resolution to define nanobody epitopes.
6. Engineered nanobodies achieve better potency and breadth (IC_{50}<0.1 ug/ml; neutralize more strains).
7. Positive therapeutic effect achieved (no symptoms or weight loss at 10 mg/kg or lower dose).

E. Industry Expertise and Regulatory Considerations:

If new lead nanobodies engineered nanobodies are identified from **Research Project 5 (RP5),** collaboration with industry and regulatory experts in **Core D** and the **Scientific Advisory Board (SAB)** will be initiated. This collaboration will involve seeking their expertise in various areas such as the identification of correlates of protection, lead optimization, stability testing, and manufacturability testing of the newly discovered nanobodies. Nanobody candidates can be engineered as desired in collaboration with investigators from **Core D**. This may involve modifications such as altered N-glycans, Fc mutations to enhance effector functions and prolong half-life, and evaluation of multimeric formats. The engineered nanobodies will then be further evaluated to correlate their mechanisms of action with maximal efficacy using the rCedV model explored in the **RP3**, and potentially transitioned to testing in authentic NiV/HeV animal models under the supervision of **Core E**. Throughout this process, stability and manufacturability assessment will be conducted to ensure the identification of a lead prototype format for further development. The goal will be to identify single domain mAb prototypes suitable for long-term intramuscular prophylaxis and/or intravenous post-exposure prophylaxis and therapy. As monoclonal antibodies are regulated by The Center for Drug Evaluation and Research (CDER), a division of the U.S. Food and Drug Administration. Therefore, all activities related to the development and evaluation of the mAbs will be performed in accordance with regulatory expectations set forth by CDER.

References:

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Luby, S. P. & Gurley, E. S. Epidemiology of henipavirus disease in humans. *Curr Top Microbiol Immunol* **359**, 25-40, doi:10.1007/82_2012_207 (2012).

3 Kerber, R. *et al.* Research efforts to control highly pathogenic arenaviruses: a summary of the progress and gaps. *J Clin Virol* **64**, 120-127, doi:10.1016/j.jcv.2014.12.004 (2015).

4 Patterson, M., Grant, A. & Paessler, S. Epidemiology and pathogenesis of Bolivian hemorrhagic fever. *Curr Opin Virol* **5**, 82-90, doi:10.1016/j.coviro.2014.02.007 (2014).

5 Kummer, S. & Kranz, D. C. Henipaviruses-A constant threat to livestock and humans. *PLoS Negl Trop Dis* **16**, e0010157, doi:10.1371/journal.pntd.0010157 (2022).

6 Gurley, E. S. *et al.* Convergence of Humans, Bats, Trees, and Culture in Nipah Virus Transmission, Bangladesh. *Emerg Infect Dis* **23**, 1446-1453, doi:10.3201/eid2309.161922 (2017).

7 Halpin, K. *et al.* Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:10.4269/ajtmh.2011.10-0567 (2011).

8 Clayton, B. A. Nipah virus: transmission of a zoonotic paramyxovirus. *Curr Opin Virol* **22**, 97-104, doi:10.1016/j.coviro.2016.12.003 (2017).

9 Luby, S. P. *et al.* Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis* **15**, 1229-1235, doi:10.3201/eid1508.081237 (2009).

10 Kulkarni, D. D., Tosh, C., Venkatesh, G. & Senthil Kumar, D. Nipah virus infection: current scenario. *Indian J Virol* **24**, 398-408, doi:10.1007/s13337-013-0171-y (2013).

11 Hsu, V. P. *et al.* Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis* **10**, 2082-2087, doi:10.3201/eid1012.040701 (2004).

12 Soman Pillai, V., Krishna, G. & Valiya Veettil, M. Nipah Virus: Past Outbreaks and Future Containment. *Viruses* **12**, doi:10.3390/v12040465 (2020).

13 Sahay, R. R. *et al.* Experiential learnings from the Nipah virus outbreaks in Kerala towards containment of infectious public health emergencies in India. *Epidemiol Infect* **148**, e90, doi:10.1017/s0950268820000825 (2020).

14 Johnson, K. M., Kuns, M. L., Mackenzie, R. B., Webb, P. A. & Yunker, C. E. Isolation of Machupo virus from wild rodent Calomys callosus. *Am J Trop Med Hyg* **15**, 103-106, doi:10.4269/ajtmh.1966.15.103 (1966).

15 Aguilar, P. V. *et al.* Reemergence of Bolivian hemorrhagic fever, 2007-2008. *Emerg Infect Dis* **15**, 1526-1528, doi:10.3201/eid1509.090017 (2009).

16 Shaffer, J. G. *et al.* Lassa fever in post-conflict sierra leone. *PLoS Negl Trop Dis* **8**, e2748, doi:10.1371/journal.pntd.0002748 (2014).

17 Asogun, D. A. *et al.* Molecular diagnostics for lassa fever at Irrua specialist teaching hospital, Nigeria: lessons learnt from two years of laboratory operation. *PLoS Negl Trop Dis* **6**, e1839, doi:10.1371/journal.pntd.0001839 (2012).

18 Günther, S. & Lenz, O. Lassa Virus. *Critical Reviews in Clinical Laboratory Sciences* **41**, 339-390, doi:10.1080/10408360490497456 (2004).

19 Salazar-Bravo, J., Ruedas, L. A. & Yates, T. L. Mammalian reservoirs of arenaviruses. *Curr Top Microbiol Immunol* **262**, 25-63, doi:10.1007/978-3-642-56029-3_2 (2002).

20 Enria, D. A. & Barrera Oro, J. G. Junin virus vaccines. *Curr Top Microbiol Immunol* **263**, 239-261, doi:10.1007/978-3-642-56055-2_12 (2002).

21 Barrera Oro, J. G. & McKee, K. T., Jr. Toward a vaccine against Argentine hemorrhagic fever. *Bull Pan Am Health Organ* **25**, 118-126 (1991).

22 Hamers-Casterman, C. *et al.* Naturally occurring antibodies devoid of light chains. *Nature* **363**, 446-448, doi:10.1038/363446a0 (1993).

23 Muyldermans, S., Atarhouch, T., Saldanha, J., Barbosa, J. A. & Hamers, R. Sequence and structure of VH domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. *Protein Eng* **7**, 1129-1135, doi:10.1093/protein/7.9.1129 (1994).

24 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annu Rev Biochem* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

25 Muyldermans, S. Applications of Nanobodies. *Annu Rev Anim Biosci* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

26 Kunz, P. *et al.* The structural basis of nanobody unfolding reversibility and thermoresistance. *Sci Rep* **8**, 7934, doi:10.1038/s41598-018-26338-z (2018).

27 Ewert, S., Cambillau, C., Conrath, K. & Plückthun, A. Biophysical properties of camelid V(HH) domains compared to those of human V(H)3 domains. *Biochemistry* **41**, 3628-3636, doi:10.1021/bi011239a (2002).

28 Omidfar, K., Rasaee, M. J., Kashanian, S., Paknejad, M. & Bathaie, Z. Studies of thermostability in Camelus bactrianus (Bactrian camel) single-domain antibody specific for the mutant epidermal-growth-factor receptor expressed by Pichia. *Biotechnol Appl Biochem* **46**, 41-49, doi:10.1042/ba20060104 (2007).

29 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

30 Schoof, M. *et al.* An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science* **370**, 1473-1479, doi:10.1126/science.abe3255 (2020).

31 Koenig, P. A. *et al.* Structure-guided multivalent nanobodies block SARS-CoV-2 infection and suppress mutational escape. *Science* **371**, doi:10.1126/science.abe6230 (2021).

32 Saelens, X. & Schepens, B. Single-domain antibodies make a difference. *Science* **371**, 681-682, doi:10.1126/science.abg2294 (2021).

33 Voss, J. E. Engineered single-domain antibodies tackle COVID variants. *Nature* **595**, 176-178, doi:10.1038/d41586-021-01721-5 (2021).

34 Bélanger, K. *et al.* Single-Domain Antibodies as Therapeutic and Imaging Agents for the Treatment of CNS Diseases. *Antibodies (Basel)* **8**, doi:10.3390/antib8020027 (2019).

35 Pothin, E., Lesuisse, D. & Lafaye, P. Brain Delivery of Single-Domain Antibodies: A Focus on VHH and VNAR. *Pharmaceutics* **12**, doi:10.3390/pharmaceutics12100937 (2020).

36 Ruiz-López, E. & Schuhmacher, A. J. Transportation of Single-Domain Antibodies through the Blood-Brain Barrier. *Biomolecules* **11**, doi:10.3390/biom11081131 (2021).

37 Wouters, Y., Jaspers, T., De Strooper, B. & Dewilde, M. Identification and in vivo characterization of a brain-penetrating nanobody. *Fluids Barriers CNS* **17**, 62, doi:10.1186/s12987-020-00226-z (2020).

38 Leitner, D. F. & Connor, J. R. Functional roles of transferrin in the brain. *Biochim Biophys Acta* **1820**, 393-402, doi:10.1016/j.bbagen.2011.10.016 (2012).

39 Johnsen, K. B. *et al.* Targeting transferrin receptors at the blood-brain barrier improves the uptake of immunoliposomes and subsequent cargo transport into the brain parenchyma. *Sci Rep* **7**, 10396, doi:10.1038/s41598-017-11220-1 (2017).

40 Zhang, X. A. *et al.* A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med* **387**, 470-472, doi:10.1056/NEJMc2202705 (2022).

41 Briese, T. *et al.* Genetic detection and characterization of Lujo virus, a new hemorrhagic fever-associated arenavirus from southern Africa. *PLoS Pathog* **5**, e1000455, doi:10.1371/journal.ppat.1000455 (2009).

42 Delgado, S. *et al.* Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. *PLoS Pathog* **4**, e1000047, doi:10.1371/journal.ppat.1000047 (2008).

43 Chan, Y. P. *et al.* Biochemical, conformational, and immunogenic analysis of soluble trimeric forms of henipavirus fusion glycoproteins. *J Virol* **86**, 11457-11471, doi:10.1128/jvi.01318-12 (2012).

44 Bradel-Tretheway, B. G., Liu, Q., Stone, J. A., McInally, S. & Aguilar, H. C. Novel Functions of Hendra Virus G N-Glycans and Comparisons to Nipah Virus. *J Virol* **89**, 7235-7247, doi:10.1128/jvi.00773-15 (2015).

45 Bonaparte, M. I. *et al.* Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A* **102**, 10652-10657, doi:10.1073/pnas.0504887102 (2005).

46 Negrete, O. A. *et al.* EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* **436**, 401-405, doi:10.1038/nature03838 (2005).

47 Negrete, O. A., Chu, D., Aguilar, H. C. & Lee, B. Single amino acid changes in the Nipah and Hendra virus attachment glycoproteins distinguish ephrinB2 from ephrinB3 usage. *J Virol* **81**, 10804-10814, doi:10.1128/jvi.00999-07 (2007).

48 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

49 Bradel-Tretheway, B. G. *et al.* Nipah and Hendra Virus Glycoproteins Induce Comparable Homologous but Distinct Heterologous Fusion Phenotypes. *J Virol* **93**, doi:10.1128/jvi.00577-19 (2019).

50 Xu, K. *et al.* Crystal Structure of the Pre-fusion Nipah Virus Fusion Glycoprotein Reveals a Novel Hexamer-of-Trimers Assembly. *PLoS Pathog* **11**, e1005322, doi:10.1371/journal.ppat.1005322 (2015).

51 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

52 Amaya, M. *et al.* A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses* **15**, doi:10.3390/v15051077 (2023).

53 Khetawat, D. & Broder, C. C. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol J* **7**, 312, doi:10.1186/1743-422x-7-312 (2010).

54 Li, S. *et al.* Acidic pH-Induced Conformations and LAMP1 Binding of the Lassa Virus Glycoprotein Spike. *PLoS Pathog* **12**, e1005418, doi:10.1371/journal.ppat.1005418 (2016).

55 Schlie, K. *et al.* Characterization of Lassa virus glycoprotein oligomerization and influence of cholesterol on virus replication. *J Virol* **84**, 983-992, doi:10.1128/jvi.02039-09 (2010).

56 Pontremoli, C., Forni, D. & Sironi, M. Arenavirus genomics: novel insights into viral diversity, origin, and evolution. *Curr Opin Virol* **34**, 18-28, doi:10.1016/j.coviro.2018.11.001 (2019).

57 Bederka, L. H., Bonhomme, C. J., Ling, E. L. & Buchmeier, M. J. Arenavirus stable signal peptide is the keystone subunit for glycoprotein complex organization. *mBio* **5**, e02063, doi:10.1128/mBio.02063-14 (2014).

58 Hastie, K. M. *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928, doi:10.1126/science.aam7260 (2017).

59 Igonet, S. *et al.* X-ray structure of the arenavirus glycoprotein GP2 in its postfusion hairpin conformation. *Proc Natl Acad Sci U S A* **108**, 19967-19972, doi:10.1073/pnas.1108910108 (2011).

60 York, J. & Nunberg, J. H. Myristoylation of the Arenavirus Envelope Glycoprotein Stable Signal Peptide Is Critical for Membrane Fusion but Dispensable for Virion Morphogenesis. *J Virol* **90**, 8341-8350, doi:10.1128/jvi.01124-16 (2016).

61 Eichler, R. *et al.* Identification of Lassa virus glycoprotein signal peptide as a trans-acting maturation factor. *EMBO Rep* **4**, 1084-1088, doi:10.1038/sj.embor.embor7400002 (2003).

62 Katz, M. *et al.* Structure and receptor recognition by the Lassa virus spike complex. *Nature* **603**, 174-179, doi:10.1038/s41586-022-04429-2 (2022).

63 Baize, S. *et al.* Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. *J Virol* **83**, 5890-5903, doi:10.1128/jvi.01948-08 (2009).

64 Fisher-Hoch, S. P., Hutwagner, L., Brown, B. & McCormick, J. B. Effective vaccine for lassa fever. *J Virol* **74**, 6777-6783, doi:10.1128/jvi.74.15.6777-6783.2000 (2000).

65 Sommerstein, R. *et al.* Arenavirus Glycan Shield Promotes Neutralizing Antibody Evasion and Protracted Infection. *PLoS Pathog* **11**, e1005276, doi:10.1371/journal.ppat.1005276 (2015).

66 Warner, B. M., Safronetz, D. & Stein, D. R. Current research for a vaccine against Lassa hemorrhagic fever virus. *Drug Des Devel Ther* **12**, 2519-2527, doi:10.2147/dddt.S147276 (2018).

67 Lukashevich, I. S. & Pushko, P. Vaccine platforms to control Lassa fever. *Expert Rev Vaccines* **15**, 1135-1150, doi:10.1080/14760584.2016.1184575 (2016).

68 Robinson, J. E. *et al.* Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits. *Nat Commun* **7**, 11544, doi:10.1038/ncomms11544 (2016).

69 Gorman, J. *et al.* Prefusion-Stabilized Lassa Virus Trimer Identifies Neutralizing Nanobodies and Reveals an Apex-Situated Site of Vulnerability. *bioRxiv*, 2022.2004.2021.488985, doi:10.1101/2022.04.21.488985 (2022).

70 Abreu-Mota, T. *et al.* Non-neutralizing antibodies elicited by recombinant Lassa-Rabies vaccine are critical for protection against Lassa fever. *Nat Commun* **9**, 4223, doi:10.1038/s41467-018-06741-w (2018).

71 Brouwer, P. J. M. *et al.* Lassa virus glycoprotein nanoparticles elicit neutralizing antibody responses and protection. *Cell Host Microbe* **30**, 1759-1772.e1712, doi:10.1016/j.chom.2022.10.018 (2022).

72 Amanat, F. *et al.* Monoclonal Antibodies with Neutralizing Activity and Fc-Effector Functions against the Machupo Virus Glycoprotein. *J Virol* **94**, doi:10.1128/jvi.01741-19 (2020).

73 Xu, K. *et al.* Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

74 Xu, K. *et al.* New insights into the Hendra virus attachment and entry process from structures of the virus G glycoprotein and its complex with Ephrin-B2. *PLoS One* **7**, e48742, doi:10.1371/journal.pone.0048742 (2012).

75 Xu, K. *et al.* Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. *Proc Natl Acad Sci U S A* **105**, 9953-9958, doi:10.1073/pnas.0804797105 (2008).

76 Steffen, D. L., Xu, K., Nikolov, D. B. & Broder, C. C. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. *Viruses* **4**, 280-308, doi:10.3390/v4020280 (2012).

77 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

78 Burri, D. J., da Palma, J. R., Kunz, S. & Pasquato, A. Envelope glycoprotein of arenaviruses. *Viruses* **4**, 2162-2181, doi:10.3390/v4102162 (2012).

79 Koma, T. *et al.* Glycoprotein N-linked glycans play a critical role in arenavirus pathogenicity. *PLoS Pathog* **17**, e1009356, doi:10.1371/journal.ppat.1009356 (2021).

80 Pennington, H. N. & Lee, J. Lassa virus glycoprotein complex review: insights into its unique fusion machinery. *Biosci Rep* **42**, doi:10.1042/bsr20211930 (2022).

81 Brisse, M. E. & Ly, H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. *Front Immunol* **10**, 372, doi:10.3389/fimmu.2019.00372 (2019).

82 Shao, J., Liang, Y. & Ly, H. Human hemorrhagic Fever causing arenaviruses: molecular mechanisms contributing to virus virulence and disease pathogenesis. *Pathogens* **4**, 283-306, doi:10.3390/pathogens4020283 (2015).

83 Sanders, R. W. & Moore, J. P. Virus vaccines: proteins prefer prolines. *Cell Host Microbe* **29**, 327-333, doi:10.1016/j.chom.2021.02.002 (2021).

84 Li, H. *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991, doi:10.1126/scitranslmed.abq0991 (2022).

85 Bhowmik, A., Khan, R. & Ghosh, M. K. Blood brain barrier: a challenge for effectual therapy of brain tumors. *Biomed Res Int* **2015**, 320941, doi:10.1155/2015/320941 (2015).

86 Pardridge, W. M. Drug transport across the blood-brain barrier. *J Cereb Blood Flow Metab* **32**, 1959-1972, doi:10.1038/jcbfm.2012.126 (2012).

87 Pandit, R., Chen, L. & Götz, J. The blood-brain barrier: Physiology and strategies for drug delivery. *Adv Drug Deliv Rev* **165-166**, 1-14, doi:10.1016/j.addr.2019.11.009 (2020).

88 Wu, D. *et al.* The blood-brain barrier: structure, regulation, and drug delivery. *Signal Transduct Target Ther* **8**, 217, doi:10.1038/s41392-023-01481-w (2023).

89 Hersh, D. S. *et al.* Evolving Drug Delivery Strategies to Overcome the Blood Brain Barrier. *Curr Pharm Des* **22**, 1177-1193, doi:10.2174/1381612822666151221150733 (2016).

90 Pulgar, V. M. Transcytosis to Cross the Blood Brain Barrier, New Advancements and Challenges. *Front Neurosci* **12**, 1019, doi:10.3389/fnins.2018.01019 (2018).

91 Zhang, W. *et al.* Differential expression of receptors mediating receptor-mediated transcytosis (RMT) in brain microvessels, brain parenchyma and peripheral tissues of the mouse and the human. *Fluids Barriers CNS* **17**, 47, doi:10.1186/s12987-020-00209-0 (2020).

92 Edavettal, S. *et al.* Enhanced delivery of antibodies across the blood-brain barrier via TEMs with inherent receptor-mediated phagocytosis. *Med* **3**, 860-882.e815, doi:10.1016/j.medj.2022.09.007 (2022).

93 Abulrob, A., Sprong, H., Van Bergen en Henegouwen, P. & Stanimirovic, D. The blood-brain barrier transmigrating single domain antibody: mechanisms of transport and antigenic epitopes in human brain endothelial cells. *J Neurochem* **95**, 1201-1214, doi:10.1111/j.1471-4159.2005.03463.x (2005).

94 Rissiek, B., Koch-Nolte, F. & Magnus, T. Nanobodies as modulators of inflammation: potential applications for acute brain injury. *Front Cell Neurosci* **8**, 344, doi:10.3389/fncel.2014.00344 (2014).

95 Tsitokana, M. E., Lafon, P. A., Prézeau, L., Pin, J. P. & Rondard, P. Targeting the Brain with Single-Domain Antibodies: Greater Potential Than Stated So Far? *Int J Mol Sci* **24**, doi:10.3390/ijms24032632 (2023).

96 Chakraborty, S. *et al.* Langya virus, a newly identified Henipavirus in China - Zoonotic pathogen causing febrile illness in humans, and its health concerns: Current knowledge and counteracting strategies - Correspondence. *Int J Surg* **105**, 106882, doi:10.1016/j.ijsu.2022.106882 (2022).

97 Simulundu, E. *et al.* Lujo viral hemorrhagic fever: considering diagnostic capacity and preparedness in the wake of recent Ebola and Zika virus outbreaks. *Rev Med Virol* **26**, 446-454, doi:10.1002/rmv.1903 (2016).

98 Cohen-Dvashi, H., Kilimnik, I. & Diskin, R. Structural basis for receptor recognition by Lujo virus. *Nat Microbiol* **3**, 1153-1160, doi:10.1038/s41564-018-0224-5 (2018).

99 Loayza Mafayle, R. *et al.* Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. *N Engl J Med* **386**, 2283-2294, doi:10.1056/NEJMoa2110339 (2022).

100 Escalera-Antezana, J. P. *et al.* Clinical features of fatal cases of Chapare virus hemorrhagic fever originating from rural La Paz, Bolivia, 2019: A cluster analysis. *Travel Med Infect Dis* **36**, 101589, doi:10.1016/j.tmaid.2020.101589 (2020).

From: Xu, Kai on behalf of Xu, Kai <xu.4692@osu.edu>
To: Haller, Sherry
Cc: Lindsey Marie Hornsby; Jianliang Xu; Workman, Jeff; Geisbert, Thomas W.; CHRISTOPHER BRODER; jxu40@gsu.edu
Subject: Re: OSU/GSU RP5 Final Documents
Date: Tuesday, June 6, 2023 10:55:20 AM
Attachments: RP5_Research Strategy_U19-UTMB_USU_final.pdf
image001.png

Hi Sherry,

RP5 Research Plan with some minor adjustments.

Thanks,
Kai

From: Xu, Kai <xu.4692@osu.edu>
Date: Monday, June 5, 2023 at 4:54 PM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Jianliang Xu <jianliang.xu@gmail.com>, Workman, Jeff <workman.45@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: Re: OSU/GSU RP5 Final Documents

Hi Sherry,

Here is the research strategy of RP5.

Thanks,
Kai

From: Haller, Sherry <shhaller@UTMB.EDU>
Date: Monday, June 5, 2023 at 1:39 PM
To: Xu, Kai <xu.4692@osu.edu>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Jianliang Xu <jianliang.xu@gmail.com>, Workman, Jeff <workman.45@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: RE: OSU/GSU RP5 Final Documents

Thanks, Kai. We do not need a narrative for your research project. This is only included in the overall component.

From: Xu, Kai <xu.4692@osu.edu>
Sent: Monday, June 5, 2023 12:08 PM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>; Jianliang Xu <jianliang.xu@gmail.com>; Workman,

Jeff <workman.45@osu.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; jxu40@gsu.edu
Subject: Re: OSU/GSU RP5 Final Documents

Here is an update for resource sharing plan.

Do we need project narrative?

Thanks,
Kai

From: Xu, Kai <xu.4692@osu.edu>
Date: Monday, June 5, 2023 at 11:42 AM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Jianliang Xu <jianliang.xu@gmail.com>, Workman, Jeff <workman.45@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: Re: OSU/GSU RP5 Final Documents

Hi Sherry,

Please see attached documents from Jianliang and me.
Including:

1. Authentication of key resource (not sure if you received this last time)
2. Abstract (not sure if you received this last time)
3. LOS from me. (not sure if you received this last time)
4. MPI plan
5. Updated vertebrate animal
6. Updated specific aims

Research plan will be ready later today.

Thanks,
Kai

From: Xu, Kai <xu.4692@osu.edu>
Date: Thursday, June 1, 2023 at 3:31 AM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Jianliang Xu <jianliang.xu@gmail.com>, Workman, Jeff <workman.45@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>,

CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: Re: OSU/GSU RP5 Final Documents

Hi Sherry,

Here are the requested documents.

Thanks,
Kai

From: Jianliang Xu <jianliang.xu@gmail.com>
Date: Thursday, June 1, 2023 at 12:20 AM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Workman, Jeff <workman.45@osu.edu>, Xu, Kai <xu.4692@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: Re: OSU/GSU RP5 Final Documents

Hi Sherry,

Thanks for the reminder. Attached please find my LOS for the application. Kai will be sending Abstract, Authentication of materials and his LOS shortly.

My GSU position starts today, June 1, 2023. I am ccing my GSU email **jxu40@gsu.edu** and I will communicate with the team from my GSU account from now on.

Best regards,
Jianliang

On Tue, May 30, 2023 at 1:31 PM Haller, Sherry <shhaller@utmb.edu> wrote:

> Hello Dr. Xu and Dr. Xu,
> This is a reminder of the documents that we still need to get from you both for RP5 on the UTMB/USUHS U19 application. Our administrative review is this coming Thursday, June 1, so we will need all of the items listed below in final form by **EOB tomorrow, Wednesday, May 31**.
>
> - Abstract- 30 lines, 1 for RP5
> - Authentication of Key Biological Materials- 1 for RP5
> - Letter of Support – If both PI's could provide letters o support for your role on this project, we can include them. This is optional, but strengthens the proposal.
>
> For the final application, please provide the following by **EOB Monday June 5** *(hard deadline!)*.

- Research Strategy – Please remember to include references for both items listed below from the RFA for your research project:

Milestone Plan: In a clearly labeled section titled "Project Milestones and Timelines" include a clear delineation of goals with measurable milestones, including detailed quantitative and qualitative criteria for Go/No-Go decision-making, and a timeline for the attainment of each goal and milestone and should be reflected in the Milestone Plan for the overall Program. This plan must include Go/No-Go criteria to be met by the end of Year 3 of the award for continuation to Phase II. Milestones must specify the outcome(s) for each activity. Milestones should be quantifiable and scientifically justified, and include the completion of major research study activities, for example, identification of protective epitopes, animal model development, vaccine or mAb candidate down-selection, identification of correlates of protection, validation of vaccine or mAb strategies for other family members, and analysis, sharing and publication of final data. Milestone criteria should not simply be a restatement of the specific aims. Using a Gantt chart or equivalent tool, describe the associated timelines and identified outcomes for the research Center.

Industry Expertise and Regulatory Considerations: For projects proposing early vaccine development, describe how industry partners will be identified and incorporated into the proposed project including a timeline for inclusion. For projects proposing IND-enabling later stage vaccine development, NIAID requires Centers to include active participation of an industry partner to ensure access to vaccine technology platforms, expertise in manufacturing, clinical development, and regulatory pathways. Applicants should describe the role of this partner in the proposed project and/or team to facilitate discovery, candidate evaluation and/or product development. For the purpose of this FOA, "industry" is defined as a large or small, domestic or foreign, pharmaceutical, biotechnology, bioengineering, or chemical company, or a related non-profit entity.

Please let me know if you have any questions about these or have any concerns about getting them back to us by the deadlines.
Thank you,

Sherry L. Haller, PhD
Associate Director
Center for Biodefense and Emerging Infectious Diseases
The University of Texas Medical Branch
Keiller Bldg., Rm. 1.104D
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RESEARCH STRATEGY:

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness [3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success [4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability [5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other Arenaviruses and Nairoviruses.

***<u>The Arenaviridae</u>*:** All Mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. [9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

<u>The *Nairoviridae*</u>: Unique from other Bunyaviruses, Orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic Orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [20]. Like Arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

<u>Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens:</u> The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine [9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

<u>Unique Nairovirus subunit vaccine strategy utilizing GP and NP immunogens:</u> CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation [20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface [20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

<u>PREP "vaccination" as a measure to control Arenaviruses:</u> For situations where a vaccine does not exist or is not appropriate (*e.g.*, immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE [34, 35] and LA [36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families.

These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select Mammarenaviruses and Orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

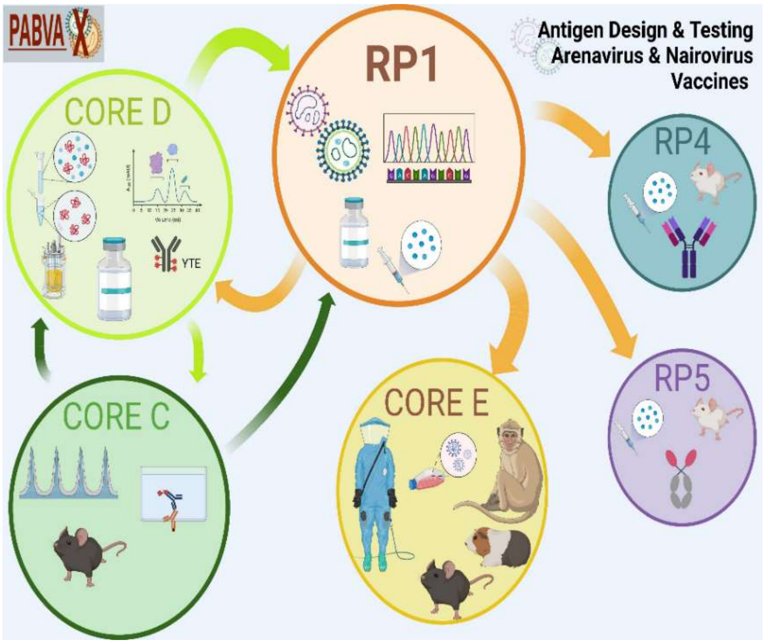


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of

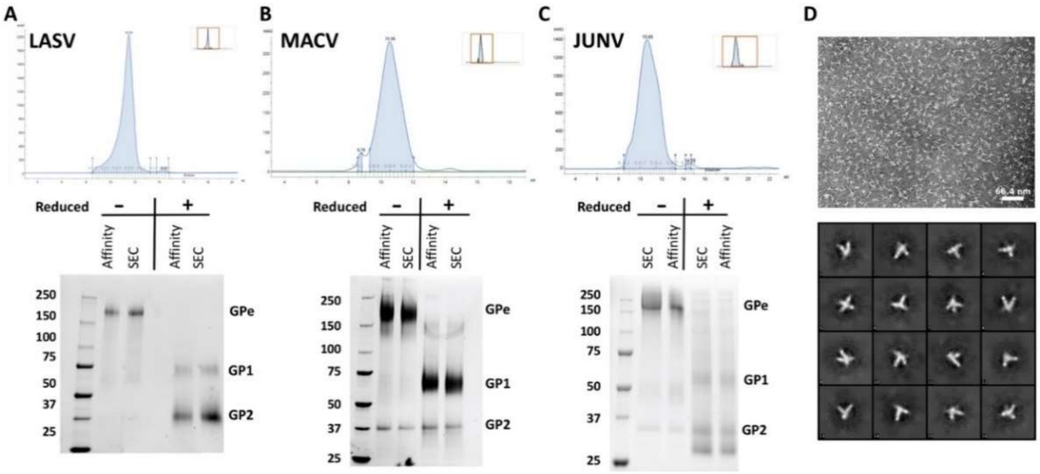


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

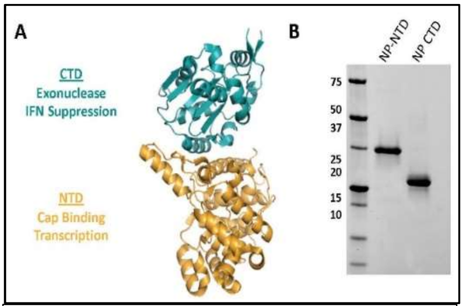
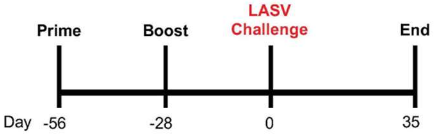


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD**



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

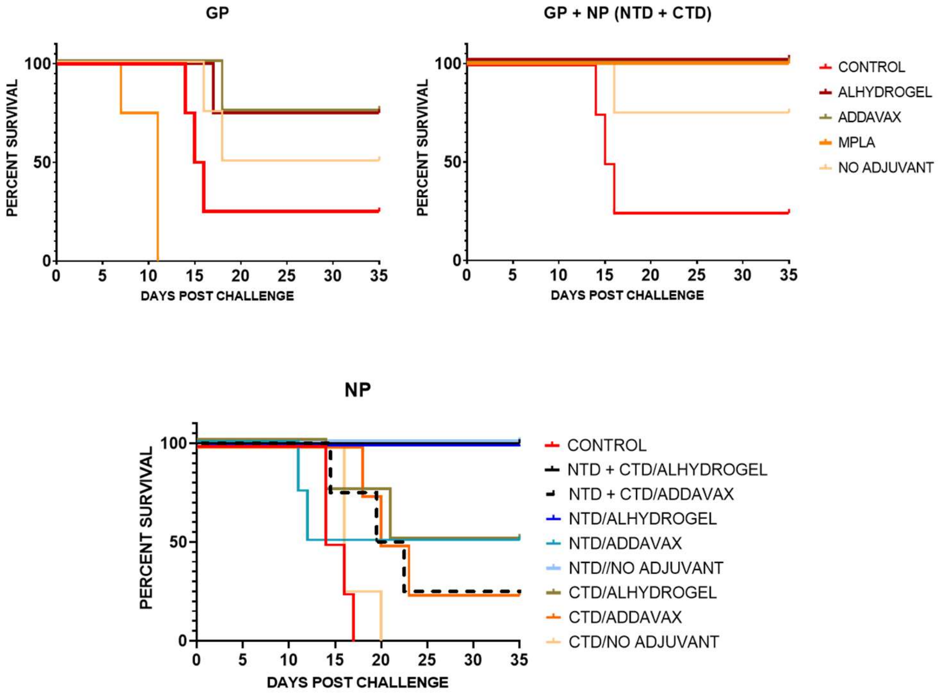


Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

subunits and any of the adjuvants were completely protected from challenge with LASV. Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

Engineering glycoproteins antigens for CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

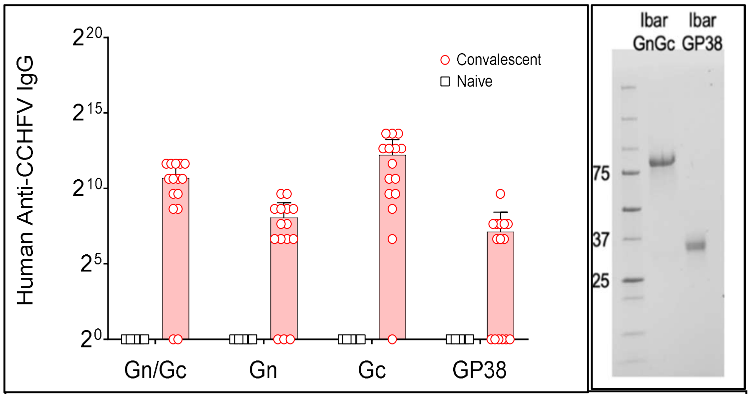


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

Scientific Approach: The choice of Arenavirus Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Arenaviruses (OWAV):* In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Arenaviruses (NWAV):* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Nairoviruses:* In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance

towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model [53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38.

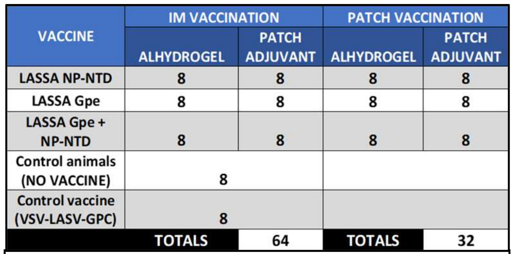
Once a candidate subunit-patch vaccine approach is selected, this RP1 will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative, “test case” human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case’s medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal “test case” OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center’s **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne Mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV as part of the Center’s **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center’s **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families [46, 61, 62] . An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared

with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.



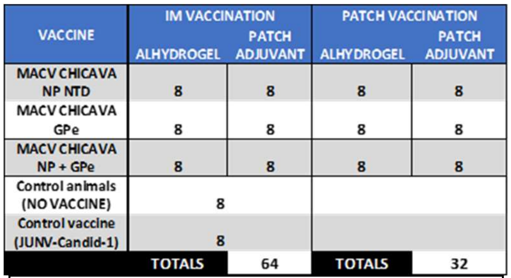
VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

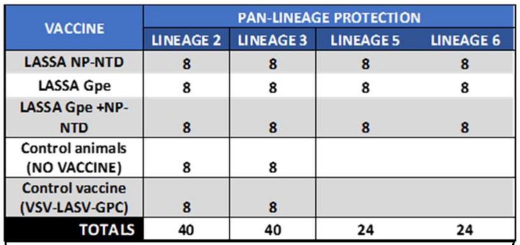
Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].



VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.B

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]**.** If Candid-1 confers protection in guinea pig study outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs [46, 47]

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	**TOTALS**	112

Table 7: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
TOTALS		128	TOTALS	112

Table 8: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10)**

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

***Milestone* 1.1.3: Determination of cellular and humoral immune correlates associated with protection.**
Milestone 1.1.3.A: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

<u>Immune subset phenotyping</u>**:** We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

<u>*Viral protein stimulation experiments*</u>: PBMC stimulations will be set up according to modifications of well established, previously published protocols.[67, 68] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

<u>*Intracellular cytokine staining*: We</u> will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

<u>*Multiplex Bead based assays:* </u>As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

<u>*IFNg/TNFa ELISPOT*</u>: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

<u>*Data Analysis:*</u> We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

<u>*Alternative Strategies:*</u> Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

***Milestone 1.1.3.A:: Systems serology Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups**:* We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument .[70, 72] Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

***Milestone* 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing Mammarenaviruses[62, 73] and Orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog [74] but is still pathogenic in mice and humans . There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months([34-37]).

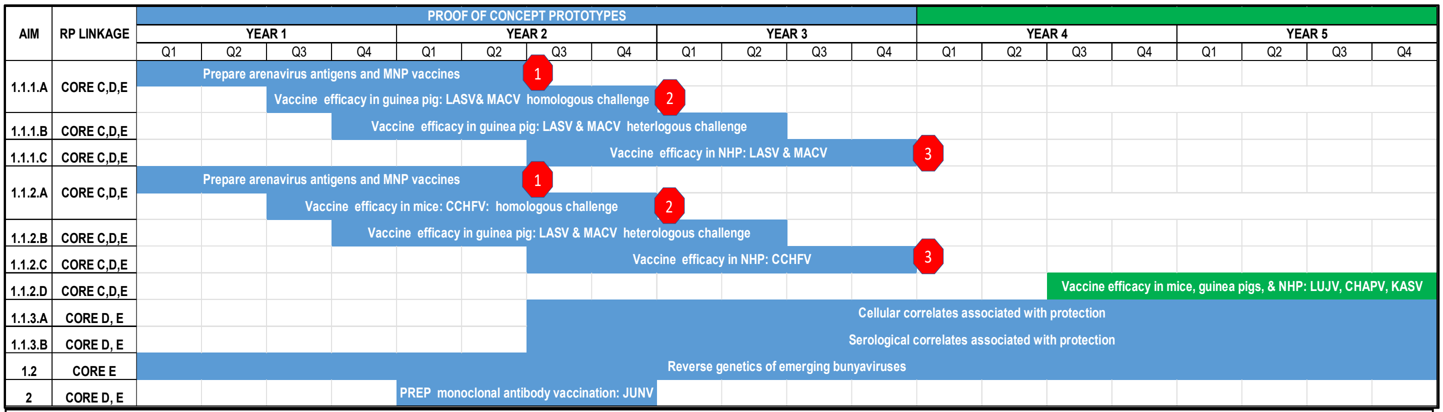
PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

***Scientific Approach**: A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine:* In conjunction with Cores D and E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11).** For further details of the NHP testing, please see **Core E**. These data will be used by the Scientific Advisory Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the "go" criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and SAC. *Please note: Discussion of expected results, other alternative approaches, and criteria for "go" or "no go" decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

References:

1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.
2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.
5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.
6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.
8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.
9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.
10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia I, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.
11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.
12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.
13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.

16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.

17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.

18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.

19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.

20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.

21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.

22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.

23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 2019/05/28. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.

24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.

25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.

26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.

27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.

28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.

29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.

30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF. Lassa fever in post-conflict sierra leone. PLoS Negl Trop Dis. 2014;8(3):e2748. Epub 20140320. doi: 10.1371/journal.pntd.0002748. PubMed PMID: 24651047; PMCID: PMC3961205.

43. Li AL, Grant D, Gbakie M, Kanneh L, Mustafa I, Bond N, Engel E, Schieffelin J, Vandy MJ, Yeh S, Shantha JG. Ophthalmic manifestations and vision impairment in Lassa fever survivors. PLoS One. 2020;15(12):e0243766. doi: 10.1371/journal.pone.0243766.

44. Ezeomah C, Adoga A, Ihekweazu C, Paessler S, Cisneros I, Tomori O, Walker D. Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. Open Forum Infect Dis. 2019;6(12):ofz512. Epub 20191203. doi: 10.1093/ofid/ofz512. PubMed PMID: 31879673; PMCID: PMC6923636.

45. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

46. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.

47. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.

48. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

49. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.

50. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.

51. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.

52. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.

53. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.

54. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.

55. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.

56. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.

57. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.

58. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.

59. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.

60. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.

61. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

62. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

63. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

64. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

65. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

66. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

67. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods. 2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

68. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

69. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

70. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

71. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

72. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

73. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

74. Walker PJ, Widen SG, Firth C, Blasdell KR, Wood TG, Travassos da Rosa AP, Guzman H, Tesh RB, Vasilakis N. Genomic Characterization of Yogue, Kasokero, Issyk-Kul, Keterah, Gossas, and Thiafora Viruses: Nairoviruses Naturally Infecting Bats, Shrews, and Ticks. Am J Trop Med Hyg. 2015;93(5):1041-51. Epub 20150831. doi: 10.4269/ajtmh.15-0344. PubMed PMID: 26324724; PMCID: PMC4703270.

75. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

76. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D,

Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

SPECIFIC AIMS

The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six Arenaviruses:Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV), are NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness. These threats are further heightened due to **lack of internationally approved vaccines** to counter natural epidemics and potential bio-weaponization. To address this unmet need, **Research Project 1 (RP1)** will leverage the combined expertise in high-containment virology, immunology, and biological product development, to develop Arenavirus and Nairovirus research tools and vaccine approaches which can be adapted across members of each viral family in a **"plug-and-play"** manner. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates concerns of efficacy as new related viruses continue to emerge and cause disease. However, recent advances in reverse genetics of viruses have allowed the generation of infectious clones and recombinant Arenaviruses (LASV, MACV, LUJV, JUNV) and Nairoviruses (CCHFV) which will make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most Arenaviruses and Nairoviruses has largely centered on the critical role of the viral glycoproteins (GP) and nucleoproteins (NP) to incur natural immunity. We recently successfully engineered a recombinant, stabilized-trimeric form of the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GPe alone, co-delivered with LASV NP subunits, or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as simple, safe, recombinant protein immunogens. Indeed, subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP), which harness the potently immunoresponsive cutaneous microenvironments, using dissolvable MNPs embedded with recombinant viral glycoprotein antigens and adjuvants to elicit robust and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease and COVID-19, however little is known of the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of **RP1** are two-fold: (**1**) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed against Old and New World Arenaviruses or emerging Nairoviruses. (**2**) Develop monoclonal antibody PREP strategies which can provide protective immunity. Specifically, we will:

Specific Aim 1: Subunit immunogens and MNP vaccination. Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches.

- **Milestone 1.1.1.A-C: Development and optimization of MNP vaccines using Arenavirus subunits in guinea pigs and non-human primates.**
- **Milestone 1.1.2.A-C: Development and optimization of MNP vaccines using Nairovirus subunits; protection in STAT-1/KO mice and non-human primates.**
- **Milestone 1.1.2.D: Determine the protective efficacy of optimized MNP vaccines against emerging Arenavirus and Nairovirus "test case" viruses in STAT-1/KO mice, guinea pigs and NHP.**
- **Milestone 1.1.3. A-B: Define the cellular and humoral immune correlates associated with protection.**
- **Milestone 1.2: Recombinantly rescue important naturally occurring Arenaviruses and Nairoviruses known only by genetic data.**

Specific Aim 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments. Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.

RESEARCH STRATEGY:

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness [3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success [4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability [5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other Arenaviruses and Nairoviruses.

***<u>The Arenaviridae</u>*: All Mammarenaviruses (Arenaviridae genus of viruses infecting mammals) share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. [9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

<u>The *Nairoviridae*</u><u>:</u> Unique from other Bunyaviruses, Orthonairoviruses (Nairoviridae genus of human pathogens) are transmitted primarily by ticks in nature. While there are a number of human pathogenic Orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [20]. Like Arenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine [9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique Nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation [20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface [20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control Arenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g.*, immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE [34, 35] and LA [36, 37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families.

These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for select Mammarenaviruses and Orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop BSL4 research tools and animal models for viruses that are otherwise not available to the research community.

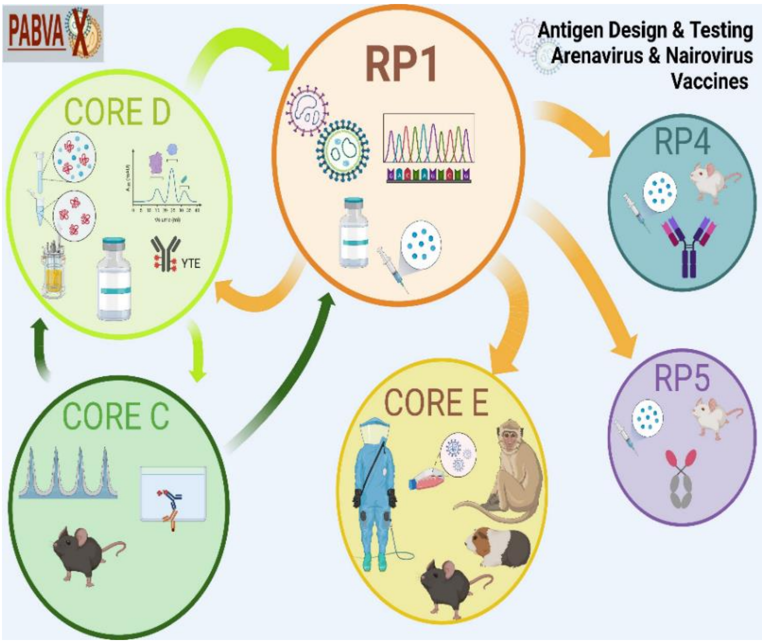


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of

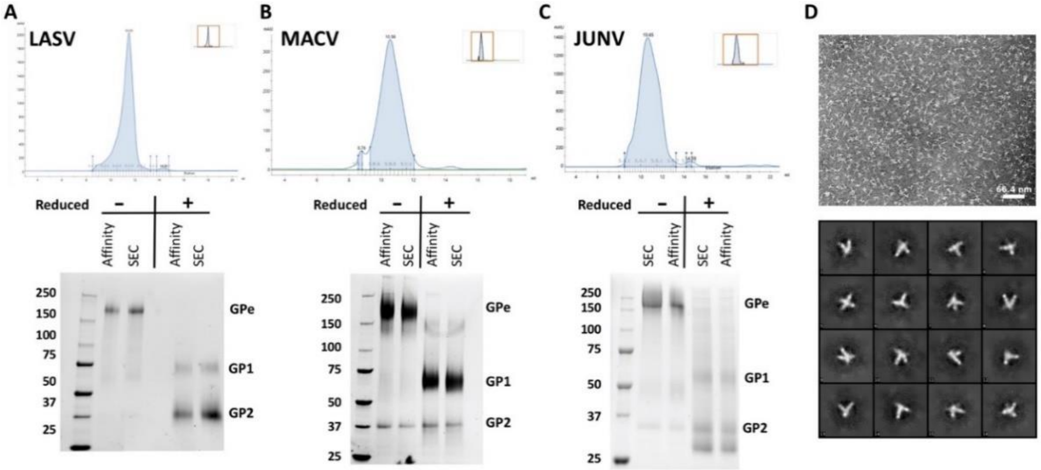


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

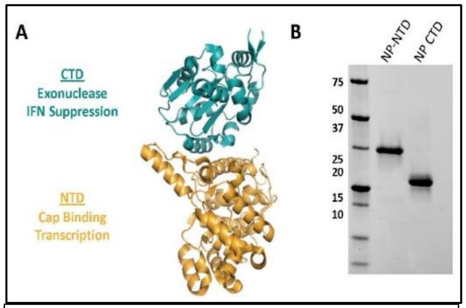
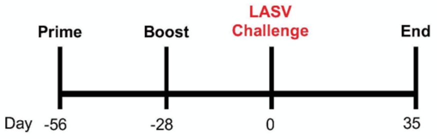


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD**



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

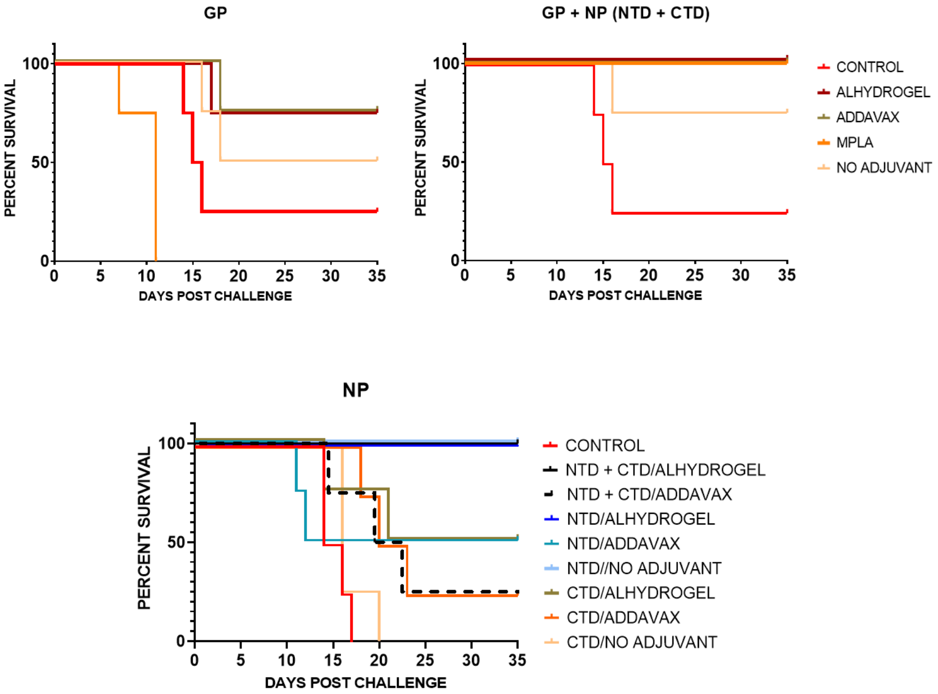


Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

subunits and any of the adjuvants were completely protected from challenge with LASV. Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

Engineering glycoproteins antigens for CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

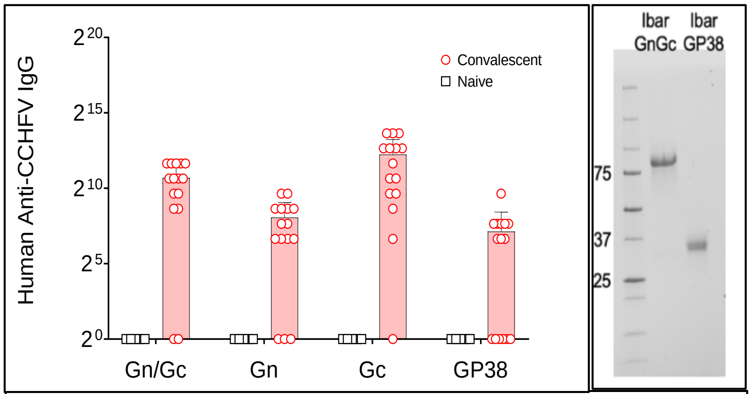


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

Scientific Approach: The choice of Arenavirus Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Arenaviridae and the Nairoviridae. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Arenaviruses (OWAV):* In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae [42-44] . There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Arenaviruses (NWAV):* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [45], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [46, 47]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[48]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Nairoviruses:* In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the Nairoviridae family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[49]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[50]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[50]; however only an MVA-CCHFV vaccine has begun to advance

towards clinical trials[51]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[52]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model [53] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38.

Once a candidate subunit-patch vaccine approach is selected, this RP1 will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative, “test case” human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case’s medical evacuation to South Africa[54]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal “test case” OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[55] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center’s **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne Mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[56, 57]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[57]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV as part of the Center’s **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [58]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[59]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[58]. (The PABVAX Center will explore **KASV** as part of the Center’s **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[60]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families [46, 61, 62] . An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared

with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[63]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[64]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[46, 47].

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[65] challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[66]**.** If Candid-1 confers protection in guinea pig study outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs [46, 47]

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.B

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[52]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
	TOTALS	128	TOTALS	112

Table 8: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10)**

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

***Milestone* 1.1.3: Determination of cellular and humoral immune correlates associated with protection.**

Milestone 1.1.3.A: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping**:** We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols.[67, 68] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.A:: Systems serology Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[69-71] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument .[70, 72] Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

***Milestone* 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing Mammarenaviruses[62, 73] and Orthonairovirus[61] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog [74] but is still pathogenic in mice and humans . There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months(34-37).

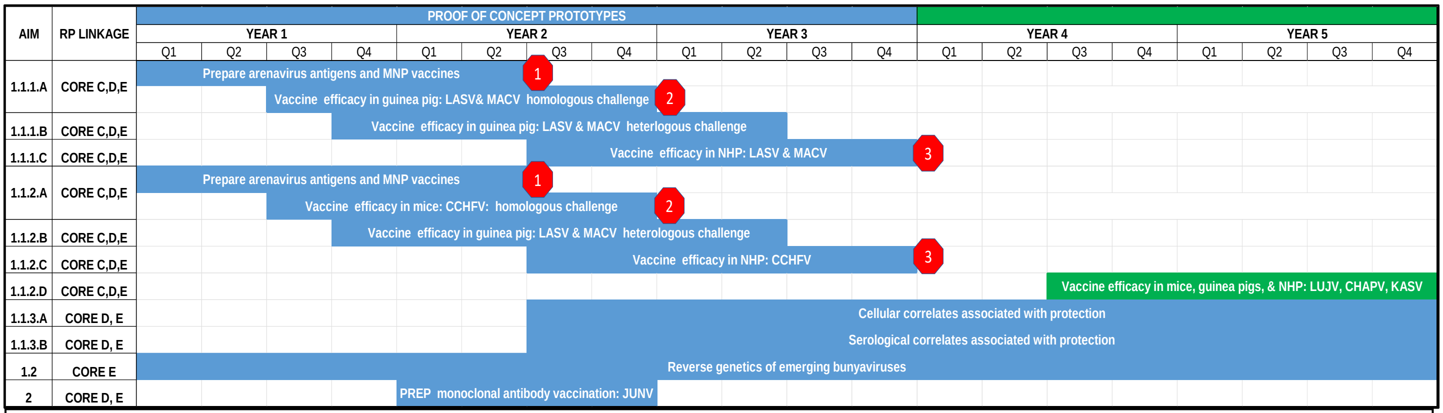
PREP	JUNV NHP CHALLENGE
5 mg/kg	**5**
15mg/kg	**5**
Control animals (NO PREP)	**5**
TOTALS	**15**

Table 11: PREP schedule outline in Milestone 2

Scientific Approach*: A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine:* In conjunction with Cores D and E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[75, 76] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11).** For further details of the NHP testing, please see **Core E**. These data will be used by the Scientific Advisory Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three “test case” viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the “go” criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and SAC. *Please note: Discussion of expected results, other alternative approaches, and criteria for “go” or “no go” decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future “plug and play” pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

References:

1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.
2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.
5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.
6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.
8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.
9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.
10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia l, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.
11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.
12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.
13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.
15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.
16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.
17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.
18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.
19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.
20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.
21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.
22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.
23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 2019/05/28. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.
24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.
25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.
26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.
27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.
28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.
29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.
30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF. Lassa fever in post-conflict sierra leone. PLoS Negl Trop Dis. 2014;8(3):e2748. Epub 20140320. doi: 10.1371/journal.pntd.0002748. PubMed PMID: 24651047; PMCID: PMC3961205.

43. Li AL, Grant D, Gbakie M, Kanneh L, Mustafa I, Bond N, Engel E, Schieffelin J, Vandy MJ, Yeh S, Shantha JG. Ophthalmic manifestations and vision impairment in Lassa fever survivors. PLoS One. 2020;15(12):e0243766. doi: 10.1371/journal.pone.0243766.

44. Ezeomah C, Adoga A, Ihekweazu C, Paessler S, Cisneros I, Tomori O, Walker D. Sequelae of Lassa Fever: Postviral Cerebellar Ataxia. Open Forum Infect Dis. 2019;6(12):ofz512. Epub 20191203. doi: 10.1093/ofid/ofz512. PubMed PMID: 31879673; PMCID: PMC6923636.

45. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

46. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.

47. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.

48. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

49. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.

50. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.

51. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.

52. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.

53. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.

54. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.

55. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.

56. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.

57. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.

58. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.

59. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.

60. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.

61. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

62. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

63. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

64. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

65. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

66. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

67. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods. 2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

68. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

69. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

70. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

71. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

72. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

73. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

74. Walker PJ, Widen SG, Firth C, Blasdell KR, Wood TG, Travassos da Rosa AP, Guzman H, Tesh RB, Vasilakis N. Genomic Characterization of Yogue, Kasokero, Issyk-Kul, Keterah, Gossas, and Thiafora Viruses: Nairoviruses Naturally Infecting Bats, Shrews, and Ticks. Am J Trop Med Hyg. 2015;93(5):1041-51. Epub 20150831. doi: 10.4269/ajtmh.15-0344. PubMed PMID: 26324724; PMCID: PMC4703270.

75. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

76. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D,

Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

ABSTRACT:

The viral order *Bunyavirales* contains several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and some are associated with significant, long-term sequelae in survivors. Of these, rodent borne arenaviruses – Lassa (LASV), Lujo, Chapare, Guanarito, Junin and Machupo viruses and one tickborne Nairovirus-Crimean-Congo Hemorrhagic Fever Virus- are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, production of significant morbidity and mortality, and have potential for major public health impact, and require special action for public health preparedness. Threats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **PABVAX RP1** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches using prototype members of each viral group which can be adapted across each viral family using a **"plug-and-play"** approach. Much of the work developing vaccines for these viruses has relied on isolates derived over 40 years ago, recent advances in viral reverse for these viral families is making vaccine testing of emerging isolates more feasible by improving access. Vaccine development for most arenaviruses and nairoviruses has centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized prefusion LASV GPe to act as an antigenic mimic of viral surface displayed GP and found this trimeric GPe alone, co-delivered with NP, or NP subunits alone, can protect guinea pigs against lethal challenge by LASV underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches like microneedle patches (MNP). MNP coupled antigens and adjuvants directly interact with the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs to elicit robust and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. In this proposal, we will develop protective protein-based subunit-MNP vaccines, PREP treatment strategies, and recombinant virus tools using prototyped arenaviruses and nairoviruses which will template development of countermeasures against other related Bunyaviridae members.

From: Cross, Robert W. on behalf of Cross, Robert W. <rwcross@UTMB.EDU>
To: Geisbert, Thomas W.; CHRISTOPHER BRODER; Haller, Sherry
Subject: RE: RE:
Date: Monday, June 5, 2023 9:43:47 PM
Attachments: 05JUN23_RP1-BUNYAVIRALES VACCINES--RESEARCH STRATEGY_RWC_2034-clean.docx
05JUN23-UTMB REVAMPP SPECIFIC AIMS-RWC-ccb-RWC-accepted edits.docx
05JUN23_RP1-BUNYAVIRALES VACCINES--RESEARCH STRATEGY_RWC_2034-clean.pdf
05JUN23 UTMB REVAMP ABSTRACT_RWC.docx

Attached are clean versions of missing three documents. I have been having an issue of things getting moved around and fonts mysteriously changing, so I added a pdf version for reference.

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Monday, June 5, 2023 4:43 PM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Cross, Robert W. <rwcross@UTMB.EDU>; Haller, Sherry <shhaller@UTMB.EDU>
Subject: RE:

I would send a near final version this evening and we can swap out tomorrow morning.

Sent via the Samsung Galaxy Note9, an AT&T 5G Evolution capable smartphone

-------- Original message --------
From: "Broder, Christopher" <christopher.broder@usuhs.edu>
Date: 6/5/23 4:41 PM (GMT-06:00)
To: "Cross, Robert W." <rwcross@UTMB.EDU>, "Geisbert, Thomas W." <twgeisbe@UTMB.EDU>, "Haller, Sherry" <shhaller@UTMB.EDU>
Subject:

hi
do we have time for Bob's RP1?
did a draft get sent for UTMB review?

if so, then Bob, can you make an accepted edits version and
we can go from there? we have time to swap in the final version
before clicknig submit

PROJECT SUMMARY – CORE D: ANIMAL MODELS AND VACCINE EVALUATION

Among viruses that cause disease in humans viral zoonoses within the family Paramyxoviridae and order Bunyavirales contain pathogens that cause significant lethality. Importantly, within the Paramyxovirdae the zoonotic henipavirus Hendra virus (HeV) is on the World Health Organizations (WHO) Priority Pathogens list. Notably, henipaviruses stand out for their impressive lethality with case fatality rates averaging about 75%. The Arenaviridae and Phelboviridae families contain viruses that cause severe hemorrhagic disease in humans. These viruses include the arenaviruses Lassa virus (LASV) and Junin virus (JUNV) and the phleboviruses Rift Valley fever virus (RVFV) and severe fever with thrombocytopenia syndrome virus (SFTSV). LASV and RVFV are also included among the WHO List of Priority Pathogens and pose threats to global public health due to the absence of approved vaccines or therapeutics. There is a clear unmet need for medical countermeasures to combat the threat of natural outbreaks, epidemics, or deliberate release. The synergistic activities of the Center partners will be focused on developing novel vaccines, treatments, and tools to support pandemic preparedness. Federal law requires that the paramyxoviruses, arenaviruses, and phleboviruses used in the Center be handled in approved Biosafety Level (BSL)-3 and BSL-4 containment laboratories. Core D provides approved BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities and a trained and highly experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support Project 2 - 5. The services provided by Core D will include 1) a secure repository of well characterized seed stocks of BSL-3 and BSL-4 paramyxoviruses, arenaviruses, and phleboviruses and know-how to rescue synthetic infectious clones (e.g. Ghana virus, GhV for this U19); 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5); development and/or optimization of animal models for GhV, Sosuga virus (SOSV) and JUNV to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BSL-3 and BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-3 and BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies. The Animal Models and Vaccine Evaluation Scientific Core (Core D) provides BSL-3 and BSL-4 resources and expertise for Projects 2 – 5. The goal of Core D is to work closely with Research Project and Scientific Core Leaders and staff, the Scientific Advisory Board, and NIAID to advance the development of countermeasures against Paramyxoviridae and Bunyaviridae.

SPECIFIC AIMS – CORE D: ANIMAL MODELS AND VACCINE EVALUATION

The main objective of the Animal Models and Vaccine Evaluation Scientific Core is to test and characterize the host response and efficacy of the vaccines and monoclonal antibody (mAb) based treatments identified in **Projects 2, 3, 4 and 5** against paramyxoviruses, arenaviruses, and phleboviruses under BSL-3/ABSL-3 and BSL-4/ABSL-4 containment. The Core will also develop select reporter viruses and conduct cell-based assays to characterize vaccine-induced neutralizing antibody responses and mAbs.

The Specific Aims of the Animal Models and Vaccine Evaluation Scientific Core are to:

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses as well as develop reporter viruses and rescue a Ghana virus synthetic infectious cloneAim 2: Provide technical expertise and conduct *in vitro* assays.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, immunology assays, virology assays, necropsies, and histopathological analysis.

Aim 4: Develop and/or optimize animal models for Ghana virus, Sosuga virus and Junin virus.

The Aims will be achieved by the following specific functions to be carried out by the Animal Models and Vaccine Evaluation Scientific Core Leader, Co-Core Leaders, and personnel:

1. Maintain records of well characterized BSL-3 and BSL-4 Hendra virus (HeV), Sosuga virus (SOSV), Lassa virus (LASV), Junin virus (JUNV), Rift valley fever virus (RVFV), and severe fever with thrombocytopenia syndrome virus (SFTSV) seed stocks including background history, passage history, genomic information, periodic monitoring of titer, mycoplasma testing, endotoxin testing, and sterility testing.
2. Perform *in vitro* antiviral activity assays. Including high-throughput BSL-2 based neutralization testing of henipavirus vaccine responses and mAbs.
3. Assist Center Investigators in designing animal studies, prepare animal protocols, and obtain IACUC approval of BSL-3 and BSL-4 animal studies.
4. Perform animal challenges using well characterized HeV, SOSV, LASV, GhV, JUNV, RVFV, and SFTSV seed stocks for **Projects 2, 3, 4 and 5.**
5. Perform vaccination and treatment of animals with vaccines and treatments provided by **Projects 3, 4 and 5.**
6. Provide samples from animals collected during the vaccination portion of studies to **Project 2** for immunological analysis.
7. Perform all duties associated with animal challenge studies including monitoring of animals, collection of samples, and necropsies.
8. Perform clinical pathology assays including hematology and clinical chemistry assays.
9. Perform virology assays on blood and/or tissue samples collected from infected animals including viral load by plaque assay and RT-PCR.
10. Perform gross pathology analysis on all animals at necropsy.
11. Perform histopathologic analysis on tissues from animals collected at necropsy.
12. Develop and/or optimize animal models for SOSV and JUNV to fill gaps; make these models available to Center Research Projects and Scientific Cores as well as external ReVAMPP Centers
13. Provide all data generated in Core D to **Data Management Core B**.

RESEARCH STRATEGY – CORE D: ANIMAL MODELS AND VACCINE EVALUATION

SIGNIFICANCE

As discussed in the Overall Component, the Center will focus in Years 1-3 on Lassa Virus (LASV), Rift Valley fever virus (RVFV), and Hendra Virus (HeV) and in Years 4-5, will target Junín virus (JUNV; *Arenaviridae*), severe fever with thrombocytopenia syndrome virus (SFTSV; *Phenuiviridae*), Sosuga virus (SOSV; *Paramyxoviridae*), and Ghanianan bat henipavirus (GhV; *Paramyxoviridae*).As HeV, LASV, JUNV, and RVFV are CDC and/or USDA select agents, Federal regulations require that these viruses be handled in Biosafety Level (BSL)-3 or BSL-4 containment. **Core D provides approved BSL-3/ABSL-3 and BSL-4/BSL-4 facilities and a trained and experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support Projects 2, 3, 4, and 5.** The services provided by Core D will include 1) a secure repository of well characterized seed stocks of BSL-3 and BSL-4 paramyxoviruses, arenaviruses, and phenuiviruses and know-how to rescue synthetic infectious clones (e.g. GhV for this U19); 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5) development and/or optimization of animal models for strains or variants of Sosuga virus (SOSV), JUNV and Ghana virus (GhV) to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BSL-3 and BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-3 and BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies. **Core D** will work closely with the **Administrative Core** to ensure all biosafety work is adequately monitored.

INNOVATION

Scientific Core D will be co-located within the Galveston National Laboratory (GNL) and Robert Shope Laboratory at the University of Texas Medical Branch (UTMB) at Galveston. The GNL is a high and maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda and stands ready to assist local, state, and national public health efforts in the event of a public health emergency. The facility is unique in that it is one of two operational BSL-4 facilities in the United States that is located on an academic campus. This unique location benefits the Center in several ways. Regarding physical resources, the GNL is a state-of-the-art facility with many specialized assets including BSL-4 imaging, telemetry, and aerobiology equipment. In addition, being located on an academic campus **Drs. Geisbert, Cross, Freiberg, and Ikegami (Core D**) are faculty members in the Departments of Microbiology and Immunology and Pathology. This academic environment is a major strength that fosters creativity and collaboration with other UTMB investigators, which will be beneficial to the overall success of Core D. **Dr. Broder**, is faculty member of the Department of Microbiology and Immunology at Uniformed Services University (USU). Drs. Broder and Geisbert have a long-standing emerging virus research collaboration, which has included the development and characterization of the first nonhuman primate (NHP) models for HeV and Nipah virus (NiV) that will be employed by **Projects 2, 3, 4 and 5**. Another strength within the Core is that **Drs. Geisbert, Broder, Cross, and Veesler** currently collaborate and lead active henipavirus projects focused on translational countermeasure development, which includes more traditional vaccine and mAb therapeutics approaches. Their shared knowledge base and experience in these areas will greatly enhance the Core's abilities to support the Center's research goals in experimentally testing advanced machine learning-enabled designs of effective prototype pathogen vaccines and antibodies. Along with animal model development and animal modeling expertise, many of the unique virological tools that will be employed by the Core have been developed from the UTMB-USU collaboration, including recombinantly generated RNA viruses, reporter gene-encoding viruses, and other pseudotype virus technologies.

APPROACH

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well-characterized seed stocks of paramyxoviruses, arenaviruses, and phenuiviruses.

Paramyxovirus, arenavirus, and phenuivirus seed stocks. UTMB will provide fully equipped BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities that are required to work with HeV, SOSV, LASV, JUNV, RVFV, SFTSV and GhV. UTMB will also provide and maintain all required approvals to work with these viruses. The BSL-3 and BSL-4 paramyxovirus, arenavirus, and phenuivirus seed stocks have been or will be titered by conventional standard plaque assay and qRT-PCR as detailed in **Aim 2** below. The virus strain identity of each seed stock has been or will be confirmed through sequencing, and deep-sequencing will be used to verify the complete viral genome of each virus seed stock used for animal challenge studies. We have or will test each seed stock for mycoplasma contamination. Endotoxin testing has been or will be performed on paramyxovirus, arenavirus, and phenuivirus stocks using the Endosafe PTS system (Charles River Laboratories) following the manufacturer's instructions. The Endosafe PTS system is a cartridge-based testing system utilizing FDA-licensed cartridges for ultrasensitive testing. Seed stocks will also be evaluated for morphological identity by transmission electron microscopy (TEM). Virus particle counts will be determined using a Virus Counter and confirmed by conventional TEM methods, if needed. The bacteriological and mycological sterility of the product will be determined by selection of aliquots of the dispensed stock, inoculation of a sterile 500 ml bottle of Dulbecco's high glucose MEM with 10% FBS, and then after 3 days subcultured to FTM and TBS media. Paramyxovirus, arenavirus, and phenuivirus seed stocks will be inventoried in the UTMB BSL-4 and BSL-4 virus stock databases and secured in dedicated controlled access alarmed -80°C freezers in the GNL or Robert Shope Laboratory. Paramyxovirus, arenavirus, and phenuivirus seed stocks are monitored periodically for any changes in virus titer.

Plan for staffing, managing, and prioritizing Core use. The Core Leader and Co-Leaders have a cadre of staff that are trained and highly experienced in all BSL-3 and BSL-4 procedures and assays needed to conduct the proposed work. In addition to the Core Leader and Co-Leaders, this staff includes an ACVP board certified veterinary pathologist, two BSL-4 contributing scientists, a BSL-4 Technical Director, a BSL-4 trained postdoctoral fellow, six BSL-4 experienced technical staff, a BSL-4 trained histotechnologist, and a BSL-4 trained graduate student. We do not anticipate any BSL-3 or BSL-4 scheduling problems regarding studies proposed in **Projects 2, 3, 4, and 5**. However, we have developed a mitigation strategy if unforeseen events cause any needed prioritization of resources. A committee consisting of the Core D Leader, the PIs of the Center, and the members of the external Scientific Advisory Board (SAB) will review the status of these Projects and any circumstances necessitating a reprioritization of work. The SAB will also hold a meeting or teleconference (Zoom, Teams) with the Project Leaders and Key Personnel to gather additional information. After careful review of all available information, the SAB will then make a recommendation on prioritization to the Center PI. The Center PI will then consult with NIAID/NIH program staff and make the final determination on prioritization.

Aim 2: Provide technical expertise and conduct *in vitro* assays.

***In vitro* antiviral activity assays. Core D** will perform authentic virus neutralization assays for **Projects 2, 3, 4, and 5** to determine the ability of sera/plasma from immunized animals and mAbs to inhibit the replication of paramyxoviruses, arenaviruses, and phenuiviruses *in vitro* using infectious viruses in the UTMB BSL-3 and BSL-4 laboratories. In brief, cultures of Vero, Vero E6, SW13, or other cell types will be infected with a range of multiplicity of infection (MOI) of paramyxoviruses, arenaviruses, and bunyaviruses and subsequently treated various dilutions of sera/plasma or antibodies. Cells and/or culture fluids will be harvested at various time points after infection and infectious virus enumerated by conventional plaque assay as detailed below.

Develop chimeric reporter-gene encoding viruses and virus neutralization assays for BSL-2 use. We have previously used reverse genetics to generate several types of replication competent VSV-GFP reporter viruses including those encoding Australian bat lyssavirus (ABLV) G glycoproteins (VSV-ABLV G-GFP)[1,2], as well as employed reverse genetics to rescue authentic ABLV and various reporter gene-encoding viruses[3,4]. In addition, we have generated a reverse genetics platform for generating recombinant Cedar virus (rCedV) and rCedV chimeras, whereby the fusion (F) and attachment (G) glycoprotein genes of rCedV were replaced with those of NiV-Bangladesh (NiV-B) or HeV, generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV), both with and without reporter genes[4-8]. The rCedV chimeras are a replication competent authentic henipavirus platform that is a high-throughput surrogate neutralization assay that is rapid, cost-effective, and can be utilized outside high containment. Similar approaches will be applied here to generate new reporter viruses for Ghana bat virus (GhV). We will design, construct, and rescue both VSV and rCedV chimeras that encode the envelope glycoproteins (fusion (F) and attachment (G) glycoproteins) of GhV. Two reporter-gene encoding versions will be developed for VSV-GhV: one encoding GFP (VSV-GhV-GFP) and the other encoding the *Photinus pyralis* (firefly) luciferase gene (VSV-GhV-Luc). We will also construct and rescue GFP and Luc reporter gene encoding versions of rCedV chimeras encoding the GhV F and G genes (rCedV-GhV-GFP and rCedV-GhV-Luc) (**Fig. 1**). The rescued viruses will be characterized using cell-based infection tropism and growth kinetics assays.

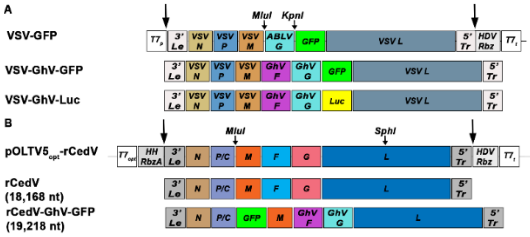


Fig. 1. Schematic of chimeric reporter viruses. (**A**)The VSV-ABLV G-GFP plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and KpnI to construct VSV-GhV-GFP and VSV-GhV-Luc. (**B**) pOLTV5$_{opt}$-rCedV plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and SphI used to construct rCedV-GhV-GFP.

The **rCedV (NiV-B and HeV) chimeric virus platform serves as a robust surrogate neutralization assay system for quantifying antibody neutralization potency of anti-NiV and anti-HeV antisera and mAbs[8].** The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G-directed mAbs against rCedV-NiV-B-GFP and rCedV-HeV-GFP are highly correlated with those obtained using authentic NiV-B and HeV when tested in parallel by plaque reduction neutralization test (PRNT) and high-throughput quantitative assay based on the reduction of GFP foci (fluorescent reduction neutralization test (FRNT) (**Fig. 2a-b**). The **Broder, Cross, Geisbert, and Veesler labs** have previously used this platform for high-throughput characterization of neutralizing mAbs and sera collaboratively[5,49].

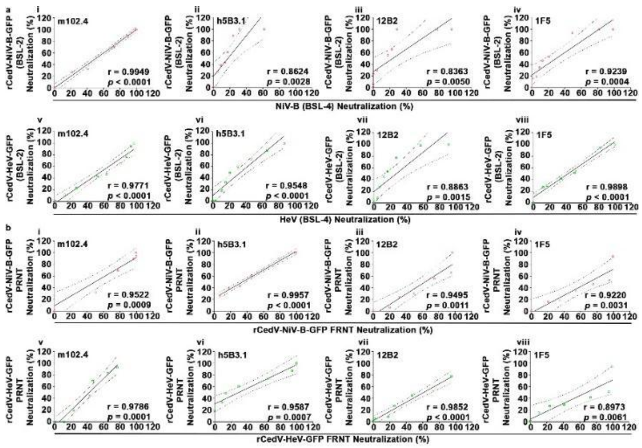


Figure 2. A) Pearson correlation for BSL-2 PRNT vs BSL-4 PRNT (rCedV-NiV-B-GFP versus NiV-B (**i-iv**) and rCedV-HeV-GFP versus HeV (**v-viii**)). **B)** Pearson correlation for PRNT vs FRNT ((rCedV-NiV-B-GFP (**i-iv**) and rCedV-HeV-GFP (**v-viii**)).

Generation of a VSV encoding GhV envelope glycoproteins. We will construct reporter gene-encoding versions of a replication-competent VSV encoding the GhV F and G glycoproteins (GFP and Luc) (**Fig. 1A)**, which are highly divergent from their HeV counterparts with which they share 55% and 30% amino acid sequence identity. A large gene cassette comprising VSV untranslated intergenic regions flanking the GhV F and G coding sequences will be synthesized. The GhV F and G coding sequences will be based on the GhV isolate Eid_hel/GH-M74a/GHA/2009 (GenBank: NC_025256.1). The GenBank accession number for GhV F is YP_009091837.1 and that for the GhV G protein is YP_009091838.1. Unique restriction enzyme sites, MluI and KpnI will facilitate the insertion of the GhV F and G gene cassette into the VSV backbone plasmid. The VSV-GhV-Luc will be similarly made. Cloning procedures are performed in *E. coli* Stbl2 and all plasmids verified by sequencing. Rescue will be performed in BSR-T7/5 cells, transfected helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-GhV-GFP or VSV-GhV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus is performed using HEK293T cells by inoculation with the supernatant from step 1. When GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above, and the virus stocks stored at -80°C. All virus stocks are deep sequenced. Virus stocks are titered by serial dilution on HEK293T cells by plaque assay[9].

Generation of rCedV encoding GhV envelope glycoproteins. rCedV-GhV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the GhV F and G coding sequences. The CedV non-coding intergenic regions are retained as is adherence to the genomic "rule of six". Unique restriction sites, MluI and SphI will facilitate the insertion of the GhV F and G gene cassette into the genome clone (pOLTV5$_{opt}$-rCedV) generating rCedV-GhV-GFP (**Fig. 1B**). Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L, and pOLTV5$_{opt}$-rCedV-GhV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (henipavirus CPE). At maximal syncytia and GFP (~2–3 days), supernatants are collected, clarified, and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[7,10,11].

Characterization of the recombinant reporter viruses in cell-based infection assays. The replication kinetics of the VSV-based and rCedV-based GhV F/G encoding viruses will be assayed by VeroE6 cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in several 24hr intervals and titered. VSV-GhV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G[1]. Similarly, rCedV-GhV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Unlike NiV and HeV, GhV G engages only the ephrin-B2 ligand to trigger membrane fusion[12]. The

GhV F/G bearing reporter viruses will be verified for this specific ephrin ligand tropism in cell infection and/or syncytia formation experiments and comparisons made to wildtype VSV or VSV-ABLV G as well as to rCedV, and rCedV NiV/HeV chimeras. The **Broder lab** has generated murine mAbs to GhV F and G (10C4-LY and 10D5-LY to GhV G; and 5B7-LY and 7C11-LY to GhV F) and these will be used in neutralization tests and assay validation. The VSV-GhV-GFP assays will be conducted by FRNTs and VSV-GhV-Luc in luciferase assays.

Rescue of a GhV infectious clone. UTMB will synthesize and rescue a GhV infectious clone, which will be propagated in VeroE6 cells and sequenced-verified. Replication kinetics will be assessed as described above and optimal infectious doses will be evaluated and determined in the hamster model based on prior work done with NiV and HeV in this challenge model[32]. All experiments will be conducted at BSL-4, as done with NiV and HeV work.

High-throughput neutralization assays. The **Veesler lab** has developed a high-throughput workflow for ELISAs and pseudovirus neutralization assays using a Biomek robotic platform enabling 24/7 automation. The neutralization assays use a safe, non-replicating vesicular stomatitis virus (VSV) encoding for luciferase instead of the native G glycoprotein or murine leukemia virus (MLV) encoding for luciferase, which we extensively used throughout the COVID-19 pandemic[54-58]. For henipaviruses, we will additionally use the chimeric recombinant CedV harboring the HeV and NiV glycoproteins developed in the **Broder lab**[5,49]. In collaboration with **Projects 2, 3, 4, and 5**, this platform will support evaluation of vaccine-elicited antibody responses to guide and iteratively refine immunogen designs against paramyxoviruses, phenuiviruses and arenaviruses, which have all been previously pseudotyped using the VSV or MLV backbones: Lassa virus[42], Junin virus[43], RVFV[44], and SFTFV[45].

Evaluation of neutralization for mAbs and vaccine-elicited antibodies. Our rCeV chimeras (rCedV-NiV-B-GFP and rCedV-HeV-GFP) will allow this Core to conduct large numbers of neutralization tests by FRNT, using vaccine-elicited sera and mAbs that will be generated in **Projects 2, 3, 4, and 5**. In Center years 1-3 the focus will be on HeV, and in years 4-5 the Core is expected to shift to GhV test case. The FRNT assay is rapid (36hrs), high throughput, and performed in 96-well plates to establish typical 7-point dose response virus neutralization data and performed at BSL-2. Typically, immune sera or mAbs are analyzed by serial dilution and mixed with virus (rCedV-NiV-B-GFP or rCedV-HeV-GFP) for 2hrs then samples used in triplicate to infect confluent Vero 76 cells in black-walled clear bottom 96-well plates and incubated 24hrs, and then plates are fixed and imaged and fluorescent foci quantitated with CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) is determined by 50% reduction in fluorescent foci versus untreated controls.

Generation and validation of neutralizing antibody titration assays using recombinant reporter phenuiviruses. We plan to generate a recombinant strain of Rift Valley fever virus (RVFV), specifically the MP-12 strain (rMP- 12), with the luciferase gene (such as NanoLuc) replacing the NSs gene utilizing our existing reverse genetics system[40,50]. By comparing the luciferase activities with the neutralizing antibody titers obtained from the plaque reduction neutralizing test[51] (PRNT), we will establish the correlation between the two assays for validation. This reporter assay will be employed for screening the antisera, while we will also perform the authentic PRNT to determine the neutralizing antibody titers of selected antisera. Additionally, we will develop a reverse genetics system for the SFTSV HB29 strain in order to establish a similar recombinant SFTSV luciferase assay. As SFTSV does not form plaques in Vero cells, we will utilize the focus reduction neutralization test (FRNT) with anti-SFTSV N antibody to determine the neutralizing antibody titers and validate the reporter assay[52,53].

Anticipated Results, Potential Pitfalls and Alternative Strategies. We anticipate the reporter viruses will replicate to titers fit for purpose based on our success with these chimeric viruses. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. If we encounter unexpected limitations in rescuing VSV-based or rCedV-based GhV F/G bearing reporter viruses; we will 1) test different ratios of helper plasmids and antigenome plasmids; 2) perform rescue using different cell lines; 3) construct alternate versions of the GhV F and G genes within the antigenome plasmids, such as truncating the GhV G protein which has been shown to enhance GhV mediated F/G membrane fusion[13]. Alternatively, fusion of the GhV F and G ectodomains with the transmembrane and cytoplasmic tail domains of the corresponding CedV F and G elements may facilitate virus particle formation and membrane fusion activity. Reporter assays for RVFV and SFTSV will enhance the throughput of screening of antisera-containing neutralizing antibodies. If the secretion of luciferase proves suboptimal for assessing relative virus replication levels, we will evaluate non-secreting luciferases, such as Renilla luciferase, utilizing cell lysates.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, immunology assays, virology assays, necropsies, and histopathological analysis. The Core D Leader and Co-Leaders will work closely with the Project Leaders and key staff of **Projects 2, 3, 4 and 5** to develop vaccination, treatment and sampling regimens, study protocols, and animal protocols that utilize countermeasures identified in **Projects 2, 3, 4 and 5**. The Core Leader and Co-Leaders will write and obtain UTMB IACUC approval for all animal protocols performed in Core D. The animal models and clinical pathology, immunology, virology, and histopathology assays employed for **Aim 3** are detailed below. All raw, graphed, and tabulated data generated by Core D will be handled and analyzed in coordination with **Core B.**

Core D will employ established animal models for HeV, LASV, JUNV, RVFV, and SFTSV. While animal models exist for SOSV and JUNV we will optimize these models to fill gaps and provide new tools as detailed in **Aim 4** below. Mice, guinea pigs, hamsters, ferrets, and cats have been used as small animal models of HeV infection[14]. This project will employ Syrian golden hamsters as the small animal model of HeV disease because they are more suitable than other small animals for screening large numbers of vaccines and mAbs. Using hamsters will allow us to select optimal vaccine and mAb formulations and regimens prior to efficacy testing in NHPs. In 2010 **Drs. Geisbert and Broder** developed the first highly lethal NHP model for henipavirus infection (Nipah virus) using African green monkeys (AGM)[15]. They subsequently developed a lethal AGM model for HeV infection[16]. Henipavirus NHP models mirror human disease with animals showing severe respiratory pathology, neurological disease, and generalized vasculitis. Based on our extensive prior experience working with NiV and HeV, we will develop a hamster challenge model for GhV, which uses the human ephrin B2 receptor[59,60] (similar to NiV and HeV). For LASV and JUNV, Core D will use established lethal outbred guinea pig models that cover several lineages of LASV[17-21] and several genetically distinct strains of JUNV[22-25] as guinea pigs are the only immunocompetent small animal model for LASV and JUNV disease. Core D will also use established cynomolgus monkey models that cover several genetically distinct lineages of LASV[26-29] and strains of JUNV[30]. For RVFV we will use established mouse models (such as BALB/c and C57/BL6 mice. In addition, we will utilize the recently described ferret model to evaluate vaccine candidates and mAbs[34]. For SFTSV, we will use the interferon a/b receptor knockout mouse model[62].

Animal vaccinations. All animals will be obtained from commercial vendors and allowed to acclimate prior to vaccination (~ 3-7 days for mice, hamsters, and guinea pigs and ~ 14 days for ferrets and NHPs). Mice, hamsters, and guinea pigs will be vaccinated by intramuscular (i.m.) or intraperitoneal (i.p.) injection of the vaccines provided by **Projects 3, 4, and 5** whereas ferrets and NHPs will be vaccinated by i.m. injection. All animals will be vaccinated in ABSL-2. Animals will be monitored daily for any reaction or adverse events caused by the vaccination. Blood will be collected for plasma, serum, and PBMC isolations before vaccination and at times after vaccination for assessment of the host immune response.

Animal challenges. Hamsters and NHPs will be challenged with infectious HeV or SOSV as previously described[31-33]. After appropriate acclimation, hamsters and ferrets will be exposed to HeV or SOSV by intranasal (i.n.) or i.p. administration of virus. NHPs will be challenged with HeV by the intratracheal (i.t.) and/or i.n. routes. Mice, guinea pigs, and ferrets will be exposed to arenaviruses and phenuiviruses by i.p. injection[19,24,25,34,35]. NHPs will be exposed to LASV or JUNV by i.m. injection[28-30]. For all animals, blood will be collected with or without anticoagulants for plasma and serum isolations, respectively, before paramyxovirus, arenavirus, or phenuivirus challenge, on the day of challenge, and daily after challenge. Animals will be monitored for clinical signs daily. We will apply a scoring sheet approved by the UTMB IACUC depicted in the Vertebrate Animals Section to assist in determining the time of euthanasia depending on clinical signs (e.g., respiratory distress, anorexia, activity, appearance, neurologic signs, etc.). Animals will be euthanized according to the 2013 AVMA Guidelines on Euthanasia. Any surviving animals will be euthanized on day 35-42 days after paramyxovirus, arenavirus, or phenuiviruses challenge.

Clinical pathology assays. For NHP studies, we will measure the following parameters at time points before and after HeV, LASV, or JUNV challenge using Abaxis VetScan HM5: total white blood cell counts (WBC), percent lymphocytes (LY), percent monocytes (MO), percent neutrophils (NEU), percent eosinophils (EOS), percent basophils (BAS), total lymphocyte counts (LY#), total monocyte counts (MO#), total neutrophil counts (NEU#), total eosinophil counts (EOS #), total basophil counts (BAS #), red blood cell counts (RBC), total hemoglobin (Hgb), hematocrit values (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV), and Plateletcrit (PCT). **Clinical chemistry on serum.** The following parameters will be measured before and after viral challenge using Abaxis Piccolo chemistry analyzer: Albumin (ALB), amylase (AMY), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), glucose (GLU), total protein (TP), blood urea nitrogen (BUN), and creatinine (CRE), C-reactive protein (CRP), Calcium (Ca2+), and uric acid (UA).

Viral load assays. *PCR Assay:* To assess viral loads in whole blood and tissues, RNA will be extracted using a virus RNA isolation kit followed by TaqMan PCR for the viral genome being studied. Qiagen buffers have been safety-tested for inactivation of paramyxoviruses, arenaviruses, and phenuiviruses. PCR assays employed for HeV, SOSV, LASV, JUNV, RVFV, and SFTSV as previously described[28-30,33,37-39].

Plaque Assay. The plaque assays employed for paramyxoviruses, arenaviruses, and phenuiviruses have previously been described[28-30,33,40]. Briefly, 10-fold dilutions (10-1 to 10-6) of serum, plasma, or whole blood samples are prepared in cell maintenance medium (DMEM/10%FBS/gentamicin) and adsorbed to 70-90% confluent monolayers of Vero E6 cells in 6-well plates. The incubation period is followed by addition of 1% agarose-containing medium (2X EBME/Hepes/10% FBS/gentamicin) to each well. After the agarose has solidified, the plates are returned to the C02 incubator and maintained for 2-14 days until plaques are visualized. At this time, PBS containing 5% neutral red and 5% FBS is added to each well incubated overnight. The following day, the liquid stain overlay is removed from each well and plaques are counted.

Bead-based multiplex assays. Plasma inflammatory mediators, thrombosis markers, and fibrinolysis analytes will be measured by FACS using Biolegend LegendPlex assays and a FACS Canto-II cytometer (Becton Dickson). Samples will be processed in duplicate for each Nonhuman Primate Inflammation 13-plex , Human Thrombosis , and Human Fibrinolysis panel as previously described[41-43].

Gross pathology and histopathology. A necropsy will be performed on all animals. We will examine the external surfaces of the body, all orifices, and the thoracic and peritoneal cavities and their contents. Core D will prepare a written report of the gross pathology findings of each animal. The following tissues will be collected at necropsy, formalin fixed, embedded in paraffin, and stained by H&E: liver, spleen, lung, kidney, adrenal gland, pancreas, axillary lymph node, inguinal lymph node, mesenteric lymph node, duodenum, ileum, colon, brain, cervical spinal cord, urinary bladder, ovary or testis, prostate or uterus, and eye. A microscopic evaluation will be performed on H&E slides of tissues collected from all animals by an ACVP board certified veterinary pathologist. Reports of the histologic findings of each tissue from each animal will be provided to the leaders of **Projects 2, 3, 4, and 5**. In consultation with them, Core D may also perform immunohistochemical and/or special stains such as PTAH for fibrin as previously described[28-30,33].

Quality systems management of all records and data collected from animal studies. Although the animal studies conducted by Core D will not be required to be performed under GLP compliance, UTMB will provide Quality Assurance that will achieve data quality and integrity when conducting animal experiments. Quality refers to whether the study was conducted in accordance with the protocol, standard operating procedures, and applicable standards of research. Integrity refers to assurance that the raw data and documentation are consistent with reported results. All animal study personnel will be trained by the UTMB Office of Regulated Nonclinical Studies (ORNcS). on Good Documentation Practices (GDP). All animal study data generated by Core D will be provided to **Core B** as detailed in the **Core B** Research Strategy.

Aim 4: Develop and/or optimize animal models for GhV, SOSV and JUNV.

For HeV and LASV Core D will employ animal models that have already been developed and are well established[32,61]. However, while animal models exist for JUNV, RVFV, and SFTSV, there are gaps that need to be addressed. Recently, it was shown that i.n. or i.p. infection of hamsters with SOSV resulted in viremia and mild clinical disease[37] but there is no lethal model for SOSV. Core D will further explore the hamster model for SOSV by 1) using higher challenge doses of SOSV which is often required to produce lethal disease in other paramyxovirus hamster models[32,44] or 2) serial passage of tissues in hamsters (i.e., repeated passage of 10% organ homogenates until uniform lethality is achieved). Core D will also assess the pathogenic potential of SOSV in ferrets as they are often used as animal models for paramyxoviruses. For arenaviruses such as JUNV, serial adaptation is often required to produce lethal disease in outbred guinea pigs. While adapted viruses have utility in assessing the protecting efficacy of medical countermeasures in animal models, non-adapted wild type viruses are preferred to better assess vaccines and treatments. We and others recently showed that for Ebolaviruses, while serial adaptation is required to produce lethality in mice, hamsters, and guinea pigs, infection of ferrets with *Zaire ebolavirus*, *Sudan ebolavirus*, *Bundibugyo ebolavirus*, or *Reston ebolavirus* resulted in uniform lethality with wild type viruses and did not require adaptation[45-48]. Therefore, Core D will assess the pathogenic potential of JUNV in ferrets. Finally, we will investigate GhV replication and pathogenicity in hamsters which is our small animal challenge model for HNVs since both HeV and GhV utilize ephrin B2 as entry receptor[59,60], to develop a suitable challenge model.

BIBLIOGRAPHY AND REFERENCES CITED – CORE D: ANIMAL MODELS AND VACCINE EVALUATION

1. Weir DL, Smith IL, Bossart KN, Wang LF, Broder CC. Host cell tropism mediated by Australian bat lyssavirus envelope glycoproteins. Virology 2013;444:21-30. PMCID: PMC6183051.

2. Weir DL, Laing ED, Smith IL, Wang LF, Broder CC. Host cell virus entry mediated by Australian bat lyssavirus G envelope glycoprotein occurs through a clathrin-mediated endocytic pathway that requires actin and Rab5. Virol J 2014;11:40. PMCID: PMC3946599.

3. Mastraccio KE, Huaman C, Warrilow D, Smith GA, Craig SB, Weir DL, Laing ED, Smith IL, Broder CC, Schaefer BC. Establishment of a longitudinal pre-clinical model of lyssavirus infection. J Virol Methods 2020;281:113882. PMCID: PMC8056983.

4. Laing ED, Sterling SL, Weir DL, Beauregard CR, Smith IL, Larsen SE, Wang LF, Snow AL, Schaefer BC, Broder CC. Enhanced Autophagy Contributes to Reduced Viral Infection in Black Flying Fox Cells. Viruses 2019;11:260. PMCID: PMC6466025.

5. Wang Z, Dang HV, Amaya M, Xu Y, Yin R, Yan L, Hickey AC, Annand EJ, Horsburgh BA, Reid PA, Smith I, Eden JS, Xu K, Broder CC, Veesler D. Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. Proc Natl Acad Sci USA 2022;119:e2122769119. PMCID: PMC9295758.

6. Laing ED, Navaratnarajah CK, Cheliout Da Silva S, Petzing SR, Xu Y, Sterling SL, Marsh GA, Wang LF, Amaya M, Nikolov DB, Cattaneo R, Broder CC, Xu K. Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. Proc Natl Acad Sci USA 2019;116:20707-20715. PMCID: PMC6789926.

7. Laing ED, Amaya M, Navaratnarajah CK, Feng YR, Cattaneo R, Wang LF, Broder CC. Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. Virol J 2018 Mar 27;15:56. PMCID: PMC5869790.

8. Amaya M, Yin R, Yan L, Borisevich V, Adhikari BN, Bennett A, Malagon F, Cer RZ, Bishop-Lilly KA, Dimitrov AS, Cross RW, Geisbert TW, Broder CC. A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. Viruses 2023;15:1077. PMCID: PMC10223282.

9. Abdelmageed AA, Ferran MC. The Propagation, Quantification, and Storage of Vesicular Stomatitis Virus. Curr Protoc Microbiol 2020;58:e110. PMCID: PMC7449588.

10. Amaya M, Cheng H, Borisevich V, Navaratnarajah CK, Cattaneo R, Cooper L, Moore TW, Gaisina IN, Geisbert TW, Rong L, Broder CC. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral Res 2021;193:105084. PMCID: PMC8631057.

11. Weingartl HM, Berhane Y, Caswell JL, Loosmore S, Audonnet JC, Roth JA, Czub M. Recombinant nipah virus vaccines protect pigs against challenge. J Virol 2006;80:7929-38. PMCID: PMC1563797.

12. Weis M, Behner L, Hoffmann M, Krüger N, Herrler G, Drosten C, Drexler JF, Dietzel E, Maisner A. Characterization of African bat henipavirus GH-M74a glycoproteins. J Gen Virol 2014;95:539-548. PMID: 24296468.

13. Voigt K, Hoffmann M, Drexler JF, Müller MA, Drosten C, Herrler G, Krüger N. Fusogenicity of the Ghana Virus (*Henipavirus*: *Ghanaian bat henipavirus*) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. Viruses 2019;11:800. PMCID: PMC6784138.

14. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. Curr Top Microbiol Immunol 2012;359:197-223. PMID: 22476556.

15. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, Smith MA, Chan YP, Wang LF, Mattapallil JJ, Geisbert JB, Bossart KN, Broder CC. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010;5:e10690. PMCID: PMC2872660.

16. Rockx B, Bossart KN, Feldmann F, Geisbert JB, Hickey AC, Brining D, Callison J, Safronetz D, Marzi A, Kercher L, Long D, Broder CC, Feldmann H, Geisbert TW. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010;84:9831-9. PMCID: PMC2937751.

17. Carrion R Jr, Patterson JL, Johnson C, Gonzales M, Moreira CR, Ticer A, Brasky K, Hubbard GB, Moshkoff D, Zapata J, Salvato MS, Lukashevich IS. A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity. Vaccine 2007;25:4093-102. PMCID: PMC1892204.

18. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A recombinant vesicular stomatitis virus-based Lassa fever vaccine protects guinea pigs and macaques against challenge with geographically and genetically distinct Lassa viruses. PLoS Negl Trop Dis 2015;9:e0003736. PMCID: PMC4401668.

19. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M, Khan SH, Robinson JE, Geisbert TW, Garry RF. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res 2016;133:218-222. PMCID: PMC5032844.

20. Maruyama J, Manning JT, Mateer EJ, Sattler R, Bukreyeva N, Huang C, Paessler S. Lethal Infection of Lassa Virus Isolated from a Human Clinical Sample in Outbred Guinea Pigs without Adaptation. mSphere 2019;4:e00428-19. PMCID: PMC6763766.

21. Deschambault Y, Soule G, Klassen L, Sloan A, Audet J, Azaransky K, Musa AS, Ahmad A, Akinpelu AM, Mba N, Stein DR, Ranson M, Almiski M, Tierney K, Fischer G, Chan M, Safronetz D. An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. Viruses 2023;15:769. PMCID: PMC10052409.

22. Kenyon RH, Green DE, Maiztegui JI, Peters CJ. Viral strain dependent differences in experimental Argentine hemorrhagic fever (Junin virus) infection of guinea pigs. Intervirology 1988;29:133-43. PMID: 2846464.

23. Yun NE, Linde NS, Dziuba N, Zacks MA, Smith JN, Smith JK, Aronson JF, Chumakova OV, Lander HM, Peters CJ, Paessler S. Pathogenesis of XJ and Romero strains of Junin virus in two strains of guinea pigs. Am J Trop Med Hyg 2008;79:275-82. PMCID: PMC2700623.

24. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci USA 2016;113:4458-63. PMCID: PMC4843420.

25. Sorvillo TE, Cross RW, Johnson DM, Dobias NS, Fenton KA, Mire CE, **Geisbert TW**. Single dose rVSVΔG-JUNVGP vaccine protects guinea pigs against lethal Junin virus challenge. NPJ Vaccines. 2021 Aug 9;6(1):96. PMCID: PMC8352877.

26. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol 2009;83:5890-903. PMCID: PMC2681932.

27. Hensley LE, Smith MA, Geisbert JB, Fritz EA, Daddario-DiCaprio KM, Larsen T, Geisbert TW. Pathogenesis of Lassa fever in cynomolgus macaques. Virol J 2011;8:205. PMCID: PMC3104370.

28. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med 2017;23:1146-1149. PMCID: PMC5719877.

29. Cross RW, Xu R, Matassov D, Hamm S, Latham TE, Gerardi CS, Nowak RM, Geisbert JB, Ota-Setlik A, Agans KN, Luckay A, Witko SE, Soukieh L, Deer DJ, Mire CE, Feldmann H, Happi C, Fenton KA, Eldridge JH,

Geisbert TW. Quadrivalent VesiculoVax vaccine protects nonhuman primates from viral-induced hemorrhagic fever and death. J Clin Invest 2020;130:539-551. PMCID: PMC6934204.

30. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci USA. 2021;118:e2023332118. PMCID: PMC7980402.

31. Mire CE, Chan YP, Borisevich V, Cross RW, Yan L, Agans KN, Dang HV, Veesler D, Fenton KA, Geisbert TW, Broder CC. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. J Infect Dis 2020;221(Suppl 4):S471-S479. PMCID: PMC7199785.

32. Doyle MP, Kose N, Borisevich V, Binshtein E, Amaya M, Nagel M, Annand EJ, Armstrong E, Bombardi R, Dong J, Schey KL, Broder CC, Zeitlin L, Kuang EA, Bornholdt ZA, West BR, Geisbert TW, Cross RW, Crowe JE Jr. Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. Cell Rep 2021;36:109628. PMCID: PMC8527959.

33. Geisbert TW, Bobb K, Borisevich V, Geisbert JB, Agans KN, Cross RW, Prasad AN, Fenton KA, Yu H, Fouts TR, Broder CC, Dimitrov AS. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. NPJ Vaccines 2021;6:23. PMCID: PMC7870971.

34. Barbeau DJ, Albe JR, Nambulli S, Tilston-Lunel NL, Hartman AL, Lakdawala SS, Klein E, Duprex WP, McElroy AK. Rift Valley Fever Virus Infection Causes Acute Encephalitis in the Ferret. mSphere 2020;5:e00798-20. PMCID: PMC7593599.

35. Johnson KN, Kalveram B, Smith JK, Zhang L, Juelich T, Atkins C, Ikegami T, Freiberg AN. Tilorone-Dihydrochloride Protects against Rift Valley Fever Virus Infection and Disease in the Mouse Model. Microorganisms 2021;10:92. PMCID: PMC8781158.

36. Smith DR, Bird BH, Lewis B, Johnston SC, McCarthy S, Keeney A, Botto M, Donnelly G, Shamblin J, Albariño CG, Nichol ST, Hensley LE. Development of a novel nonhuman primate model for Rift Valley fever. J Virol 2012;86:2109-20. PMCID: PMC3302397.

37. Welch SR, Ritter JM, Schuh AJ, Genzer SC, Sorvillo TE, Harmon JR, Coleman-McCray JD, Jain S, Shrivastava-Ranjan P, Seixas JN, Estetter LB, Fair PS, Towner JS, Montgomery JM, Albariño CG, Spiropoulou CF, Spengler JR. Tissue replication and mucosal swab detection of Sosuga virus in Syrian hamsters in the absence of overt tissue pathology and clinical disease. Antiviral Res 2023;209:105490. PMID: 36521633

38. Lokugamage N, Ikegami T. Genetic stability of Rift Valley fever virus MP-12 vaccine during serial passages in culture cells. NPJ Vaccines 2017;2:20. PMCID: PMC5627234.

39. Yoshikawa T, Fukushi S, Tani H, Fukuma A, Taniguchi S, Toda S, Shimazu Y, Yano K, Morimitsu T, Ando K, Yoshikawa A, Kan M, Kato N, Motoya T, Kuzuguchi T, Nishino Y, Osako H, Yumisashi T, Kida K, Suzuki F, Takimoto H, Kitamoto H, Maeda K, Takahashi T, Yamagishi T, Oishi K, Morikawa S, Saijo M, Shimojima M. Sensitive and specific PCR systems for detection of both Chinese and Japanese severe fever with thrombocytopenia syndrome virus strains and prediction of patient survival based on viral load. J Clin Microbiol 2014;52:3325-33. PMCID: PMC4313158.

40. Ikegami T, Won S, Peters CJ, Makino S. Rescue of infectious rift valley fever virus entirely from cDNA, analysis of virus lacking the NSs gene, and expression of a foreign gene. J Virol 2006;80:2933-40. PMCID: PMC1395455.

41. Woolsey C, Borisevich V, Prasad AN, Agans KN, Deer DJ, Dobias NS, Heymann JC, Foster SL, Levine CB, Medina L, Melody K, Geisbert JB, Fenton KA, Geisbert TW, Cross RW. Establishment of an African green monkey model for COVID-19 and protection against re-infection. Nat Immunol 2021;22:86-98. PMCID: PMC7790436.

42. Woolsey C, Borisevich V, Agans KN, Fenton KA, Cross RW, Geisbert TW. *Bundibugyo ebolavirus* Survival Is Associated with Early Activation of Adaptive Immunity and Reduced Myeloid-Derived Suppressor Cell Signaling. mBio 2021;12:e0151721. PMCID: PMC8406165.

43. Woolsey C, Fears AC, Borisevich V, Agans KN, Dobias NS, Prasad AN, Deer DJ, Geisbert JB, Fenton KA, Geisbert TW, Cross RW. Natural history of *Sudan ebolavirus* infection in rhesus and cynomolgus macaques. Emerg Microbes Infect 2022;11:1635-1646. PMCID: PMC9225728.

44. Rockx B, Brining D, Kramer J, Callison J, Ebihara H, Mansfield K, Feldmann H. Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. J Virol 2011;85:7658-71. PMCID: PMC3147900.

45. Cross RW, Mire CE, Borisevich V, Geisbert JB, Fenton KA, Geisbert TW. The domestic ferret (*Mustela putorius furo*) as a lethal infection model for 3 species of Ebolavirus. J Infect Dis 2016;214:565-9. PMCID: PMC4957446.

46. Kozak R, He S, Kroeker A, de La Vega MA, Audet J, Wong G, Urfano C, Antonation K, Embury-Hyatt C, Kobinger GP, Qiu X. Ferrets Infected with Bundibugyo Virus or Ebola Virus Recapitulate Important Aspects of Human Filovirus Disease. J Virol 2016;90:9209-23. PMCID: PMC5044830.

47. Kroeker A, He S, de La Vega MA, Wong G, Embury-Hyatt C, Qiu X. Characterization of Sudan Ebolavirus infection in ferrets. Oncotarget 2017;8:46262-46272. PMCID: PMC5542265.

48. Yan F, He S, Banadyga L, Zhu W, Zhang H, Rahim MN, Collignon B, Senthilkumaran C, Embury-Hyatt C, Qiu X. Characterization of Reston virus infection in ferrets. Antiviral Res 2019;165:1-10. PMID: 30836107.

49. Wang, Zhaoqian, Moushimi Amaya, Amin Addetia, Ha V. Dang, Gabriella Reggiano, Lianying Yan, Andrew C. Hickey, Frank DiMaio, Christopher C. Broder, and David Veesler. 2022. “Architecture and Antigenicity of the Nipah Virus Attachment Glycoprotein.” *Science* 375 (6587): 1373–78.

50. Nishiyama S, Slack OAL, Lokugamage N, Hill TE, Juelich TL, Zhang L, Smith JK, Perez D, Gong B, Freiberg AN, Ikegami T. Attenuation of pathogenic Rift Valley fever virus strain through the chimeric S-segment encoding sandfly fever phlebovirus NSs or a dominant-negative PKR. Virulence. 2016 Nov 16;7(8):871–881. PMCID: PMC5160418

51. Ikegami T, Jurado-Cobena E, Alkan C, Smith JK, Zhang L, Kalveram B, Juelich TL, Esterly AT, Bhaskar JR, Thangamani S, Freiberg AN. Evaluations of rationally designed rift valley fever vaccine candidate RVax-1 in mosquito and rodent models. NPJ Vaccines. 2022 Sep 21;7(1):109. PMCID: PMC9492667

52. Tamin A, Harcourt BH, Lo MK, Roth JA, Wolf MC, Lee B, Weingartl H, Audonnet JC, Bellini WJ, Rota PA. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods. 2009 Sep;160(1-2):1–6. PMCID: PMC2704486

53. Buck TK, Enriquez AS, Schendel SL, Zandonatti MA, Harkins SS, Li H, Moon-Walker A, Robinson JE, Branco LM, Garry RF, Saphire EO, Hastie KM. Neutralizing Antibodies against Lassa Virus Lineage I. MBio. 2022 Aug 30;13(4):e0127822. PMCID: PMC9426445

54. Bowen JE, Addetia A, Dang HV, Stewart C, Brown JT, Sharkey WK, Sprouse KR, Walls AC, Mazzitelli IG, Logue JK, Franko NM, Czudnochowski N, Powell AE, Dellota E Jr, Ahmed K, Ansari AS, Cameroni E, Gori A, Bandera A, Posavad CM, Dan JM, Zhang Z, Weiskopf D, Sette A, Crotty S, Iqbal NT, Corti D, Geffner J, Snell G, Grifantini R, Chu HY, Veesler D. Omicron spike function and neutralizing activity elicited by a comprehensive panel of vaccines. Science. 2022 Aug 19;377(6608):890–894. PMCID: PMC9348749

55. Cameroni E, Bowen JE, Rosen LE, Saliba C, Zepeda SK, Culap K, Pinto D, VanBlargan LA, De Marco A, di Iulio J, Zatta F, Kaiser H, Noack J, Farhat N, Czudnochowski N, Havenar-Daughton C, Sprouse KR, Dillen JR, Powell AE, Chen A, Maher C, Yin L, Sun D, Soriaga L, Bassi J, Silacci-Fregni C, Gustafsson C, Franko NM, Logue J, Iqbal NT, Mazzitelli I, Geffner J, Grifantini R, Chu H, Gori A, Riva A, Giannini O, Ceschi A, Ferrari P, Cippà PE, Franzetti-Pellanda A, Garzoni C, Halfmann PJ, Kawaoka Y, Hebner C, Purcell LA, Piccoli L, Pizzuto MS, Walls AC, Diamond MS, Telenti A, Virgin HW, Lanzavecchia A, Snell G, Veesler D, Corti D.

Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. Nature. 2022 Feb;602(7898):664–670. PMCID: PMC9531318

56. Walls AC, Sprouse KR, Bowen JE, Joshi A, Franko N, Navarro MJ, Stewart C, Cameroni E, McCallum M, Goecker EA, Degli-Angeli EJ, Logue J, Greninger A, Corti D, Chu HY, Veesler D. SARS-CoV-2 breakthrough infections elicit potent, broad, and durable neutralizing antibody responses. Cell. 2022 Mar 3;185(5):872–880.e3. PMCID: PMC8769922

57. Starr TN, Czudnochowski N, Liu Z, Zatta F, Park YJ, Addetia A, Pinto D, Beltramello M, Hernandez P, Greaney AJ, Marzi R, Glass WG, Zhang I, Dingens AS, Bowen JE, Tortorici MA, Walls AC, Wojcechowskyj JA, De Marco A, Rosen LE, Zhou J, Montiel-Ruiz M, Kaiser H, Dillen JR, Tucker H, Bassi J, Silacci-Fregni C, Housley MP, di Iulio J, Lombardo G, Agostini M, Sprugasci N, Culap K, Jaconi S, Meury M, Dellota E Jr, Abdelnabi R, Foo SYC, Cameroni E, Stumpf S, Croll TI, Nix JC, Havenar-Daughton C, Piccoli L, Benigni F, Neyts J, Telenti A, Lempp FA, Pizzuto MS, Chodera JD, Hebner CM, Virgin HW, Whelan SPJ, Veesler D, Corti D, Bloom JD, Snell G. SARS-CoV-2 RBD antibodies that maximize breadth and resistance to escape. Nature. 2021 Sep;597(7874):97–102. PMCID: PMC9282883

58. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020 Apr 16;181(2):281–292.e6. PMCID: PMC7102599

59. Pernet O, Beaty S, Lee B. Functional rectification of the newly described African henipavirus fusion glycoprotein (Gh-M74a). J Virol. 2014 May;88(9):5171–5176. PMCID: PMC3993810

60. Lee B, Pernet O, Ahmed AA, Zeltina A, Beaty SM, Bowden TA. Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. Proc Natl Acad Sci U S A. 2015 Apr 28;112(17):E2156–65. PMCID: PMC4418902

61. Guillaume V, Wong KT, Looi RY, Georges-Courbot MC, Barrot L, Buckland R, Wild TF, Horvat B. Acute Hendra virus infection: Analysis of the pathogenesis and passive antibody protection in the hamster model. Virology. 2009 May 10;387(2):459–465. PMID: 19328514

62. Park A, Iwasaki A. Type I and Type III Interferons - Induction, Signaling, Evasion, and Application to Combat COVID-19. Cell Host Microbe. 2020 Jun 10;27(6):870–878. PMCID: PMC7255347

From:	Neil King on behalf of Neil King <neilking@uw.edu>
To:	Geisbert, Thomas W.
Cc:	Cross, Robert W.; David Veesler; Freiberg, Alexander N.; Ikegami, Tetsuro; Lynda Stuart; kherrera; CHRISTOPHER BRODER
Subject:	submitting edited Core D documents
Date:	Monday, June 5, 2023 7:12:13 PM
Attachments:	Core_D_ProjectSummary.pdf Core_D_SpecificAims_FINAL.pdf Core_D_Research_Strategy_FINAL.pdf Core_D_Refs_FINAL.pdf

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Hi Tom, Bob, Chris, Alex, and Tetsuro,

Please find attached the documents we will be submitting within the next hour for Core D. The documents required some editing to fit within the required page limits and to introduce synergies with other parts of the Center.

Please let me know within the next 45 minutes or so if you see anything that needs to be fixed.

We'll report back once UW has submitted the proposal to the sponsor.

Hope everything is going well finishing up your application too!

Best,
Neil

A. Significance

Henipaviruses (HNV) and arenaviruses (ARV) are two families of viruses that pose significant global public health concerns[1-5]. HNV, which includes Nipah virus (NiV) and Hendra virus (HeV), has caused outbreaks in Asia and Australia, resulting in severe respiratory and central nervous system (CNS) illnesses in humans[6-8]. These infections have been associated with high mortality rates ranging from 40% to 90%[4,9-13]. Similarly, ARV, such as Lassa virus (LASV) and Machupo virus (MACV), can cause severe illness and death in humans, with LASV being endemic in West Africa and MACV responsible for Bolivian hemorrhagic fever[14-19]. The geographical distribution and diversity of these viruses make them a global public health threat. While there are some vaccines and antiviral drugs available[4,13,20,21], there are no licensed treatments specifically targeting these viruses. Thus, there is a pressing need to develop new therapies that can effectively neutralize and prevent the spread of these viruses.

Significance of Developing Nanobody Therapy

The urgent need for effective treatments against HNV and ARV infections, highlighted by the ongoing COVID-19 pandemic, emphasizes the importance of rapid drug development and innovative antiviral therapies. Nanobodies, also known as VHH or single-domain antibodies, derived from camelid animals, offer unique advantages that make them promising candidates for antiviral therapy[22-25].

One significant advantage of nanobodies is their inherent stability[26-28]. Their small size and single-domain structure make them highly resistant to denaturation and degradation, allowing them to maintain their binding activity under various conditions. Nanobodies also offer ease of engineering. Their single-domain format simplifies the modification and optimization of their binding properties. Through techniques such as affinity maturation and structure-guided design, nanobodies can be readily engineered to enhance their affinity, specificity, and neutralization potency against specific viral targets. This flexibility in engineering enables the development of tailored nanobodies with improved therapeutic properties[29,30].

Also, nanobodies exhibit modular characteristics[26]. Their small size and independent folding domains allow for the construction of multi-specific or bi-specific nanobodies, where different specificities can be combined into a single molecule[31,32]. This modularity opens up possibilities for targeting multiple epitopes on viral proteins or simultaneously targeting different viral strains, providing enhanced breadth and efficacy in combating HNV and ARV infections.

Combined with their specificity, small size, stability, ease of engineering, and modular nature, nanobodies represent a promising approach for developing effective antiviral therapies against HNV and ARV infections.

Three nanobody development platforms to enhance diversity and coverage

The use of multiple nanobody development platforms, including camelid animals, nanomice, and low-immunogenic human nanobody libraries (**Fig.1**), enhances the diversity and coverage of nanobody libraries, increasing the likelihood of identifying potent nanobody candidates. Notably, **Dr. Jianliang Xu**, a nanobody research expert, is **the inventor of the "nanomice" platform**[29], which was commented by *Nature News and Views* that "*Just as the development of mice with antibodies containing human variable domains (such as Regeneron's VelocImmune mouse) has helped to deliver the 100th FDA-approved monoclonal antibody, perhaps the nanomouse will give nanobody-based therapeutics a push in the same direction*"[33]. In addition, we have recently cloned the CDR regions of human antibodies (IgM, IgD, IgG1-4, IgE, IgA1-2) heavy chain from 60 healthy donors of various demographic backgrounds, assembled them on Ighv3 family framework, and generated a human nanobody library that is close to natural human antibody heavy chain variable domain. The potentially low immunogenicity of such a library will enable us to quickly identify nanobody leads that need minimum optimization for clinical use.

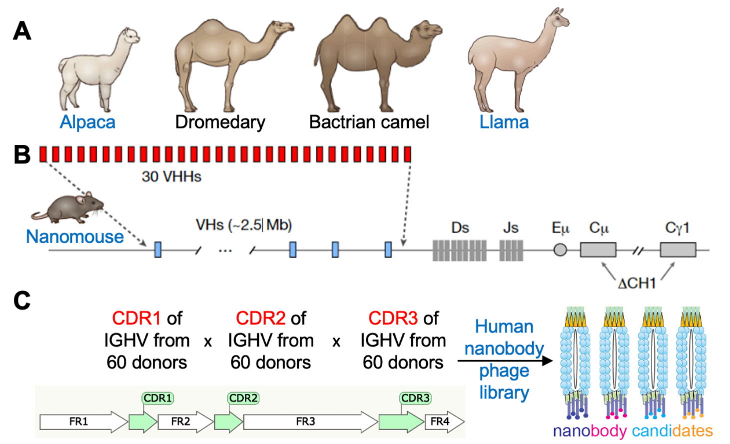


Figure 1: Three nanobody development platforms. (A) Camelid animals; (B) Nanomouse; (C) Human nanobody phage library.

The deep sequencing of antigen-specific enriched nanobody libraries ensures a comprehensive exploration of the nanobody repertoire[29], allowing for the rapid identification of leads with minimal optimization required for clinical use. This expedited development process is critical in responding to emerging viral threats and addressing the urgent need for effective antiviral therapies.

Targeting CNS diseases caused by viral infection

Nanobodies hold great promise for targeted therapy of CNS diseases as they can efficiently cross the blood-brain barrier (BBB) due to their small size[34-37]. To further enhance brain delivery, we have developed a bi-specific nanobody strategy by coupling another nanobody that targets TfR1[38,39], a receptor expressed at the BBB. This bi-specific nanobody can cross-react with both human and mouse TfR1, facilitating enhanced cargo delivery to the brain. In our upcoming study, we will compare the brain delivery and therapeutic efficacy of un-engineered nanobodies with the bi-specific nanobodies using animal models in **RP3 and Core E**. This study aims to evaluate the abilities of these two approaches to effectively penetrate the BBB and deliver therapeutic nanobodies to the central nervous system. Through this evaluation, we hope to understand the benefits of utilizing bi-specific nanobodies for targeted therapy of CNS diseases caused by HNV and ARV infections.

Generalizable research strategy

The significance of developing nanobody therapy extends beyond the four selected prototypic viruses in HNV and ARV. By applying and testing the research strategy developed in this project during **phase 2** of the project, the scope can be expanded to include other members of these viral groups, such as Langya virus (LayV)[40], Lujo virus (LUJV)[41], and Chapare virus (CHAV)[42]. This approach expands the scope of nanobody-based therapies, tackling global health risks from emerging viral diseases.

B. Innovation

The study incorporates several innovative aspects in the development of nanobody-based therapies for HNV and ARV infections:

- Utilization of diverse nanobody sources: The study leverages nanobodies derived from camelid animals, nanomice, and low-immunogenic human nanobody libraries. This approach expands the repertoire of nanobodies available for antiviral therapy development.
- Targeting viral entry machinery: Nanobodies are employed to target and neutralize viral proteins involved in viral attachment and entry. This innovative approach provides a novel avenue to inhibit viral infection at the early stages.
- Integration of structural characterization techniques: The study integrates advanced structural characterization techniques such as X-ray crystallography and Electron Microscopy Single Particle Analysis. This enables a detailed understanding of nanobody epitopes and viral neutralization mechanisms, facilitating the rational design of nanobody-based therapies.
- Development of BBB-penetrating nanobodies: The study focuses on designing nanobodies with the ability to penetrate the BBB, enabling effective targeting of the central nervous system. This is particularly relevant for combating the severe neurological manifestations associated with HNV and ARV infections.
- Generalizable research strategy: The research strategy developed in this study can be applied to other members of the HNV and ARV viral groups, such as Langya virus, Lujo virus, and Chapare virus. This expands the potential impact of nanobody therapies to address a broader range of emerging viral diseases.
- Deep sequencing for comprehensive coverage: The study utilizes deep sequencing to ensure comprehensive coverage of antigen-specific enriched nanobody libraries. This approach facilitates the rapid identification of potent nanobody leads with minimal optimization required for clinical use.
- Rapid and efficient therapy development: By leveraging the advantages of nanobodies and employing a well-designed research strategy, the study aims to rapidly and efficiently develop nanobody-based therapies. This contributes to the field of antiviral research and addresses the urgent need for effective treatments against HNV and ARV infections, thereby enhancing public health protection.

C. Approach

Scientific Premise: Our hypothesis is that nanobodies targeting viruses' key entry machinery can effectively inhibit virus infection. By utilizing structure-based analysis and rational design, we can enhance the efficacy of nanobody therapy by increasing its potency and breadth. Through the design of bi-specific nanobodies coupled with brain receptor-mediated transcytosis, we aim to enhance their ability to penetrate the blood-brain barrier, thus effectively combating central nervous system diseases caused by HNVs and ARVs.

Aim 1: To identify cross-reactive nanobodies to HNVs.

Rationale and Preliminary Data:

Envelope fusion glycoprotein F and the attachment glycoprotein G of Hendra virus (HeV) and Nipah virus (NiV) play crucial roles in host cell receptor engagement and membrane fusion, making them the main targets of neutralizing antibodies[43-46]. The high degree of similarity, approximately 80-90%, between the glycoproteins of HeV and NiV presents an attractive opportunity to identify cross-reactive neutralizing nanobodies capable of providing broad protection against both viruses[1,47-49]. To achieve this goal, we conducted immunization experiments using four groups of nanomice, with each group consisting of four mice. The mice were immunized with soluble F and G proteins of HeV and NiV, namely HeV_sF, HeV_sG, NiV_sF, and NiV_sG. These proteins, designed by **Dr. Kai Xu** and produced by our collaborator **Dr. Christopher Broder's** group, served as the immunogens[43,50,51] (**Fig. 2A**).

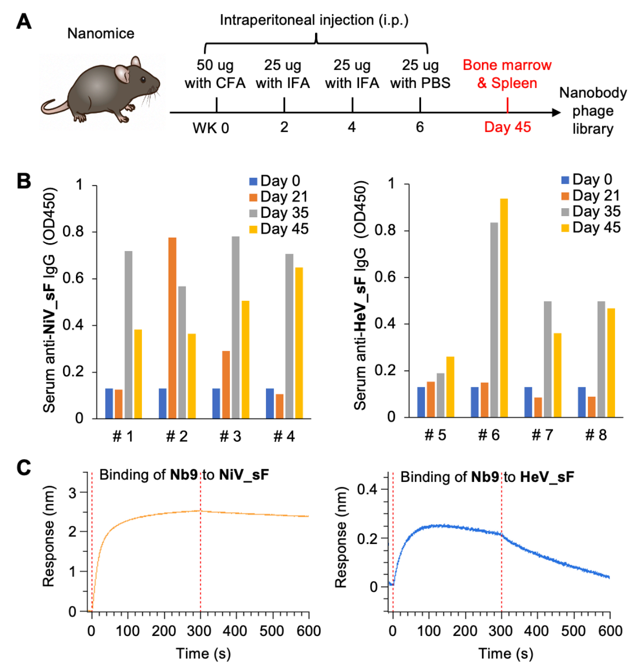


Figure 2: Nanomouse immunization and NiV_sF_Nb9 characterization. (A) Nanomouse immunization scheme. (B) HNV F proteins immunized nanomice serum response to the respective immunogen determined by ELISA; (C) Binding of NiV_sF_Nb9 to NiV_sF and HeV_sF determined by Octet.

Following four injections in the nanomice, we observed various levels of serum antibody responses against all four immunogens, as depicted in **Figure 2B** for F proteins (data for G proteins not shown). To validate our approach, we isolated RNA from the bone marrow and spleen of mice immunized with NiV_sF and constructed nanobody phage libraries. Through a series of three rounds of phage panning using NiV_sF, subsequent individual colony picking, ELISA screening, and Octet affinity validation, we successfully identified several nanobody binders specific to NiV_sF. Of particular significance, one of the identified nanobodies, NiV_sF_Nb9, exhibited the ability to bind not only to NiV-sF protein but also to HeV-sF protein (**Fig. 2C**). This finding suggests that NiV_sF_Nb9 recognizes an epitope shared by the F proteins of both NiV and HeV. The results obtained from these experiments clearly demonstrate the potential of nanomice as a convenient and efficient model for the development of nanobodies. Consequently, we are confident in our ability to identify nanobodies against other F/G proteins of HeV and NiV using the same approach applied to the immunized mice.

In addition to the nanomice immunization strategy, we have also made significant progress using a **human library version 1.0 (Fig. 1C)** for screening purposes. Notably, we sequentially screened this human nanobody library with spikes of omicron variants (BA.4/5, BA.1) and WA-1, leading to the enrichment of numerous nanobody candidates. Among them, nine nanobodies exhibited binding affinity to the receptor-binding domain (RBD) of BA.4/5, BA.1, WA-1, SARS, and WIV16, indicating their ability to recognize a range of sarbecoviruses (**Fig. 3**). Importantly, all nine of these human nanobodies demonstrated potent neutralization against WA-1, and five of them exhibited cross-neutralization of Omicron subvariants (BA.4/5, BQ.1.1, XBB.1), SARS1, and Bat-WIV1. These results underscore the power and versatility of the human nanobody library platform, which holds significant potential for targeting various other antigens.

human nanobodies	WA1	Omicron (BA.4/5)	SARS1	Bat-WIV1	BQ.1.1	XBB.1
CoV2.hNb3	0.056	9.1	0.2	30.0	16.4	17.9
CoV2.hNb6	0.027	4.5	0.3	33.1	17.9	19.8
CoV2.hNb17	0.228	16.3	0.8	20.9	44.7	21.2
CoV2.hNb18	0.158	13.9	0.4	27.5	18.4	45.6
CoV2.hNb31	0.089	9.7	0.2	15.2	15.0	28.0
CoV2.hNb35	0.028	10.9	0.4	>50		
CoV2.hNb9	0.011	>50	4.4	>50		
CoV2.hNb28	0.123	>50	>50	>50		
CoV2.hNb30	0.122	>50	>50	>50		

Figure 3: SARS-CoV-2 neutralizing nanobodies identified by human nanobody library. Neutralization IC50 (ug/ml) of 9 human nanobodies against various pseudotyped sarbecoviruses.

Experimental Design:

To expand our search for cross-reactive nanobodies against HeV and NiV glycoproteins, we will immunize additional groups of nanomice sequentially with either HeV or NiV F or G proteins. Each group will serve as a source for constructing *in vivo* matured nanobody phage libraries, using bone marrow, spleen, and/or peripheral blood mononuclear cells (PBMCs). These libraries will undergo sequential screening with HeV and NiV F or G proteins to identify nanobodies that exhibit cross-recognition of the F or G proteins of both viruses. To ensure comprehensive coverage of potential binders, we will employ a two-pronged approach. Firstly, we will randomly pick colonies from the libraries, and secondly, we will subject the enriched libraries to deep sequencing using the MiSeq platform. This approach will enable us to identify nanobody candidates from all complementarity-determining region 3 (CDR3) families. Subsequently, we will assess the target specificity of individual nanobodies through enzyme-linked immunosorbent assay (ELISA). Moreover, the neutralization activity of the identified nanobody candidates will be evaluated using RP2 assays.

While nanomice have proven to be valuable for generating in vivo matured nanobodies, it is important to consider the potential immunogenicity associated with animal-derived nanobodies. Therefore, in parallel with the nanomice immunization strategy, we will also screen for F or G protein binders using a human nanobody library. By utilizing the human nanobody library, we can significantly minimize the risk of immunogenicity and obtain nanobody candidates that are better suited for therapeutic testing in human subjects.

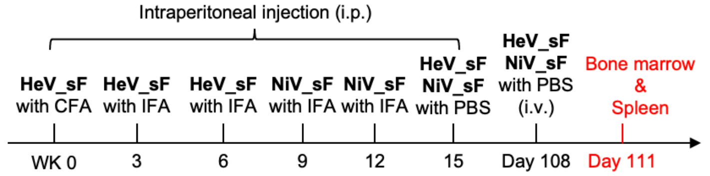


Figure 4: Nanomice sequential immunization scheme for HNV F proteins.

1.1: Nanomice immunization. Four groups of nanomice have already been immunized with soluble HNV F and G separately. Our preliminary data showed that almost all mice developed good immune response after 3 injections. We will next immunize 2 more groups, 8 mice each, sequentially with HNV F or G proteins (**Fig.4**). Injections (i.p.) will be performed at 3 weeks interval to allow more time for mouse immune system to react to each immunization. Specifically, for F proteins, mice will be immunized with HeV_sF at week 0, 3, 6, NiV_sF at week 9, 12, and a mixture of HeV_sF and NiV_sF at week 15, followed by a final boost (i.v.) with HeV_sF and NiV_sF mixture 3 days later. Bone marrow, spleen and PBMCs will be harvested 3 days after the final i.v. injection. HeV_sG and NiV_sG proteins immunization will follow the same scheme. Test blood/serum will be collected one week after each injection except for the first and last two injections, and serum antibody response to F or G proteins will be monitored by ELISA. The same serum will be used for HNV neutralization test by RP2.

1.2: Nanobody phage library construction. We have extracted RNA from bone marrow and spleen from NiV_sF immunized mice, constructed nanobody phage library, screened for NiV_sF binders, and identified NiV_sF_Nb9 to be NiV_sF and HeV-sF cross binder (**Fig.2**). We will complete the nanobody phage library construction for all immunization groups and perform sequential screening to identify cross binders. Specifically, total RNA will be extracted from bone marrow, spleen and PBMCs from the best responding mice based on ELISA and neutralization test results. Two-step PCR will be performed to amply VHH-DJ (nanobody sequence) from the matured heavy chain-only IgG1 mRNA molecules (**Fig. 5**). First, we will amplify VHH-DJ-CH2 domain from IgG1 using 30 forward primers aligning to the leading exons of 30 VHH genes and one reverse primer aligning to CH2 sequence of IgG1. Next, a mixture of 30 forward primers aligning to 5' end of individual VHH exon and 4 reverse primers aligning to JH1~4 will be used to amplify nanobody sequences, which will be cloned to pMES4 phagemid and subsequently transformed to TG1 E.coli cells.

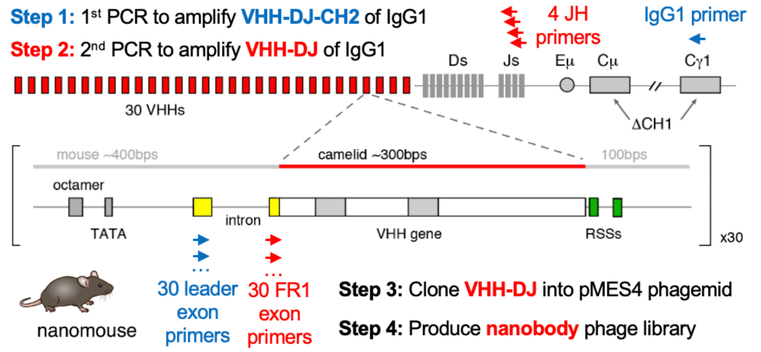


Figure 5: Nanobody phage library construction with nested PCR.

1.3: Screening nanobody libraries for HNV F/G binders. We will produce phages expressing individual nanobodies fused to their coat protein by infecting TG1 cells with VCSM13 helper phages. To identify nanobodies that recognize both HeV_sF and NiV_sF, libraries from F proteins immunized mice will be sequentially screened with HeV_sF and NiV_sF proteins in wells of 96-well Maxisorp plate; to identify nanobodies that recognize both HeV_sG and NiV_sG, libraries from G proteins immunized mice will be sequentially screened with HeV_sG and NiV_sG proteins. Multiple rounds of screening (phage panning) will be performed to ensure ideally over 100 times elution of phages from F or G protein coated wells than control wells (**Fig. 6A**). For each TG1 cell sub library from single or sequential F or G protein screening, 96 ~192 individual colonies will be picked and cultured in deep-well plates. Nanobodies (fused to phage coat protein pIII) induced by IPTG will be extracted from

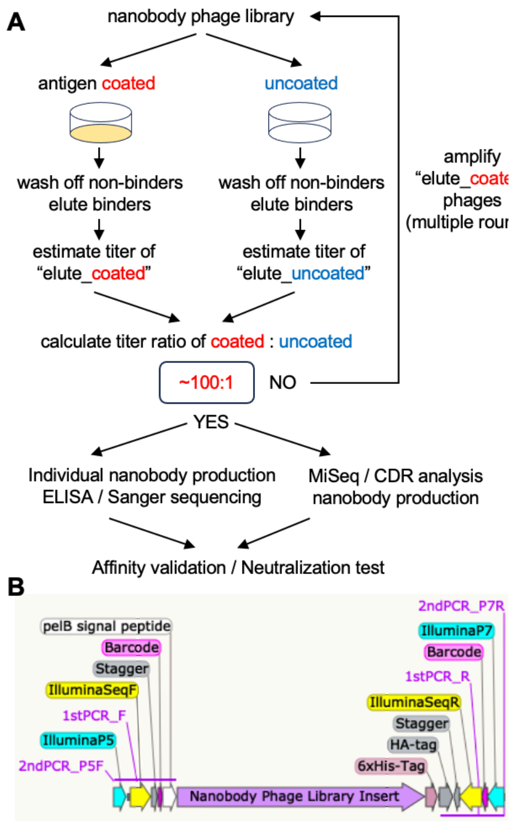


Figure 6: Phage screening, sanger sequencing, and MiSeq sequencing. (A) Nanobody phage screening strategy. (B) MiSeq sequencing library preparation. 1st round PCR will attach MiSeq F/R sequences to nanobodies, and 2nd round PCR will introduce P5/P7 adaptors to amplicon 5' and 3' ends.

periplasmic region of TG1 cells and tested for binding to HNV F or G proteins by ELISA. Phagemid of ELISA positive clones will be extracted and Sanger sequenced, and then transformed into WK6 E.coli cells to produce individual nanobodies (monomer, 13 kDa). In addition, sub libraries will be deep sequenced (**Fig. 6B**) and nanobody sequences analyzed as described before[29]. Nanobodies will be clustered based on their CDR3 differences and cross checked with the sequences of ELISA-positive clones. Representative unique nanobodies from each CDR3 class will be codon optimized and synthesized, cloned into pMES4 phagemid, and nanobodies will be produced in WK6 cells. <u>We will perform step 1.3 with our newly designed and constructed human nanobody library to identify less immunogenic nanobodies against HNV F and G protein.</u>

1.4: Nanobody binding validation and neutralizing activity assessment. To validate the binding of nanobodies to HNV F/G proteins, we will employ two methods: ELISA and Octet Bio-Layer Interferometry (BLI) affinity analysis. ELISA will allow us to determine the binding of nanobodies to the target proteins, while Octet BLI will provide detailed information on affinity. Additionally, we will perform Octet epitope binning assays using available F/G antibodies to map the epitopes recognized by the nanobodies. Furthermore, we will assess the neutralizing activity of the identified nanobodies with the neutralization assay developed by **RP2**[52,53]. These comprehensive analyses will enable us to evaluate the binding specificity, affinity, and neutralizing capability of the nanobodies. We will further select representative neutralizing nanobodies for structural analysis (**Aim 3**) and consider various engineering approaches such as Fc conjugation and multimerization (**Aim 4**) to further optimize the properties and efficacy of the nanobodies.

<u>Expected results, potential pitfalls and alternatives:</u>

We expect to be able to produce 5-10 HNV F or G nanobodies that either specifically recognize and neutralize HeV or NiV, or cross-bind to HeV and NiV proteins and cross neutralize the two viruses. We also expect to identify nanobodies with novel epitopes compare to currently available human antibodies.

Based on our success in identifying HeV_sF and NiV_sF cross binder (NiV_sF_Nb9 from nanomouse immunization) and SARS-CoV-2/SARS/WIV1 cross neutralizers (hNb3, 6, 17, 18 and 31 from human nanobody library screening), we do not anticipate any major technical or methodological pitfalls. While we have high expectations for our study, there are potential pitfalls that we need to consider. One challenge could be the limited number of nanobody candidates that meet our criteria, particularly if their affinity or neutralizing activity is not optimal. In such cases, we have a strategy in place to perform in vitro PCR-based CDR maturation to generate nanobody variants with improved properties. Additionally, there is a possibility that our efforts to identify HNV F or G cross binders/neutralizers using nanomice or the human nanobody library may not yield satisfactory epitope diversity. To overcome this limitation, an alternative approach would involve immunizing camelid animals and applying the same selection and screening processes to identify target-specific nanobodies.

Aim 2: Identification of nanobodies to ARVs.

***<u>Rationale and Preliminary Data</u>*:**

The surface of LASV and MACV virions is covered by the heavily glycosylated trimeric type-1 fusion glycoprotein precursor (GPC)[54-57]. The GPC trimer consists of receptor-binding GP1, fusion machinery GP2, and the associated stable signal peptide (SSP)[57-62]. The extensive glycan shield on GP1 and GP2 poses a challenge for the immune system to generate a robust GPC-directed antibody response, resulting in weak and inconsistent neutralizing antibody development during natural infection[63-67]. Only a few LASV-neutralizing antibodies have been isolated from convalescent patients to date[68]. While LASV GPC has shown neutralizing response in rabbit and guinea pig studies, serum neutralization has not been achieved in mouse models[69-71]. In contrast, MACV neutralizing antibodies have been successfully isolated from GPC immunized mice[72]. However, there are currently no FDA-approved treatments or vaccines for LASV or MACV infections. In our study, we aim to leverage the small size of nanobodies to develop glycan shield-penetrating neutralizing nanobodies against LASV and MACV.

In our experiments, we immunized 8 nanomice with a stabilized LASV GPC trimer, following a modified scheme. We performed an intravenous boost injection on day 45 and harvested bone marrow and spleen on day 48. With one mouse accidentally died on day 46, 7 mice remain. Over time, 6 out of 7 mice developed an antibody response against GPC, reaching their highest titers on day 48 (**Fig.7**). Importantly, the serum from nanomouse #3 on day 48 showed weak but detectable neutralization activity against LASV Josiah strain. These findings demonstrate the potential of nanomice as a valuable model for developing neutralizing nanobodies against LASV and MACV.

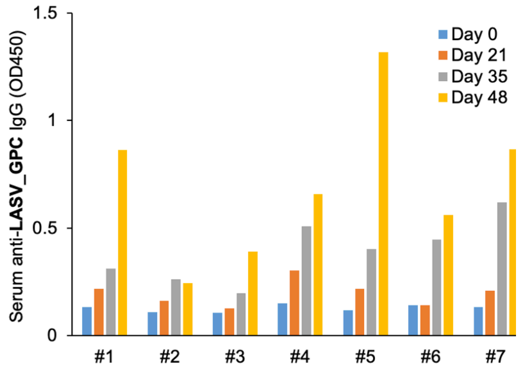


Figure 7: Antibody response aginst LASV GPC in immunized nanomice sera.

Experimental Design:

We will construct nanobody phage library using the top responders from the LASV immunized nanomice, with a particular focus on nanomouse #3 due to its detectable neutralization activity. We will immunize nanomice with alternative versions of soluble stabilized trimeric LASV GPC and MACV GPC proteins **provided by RP1** and construct *in vivo* matured nanobody phage libraries. These libraries and the human nanobody library will be screened with GPC proteins to identify nanobody binders. Individual nanobodies will be assessed for target specificity by ELISA and Octet, and neutralization activity of nanobody candidates will be **evaluated by RP1**.

2.1: Nanomice immunization and nanobody phage library construction. In our previous LASV GPC immunization attempt, 1 out of 8 nanomice developed serum neutralization titer at day 48. We will immunize two groups of nanomice separately with LASV GPC or MACV GPC designed by RP1 with slightly modified immunization regimen. For both LASV and MACV, injections (i.p.) will be performed once every 3 weeks, and test blood/serum will be collected one week after each injection except for the first injection. Serum antibody response to GPC proteins will be monitored by ELISA and send to RP1 for neutralization test. Immunization (i.p.) will stop until serum neutralization titer is observed, or the 6th injection is performed on week 15. Three days after a final boost (i.v.), bone marrow, spleen and PBMCs will be harvested for nanobody phage library construction following the same strategy listed in Aim1.

2.2: Screening nanobody libraries for LASV GPC or MACV GPC binders. Nanobody phage library screening using LASV GPC or MACV GPC will be performed for multiple rounds until the ideally 100:1 ratio of eluted phages from coated vs uncoated wells is achieved. We will carry out random colony picking followed by ELISA and Sanger sequencing, as well as Miseq for comprehensive analysis of enrich sub libraries. Same screening will be applied to human nanobody library too. 13 kDa nanobody candidates will be produced in WK6 cells for further tests.

2.3: Nanobody binding analysis and neutralizing activity assessment. Individual nanobodies will be validated using ELISA and Octet assays to confirm their interaction with GPC proteins. Precise affinity will be measured by BLI method. Epitope competition mapping and binning will be performed using known human/mouse GPC antibodies. Nanobodies with positive binding will be evaluated for neutralization activity by **RP1**.

***Expected results, potential pitfalls and alternatives*:** We expect to be able to produce 5-10 LASV or MACV neutralizing nanobodies targeting GPC proteins. The monoclonal antibodies that are currently known to have LASV neutralizing activity are categorized into four competition groups: GP1-A, GPC-A, GPC-B, and GPC-C[68]. We expect to identify LASV neutralizing nanobodies lie within these 4 groups. Given the small size of nanobodies and their potential to target hidden epitopes, we also expect to identify novel epitopes of vulnerability on LASV and MASC GPC surface. Even though the immunogenicity of LASV GPC seems to be very poor in mouse, nanomouse #3 developed serum neutralizing response to LASV Josiah strain. Furthermore, a nanobody neutralizer D5 was identified by screening the same LASV GPC with a naïve llama nanobody library[69]. Therefore, we do not anticipate any major technical or methodological pitfalls with nanomouse and human nanobody library platforms. However, if we are unsuccessful at isolating LASV/MACV binders and neutralizers using nanomice or human nanobody library, we will immunize camelid animals instead. We have a standing by llama from Capralogics Inc. ready for immunization. In the past a few years, we have immunized this animal with HIV envelop trimer, SARS-CoV-2 spike and HMPV F proteins, and all three studies led to the identification of potent nanobody neutralizers against these virus targets. We expect this llama will perform well against LASV and MACV. If our selected nanobody candidates are less-than-optimal, *in vitro* PCR-based CDR maturation will be performed and nanobody variants with improved activity will be screened.

Aim 3: Structural characterization.

Rationale and Preliminary Data.

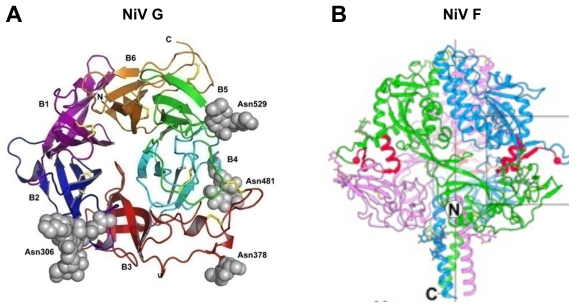


Figure 8: Structures of NiV G and F glycoproteins by X-ray crystallography. (A) NiV G head domain; (B) NiV F in the native prefusion conformation.

Dr. Kai Xu is a distinguished researcher with a proven track record in the structural determination of Henipavirus (HNV) glycoproteins and their complexes with receptors and monoclonal antibodies[50,73-75]. Notably, he achieved a significant milestone by determining the first crystal structures of the Nipah virus (NiV) G protein head domain and NiV F protein in the prefusion conformation (**Fig.8**). Moreover, Dr. Xu possesses exceptional expertise in designing HNV protein constructs that faithfully present their native conformation[43,74]. This capability enables him to create accurate representations of HNV glycoproteins, facilitating a deeper understanding of their functional properties and enabling investigations into their interactions with receptors and antibodies[51,76,77]. With his comprehensive skill set in protein design and structural determination, Dr. Xu stands as a leading authority in the field of HNV research.

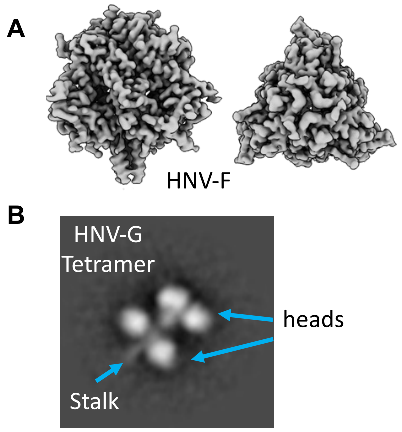


Figure 9: Validation of HNV F and G protein used in this study by EM single particle analysis. (A) Orthogonal views of HNV F protein cryo-EM 3D reconstruction at 2.5 A resolution; (B) 2D analysis of HNV G protein tetramer.

Our proposed research is driven by the need to present the native conformation of viral antigens, which is essential for antigenicity, immunogenicity, and the induction of protective immune responses. Glycoproteins of HNVs and ARVs are particularly prone to conformational flexibility, resulting in sample heterogeneity. To overcome this challenge, our preliminary data demonstrate the feasibility of creating viral antigens that display their native protein conformation. We have successfully stabilized viral antigens in their native states by designing prefusion conformation stabilizing mutations and incorporating specific stabilizing tags, such as GCNt and GCN4, to F and G proteins, respectively[50,73] (described in **RP1, 2 and Core D**). This stabilization approach has been validated through structural analysis (**Fig.9**), biochemical and biophysical characterization, and functional assessments, including receptor binding and the induction of neutralizing antibody responses.

Additionally, we have extensive experience in determining high-resolution structures of nanobodies in complex with viral antigens, such as SARS-CoV-2 spike proteins[29] (**Fig.10**). Leveraging advanced structural and functional characterization techniques such as X-ray crystallography, cryo-electron microscopy, and binding assays, we aim to unravel the detailed structural features and functional properties of the native-like viral antigens. This comprehensive understanding will provide critical insights into the interactions between antigens and antibodies, as well as the mechanisms of immune recognition. Ultimately, this knowledge will guide the rational design of effective vaccines and therapeutics targeting viral infections.

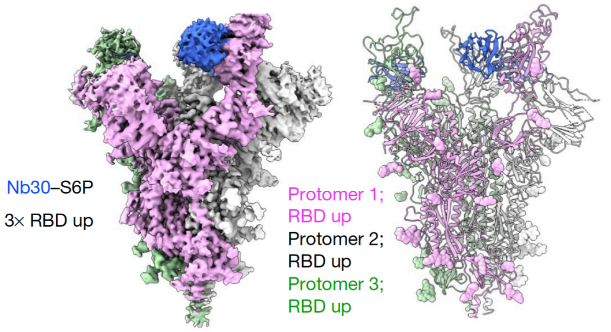


Figure 10: Cryo-EM single particle analysis of broadly protective nanobody against SARS-CoV2. 2.6 Angstrom high-resolution cryo-EM map (left) and structural model (right) of nanobody in complex with SARS-CoV2 spike protein.

Experimental Design:

Our experimental design aims to understand how nanobodies neutralize HNV and ARV viruses by analyzing their structure and interactions. Using X-ray crystallography and EM methods, we will determine the structures of nanobody-viral glycoprotein complexes, providing insights into their binding. Additionally, we will employ structure-based mutagenesis techniques to investigate the impact of specific mutations on viral entry and neutralization **in RP1 and 2**. By combining these approaches, we will gain detailed knowledge of nanobody epitopes and their interactions, contributing to the development of antiviral therapeutics for HNV and ARV.

3.1. Structural basis of nanobody cross-neutralization of both NiV and HeV. To determine the structure of the NiV or HeV F or G protein complexed with cross-neutralizing nanobodies, a series of steps will be undertaken. Initially, the NiV and HeV F and G proteins will be prepared in their native prefusion conformation by **Core D**. Subsequently, the purified nanobodies will be incubated with the F or G proteins to form stable complexes, optimizing the incubation conditions. High-resolution structures of the F or G protein complexes bound to the cross-neutralizing nanobodies will be determined using both X-ray crystallography and cryo-EM single particle analysis. Epitope analysis, including sequence analysis to identify conserved regions between the NiV and HeV F proteins, will provide insights into the potential neutralization mechanism. Additional experiments, such as site-directed mutagenesis, binding assays, and functional studies, will be conducted to validate the interaction between the nanobodies and the F proteins, confirming the importance of specific residues and regions identified in the structural analysis. Notably, a negative stain electron microscopy (ns-EM) analysis of a cross-neutralizing nanobody targeting both HeV and NiV G protein head domain has been performed, employing a compatible monoclonal antibody hAH1.3 to enhance particle 2D alignment (**Fig.11A**) and 3D reconstruction (**Fig. 11B**). This study demonstrates the feasibility of obtaining high-resolution cryo-EM structures. Ultimately, this study will yield crucial insights into the molecular mechanisms of neutralization and facilitate the development of effective antiviral therapies against HNVs.

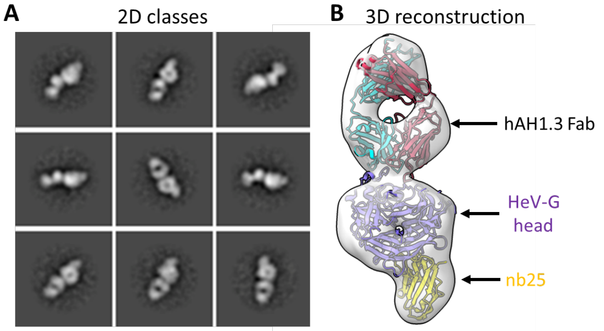


Figure 11: Anti-HeV-G nanobody epitope mapping by ns-EM analysis. (A) 2D classification; (B) 3D reconstruction showing a ternary complex of HeV-G head domain with a murine neutralizing antibody hAH1.3 and a nanobody nb25.

3.2. Structural analysis of LASV or MACV GPC proteins in complex with neutralizing nanobodies*.* The arenavirus glycoprotein precursor (GPC) proteins are key components of the viral envelope and play a crucial role in the entry and infection process of arenaviruses[78-80]. Arenaviruses are a family of RNA viruses that can cause severe diseases in humans, including hemorrhagic fevers[81,82]. The GPC proteins are synthesized as a single polypeptide chain, which undergoes post-translational processing to generate mature glycoproteins. The mature glycoproteins consist of two subunits, GP1 and GP2, that are responsible for receptor recognition and membrane fusion, respectively. The GP1 subunit is involved in receptor binding and determines the host tropism of the virus, while the GP2 subunit mediates membrane fusion between the viral envelope and host cell membrane. The GPC proteins are highly immunogenic and are targets for neutralizing antibodies. Understanding the structure and function of ARV GPC proteins is essential for developing effective antiviral strategies and vaccines against arenavirus infections. To investigate the structure of LASV and MACV GPC complexed with nanobodies using EM and X-ray crystallography, the experimental procedure involves preparation of stabilized GPC constructs, such as GPCysR4 and GPCysRRLL by **Core D**[58,83,84]. These constructs, developed with specific mutations, have been extensively used in structural studies of GPC-neutralizing antibodies. The purified GPC constructs are then mixed with the nanobodies of interest to form stable GPC-nanobody complexes. Cryo-EM analysis is performed by preparing cryo-EM grids, imaging them using a cryo-electron microscope, and generating a 3D reconstruction of the complex. X-ray crystallography is employed to crystallize the GPC-nanobody complex, collect diffraction data, and determine the crystallographic structure. The obtained structures are analyzed to identify nanobody-viral glycoprotein interactions and understand neutralization mechanisms. This combined approach provides valuable insights into the complex structures and guides the optimization of nanobody-based antiviral strategies.

Expected results, potential pitfalls and alternatives:

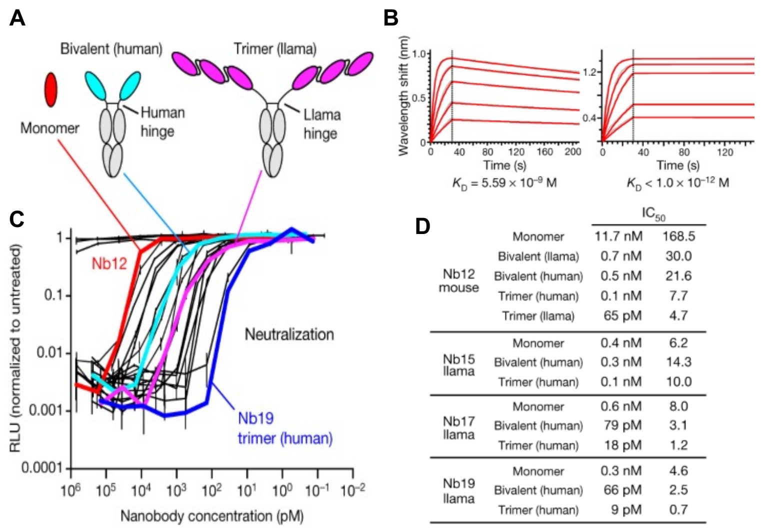
We anticipate that our research efforts will result in the generation of high-resolution structures of nanobody-antigen complexes, providing detailed insights into the precise epitope information and neutralization mechanisms. However, obtaining high-quality crystals suitable for X-ray analysis can be a challenging task. We are aware that extensive optimization of crystallization conditions may be necessary to promote crystal formation. To overcome this potential pitfall, we will employ various strategies and screening methods to enhance crystal quality and obtain the desired structural information. The conformational flexibility and heterogeneity of viral antigens and their complexes pose another challenge. In such cases, cryo-EM SPA can serve as a valuable alternative to X-ray crystallography. Through the application of computational algorithms, we can classify different particle conformations and extract high-resolution structural information from cryo-EM data. This approach will provide valuable insights into the structural features and dynamics of the viral antigens, despite their inherent flexibility. While our GPC stabilization strategy is primarily derived from LASV antigens, it may not be equally effective for MACV antigens due to their distinct structural and stability characteristics. As an alternative approach, we propose co-expressing MACV GPC with specific antibodies or nanobodies that can assist in stabilizing the native conformation of the antigens. This co-expression strategy will enhance the structural integrity of MACV antigens, enabling their characterization using various structural biology techniques.

Aim 4: Enhancing efficacy and CNS bioavailability of nanobodies.

Rationale and Preliminary Data.

Our aim is to enhance the therapeutic efficacy of nanobodies through engineering strategies. We will achieve this by extending the half-life of nanobodies, enhancing their interaction with immune effector cells, and increasing their binding avidity and neutralization potency. In addition, we will focus on improving the therapeutic effectiveness and availability of nanobodies within the CNS. The BBB presents a challenge in delivering therapeutic substances to the brain and spinal cord[85,86]. This barrier, formed by specialized brain capillary endothelial cells, tightly regulates the passage of molecules into the CNS, limiting the transport of drugs and antibodies[87-89]. Nanobodies, with their small size, show promise for targeted therapy of CNS diseases as they have demonstrated the ability to cross the BBB in recent studies[34-37]. In Aim 4, our research focuses on enhancing the therapeutic effectiveness and availability of nanobodies within the CNS, specifically for addressing viral infections that affect the CNS. We propose utilizing receptor-mediated transcytosis (RMT) as a strategy to facilitate the transport of nanobodies across the BBB[90,91]. RMT has emerged as a promising approach for delivering biologics, including antibodies, to the CNS[92]. RMT involves targeting specific receptors or transporters on brain capillary endothelial cells to enhance the uptake of nanobodies into the CNS. By engineering bi-specific nanobodies to selectively bind to these receptors or transporters, we can improve their transport across the BBB.

Our goal is to optimize the targeting and transcytosis efficiency of nanobodies through RMT, thereby improving their delivery and distribution within the CNS. This optimization will ultimately enhance the therapeutic efficacy of nanobodies in combating viral infections and CNS-related diseases.



		IC_{50}	
Nb12 mouse	Monomer	11.7 nM	168.5
	Bivalent (llama)	0.7 nM	30.0
	Bivalent (human)	0.5 nM	21.6
	Trimer (human)	0.1 nM	7.7
	Trimer (llama)	65 pM	4.7
Nb15 llama	Monomer	0.4 nM	6.2
	Bivalent (human)	0.3 nM	14.3
	Trimer (human)	0.1 nM	10.0
Nb17 llama	Monomer	0.6 nM	8.0
	Bivalent (human)	79 pM	3.1
	Trimer (human)	18 pM	1.2
Nb19 llama	Monomer	0.3 nM	4.6
	Bivalent (human)	66 pM	2.5
	Trimer (human)	9 pM	0.7

Figure 12: Fc fusion and multimerization improve potency of nbs against SARS-CoV2. (A) Diagrams show nanobody monomers, dimer, and trimers (fused to human IgG1 Fc) (B). Comparison BLI binding of monomer and trimer binding 1000x affinity improvement due to avidity. (C). Neutralization shows of SARS-CoV-2 pseudovirus by various nbs and multimers. (D). Table summarizes pseudovirus neutralization potency of selected nanobodies. Values given in molarity and ng/ml.

Experimental Design:

4.1. Generate nanobody Fc-fusion and tri-valent nanobodies*:* In our previous study focusing on SARS-CoV-2 nanobody therapy development, we successfully implemented two crucial engineering strategies: Human IgG1 Fc fusion and nanobody multimerization[29] (**Fig.12**). These strategies have shown significant advancements in improving the effectiveness of nanobody-based therapeutics.

To extend the half-life of nanobodies, we incorporated Human IgG1 Fc fusion through a flexible hinge region (**Fig.12A**). This fusion strategy substantially increased the duration of nanobody activity within the body by improving their stability and protection against degradation. The Fc region of the antibody played a crucial role in enhancing the therapeutic effect by providing enhanced durability. Furthermore, we designed nanobody multimers, specifically tri-valent structures, to enhance the binding affinity (**Fig.12B**) and neutralization potency of the nanobodies (**Fig.12C and D**). The multimeric arrangement allowed for stronger and more effective binding interactions with the target viral proteins, leading to improved neutralization of the virus and ultimately enhancing the therapeutic efficacy of the nanobodies.

Our experimental strategy involves extending the half-life and enhancing the therapeutic efficacy of our top nanobody candidates through the generation of nanobody Fc-fusion proteins and tri-valent nanobodies. To extend the half-life, we will fuse the Fc region of IgG1 to the nanobodies using either the human or mouse IgG1 hinge region or the llama IgG2a hinge region, taking advantage of the FcRn-mediated recycling process and stability conferred by these hinge regions, respectively. For the generation of nanobody trimers, we will design rational linkers based on structural information of viral glycoproteins and the approaching angle of nanobody recognition. This will ensure proper orientation and spatial arrangement of the nanobodies within the trimeric structure, leading to increased avidity, binding affinity, and improved target engagement. By implementing these strategies, we aim to enhance the stability, therapeutic potential, and neutralization potency of our nanobodies, ultimately contributing to the development of more effective therapeutic interventions against viral infections.

4.2. Design multi-specific nanobodies by combining nanobodies with non-overlapping epitopes: In this aim, we will design nanobodies that target distinct and non-overlapping epitopes on the viral glycoproteins. These nanobodies will be linked together in a tandem fashion to create a synergistic effect, enhancing their overall binding affinity and neutralization potency against the target viruses. The design of these multi-specific nanobodies will involve careful consideration of the orientation and flexibility of the linker regions, ensuring optimal cooperative binding properties. Through this approach, we aim to create a robust and comprehensive neutralizing response against viral infections, ultimately advancing the development of more effective therapeutic interventions. (**Fig.13A**)

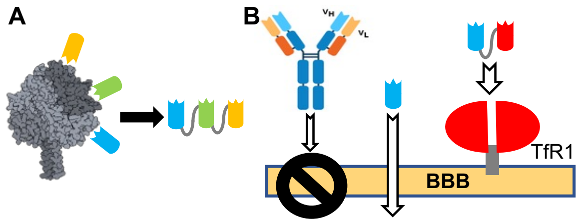


Figure 13: Bi-specific and multi-specific nanobody engineering. (A) Combination of epitope specificities to enable synergy, (B) Bi-specific nb with TfR1 specificity to improve brain targeted delivery.

4.3. Design Bi-specific nanobody by coupling with TfR1-targeting specificity to facilitate CNS delivery: In this aim, we will explore the incorporation of TfR1-specific nanobodies to enhance the delivery of nanobodies to the CNS (**Fig.13B**). The high expression of TfR1 on the blood-brain barrier makes it an attractive target for facilitating CNS delivery. To improve the penetration of nanobodies across the blood-brain barrier and enhance their bioavailability within the CNS, we will design bi-specific nanobodies by genetically fusing our candidate nanobodies against highly pathogenic neurotropic HNVs or ARVs with TfR1-specific nanobodies. By combining the targeting specificity of both nanobodies, we aim to achieve enhanced CNS delivery and therapeutic efficacy. We will evaluate several published TfR1-specific nanobodies, including FC5, a camelid single-domain antibody with known sequence[93]. FC5 has demonstrated the ability to transcytose across the blood-brain barrier and effectively deliver bioactive molecules to the CNS[36,94,95]. With its cross-reactivity across species and strong binding affinity to brain endothelial cells, FC5 holds promise for enhancing the CNS delivery of our nanobodies.

4.4. Efficacy test in both in vitro neutralization assay and animal studies. We will assess the efficacy of the original nanobodies identified in aims 1 and 2 and compare them with the engineered nanobodies in both *in vitro* neutralization assays and animal studies. Our goal is to evaluate the performance of the engineered nanobodies and determine their potential for therapeutic applications. The *in vitro* neutralization assays will assess the ability of the nanobodies to neutralize the target viruses, providing valuable insights into their antiviral potency. By measuring the reduction in viral replication or the inhibition of viral entry, we can determine the neutralization potency of the nanobodies. This will provide valuable insights into their antiviral activity and effectiveness. To further evaluate the efficacy of the nanobodies *in vivo*, we will utilize the rCedV-NiV-B chimera mouse model at the Uniformed Services University (USU) **in RP3** for HNVs, as well as animal models of ARVs developed at the University of Texas Medical Branch (UTMB) in **Core E**. These animal models will allow us to assess the therapeutic efficacy of the original and engineered nanobodies in terms of nanobody brain delivery efficiency, viral load reduction, disease progression, and overall survival rates. By comparing the outcomes between the two groups, we can determine the superiority of the engineered nanobodies in terms of therapeutic effectiveness.

Through these comprehensive in vitro and in vivo evaluations, we aim to demonstrate the improved efficacy of the engineered nanobodies compared to the original nanobodies. This assessment will provide crucial data for further development and optimization of nanobody-based therapeutics targeting HNVs and ARVs, with the ultimate goal of improving patient outcomes and combating viral infections.

<u>Expected results, potential pitfalls and alternatives</u>:

We anticipate that the design of tandemly linked nanobodies will result in enhanced neutralization potency and viral inhibition. The incorporation of a hinge and Fc region is expected to improve the half-life and stability of nanobodies, increasing their therapeutic potential. Coupling with TfR1-specific nanobodies is likely to improve brain delivery efficiency. However, potential pitfalls of using VHH FC5 as bi-specific nanobodies include short half-life due to susceptible to proteolytic degradation, thus limiting its development for clinical use. Alternative strategies, such as utilizing alternative receptor targeting or seeking to identify an antibody that binds to the same epitope, but that is derived from a fully human antibody library and has the requisite properties for clinical development, will be explored if necessary. Overall, these enhancements in efficacy and CNS bioavailability of nanobodies will significantly contribute to the development of potent antiviral therapies against CNS infections.

Aim 5: Expanding to other members of the viral groups.

<u>Rationale</u>

Building upon the valuable insights and progress achieved in Aims 1-4, Aim 5 aims to expand our nanobody-based therapeutic approach to target other members of the HNV and ARV in the **2nd phase** of this project. This expansion is based on the premise that the knowledge gained from studying NiV, HeV, LASV, and MACV can be extrapolated to other related viruses within these groups.

<u>Experimental Design:</u>

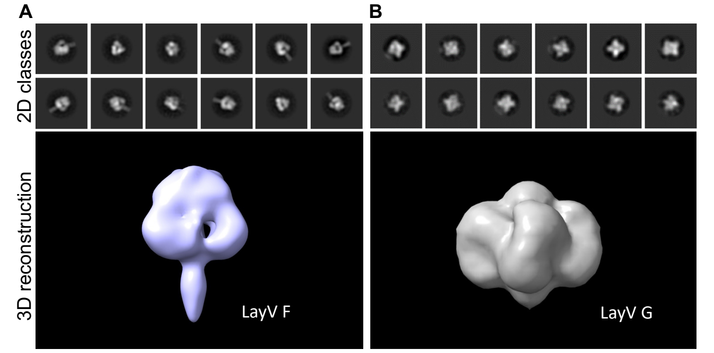


Figure 14: LayV F and G protein analysis. 2D classification and 3D reconstruction of LayV prefusion F (A), and G tetramer (B)

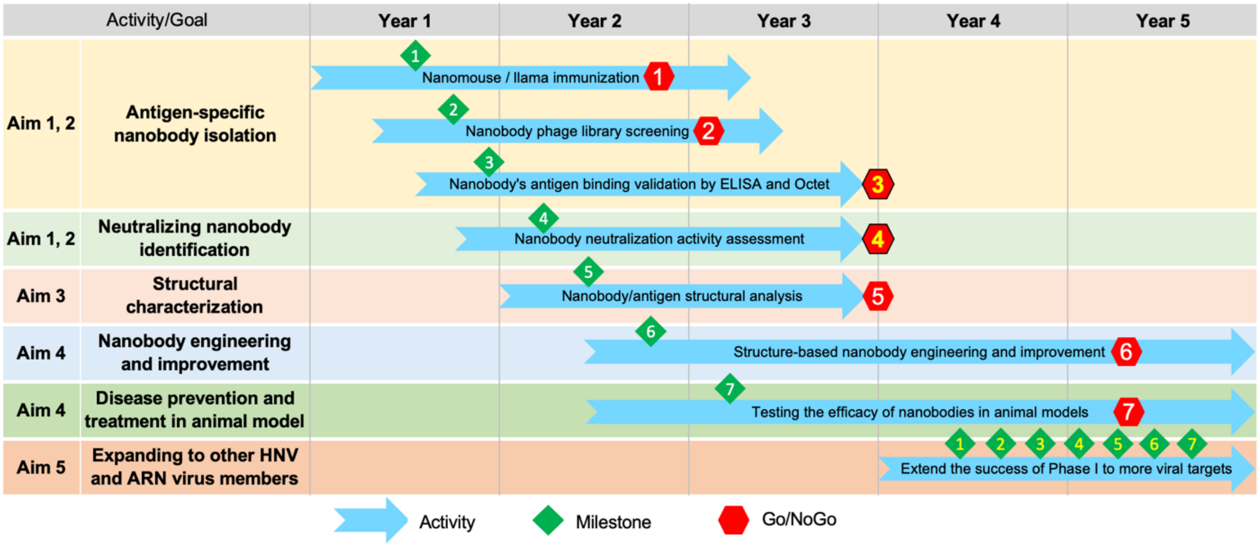
5.1. Langya virus: Langya virus (LayV) is a newly emerged member of the Henipavirus (HNV) family that has been linked to respiratory illness in humans[40,96]. LayV shares a close genetic relationship with Nipah virus and Hendra virus, both of which are known to cause severe disease in humans. To develop effective nanobody therapies against LayV infection, we have successfully generated native viral glycoprotein antigens by design, specifically the G and F glycoproteins (**Fig. 14**). By following our established research strategy, we will utilize nanobody development platforms, employ structural characterization techniques, and implement optimization approaches to identify nanobodies with protective properties against LayV. This process will involve evaluating the selected nanobodies with the neutralization assay and the animal disease model developed in collaboration with **RP2 and Core E**, and ready during the **phase 2** of this project. These efforts will not only contribute to development of nanobody-based therapies targeting LayV but also demonstrate the plug-and-play nature and rapid generalizability of our research strategy. By successfully applying our established approach to a newly emerged member of the Henipavirus family, we will showcase the versatility and effectiveness of our methodology in identifying and optimizing nanobodies against emerging viral pathogens. This will have broader implications for future studies on other viral infections, as our research strategy can be readily adapted and applied to different viral targets.

5.2. Lujo virus and Chapare virus: In our continued research, we will apply a similar strategy to the one used in Aim 2-4 for Lujo virus (LUJV)[41,97,98] and Chapare virus (CHAV)[42,99,100], which are closely related to the prototype LASV and MACV Arenaviruses. This strategy involves stabilizing the glycoprotein complex (GPC) using existing mutation designs transferred from LASV, isolating nanobodies specific to LUJV and CHAV, and performing structural characterization. By leveraging the knowledge and techniques gained from previous aims, we aim to identify nanobodies that can effectively neutralize LUJV and CHAV.

<u>Expected results, potential pitfalls and alternatives</u>:

We anticipate that our research strategy, combined with the knowledge gained from the phase 1 study, will enable us to identify potent neutralizing nanobodies against LayV, LUJV, and CHAV, providing promising therapeutic options for these viral infections. However, it is important to acknowledge potential challenges, such as limited cross-reactivity due to the genetic and antigenic differences between these additional viral targets and the prototypes studied in phase 1. In such cases, alternative approaches, including structure-guided rational design, may be explored to combine viral specificity and expand the protection breadth. Another potential limitation could be the difficulty to generate native prefusion GPC for the additional ARVs. In such instances, we will focus on utilizing the immune-dominant GP1 subunit, which is more stable and contains vulnerable sites for neutralizing antibody recognition. Rational design will be employed to shield non-native surfaces and minimize the generation of non-neutralizing nanobodies. Overall, the successful expansion of our research to other members of the HNV and ARV groups will make a significant contribution to broadening the effectiveness of nanobody-based therapies and addressing the global health risks associated with these viral pathogens.

D. Project Milestones and Timelines



<u>Milestones</u>: <u>(Same milestones are expected to be achieved during phase II with a faster pace).</u>

1. Serum neutralization activity observed (ID_{50}>10).
2. Nanobody phage library panning good enrichment achieved (enrichment>100:1).
3. High affinity viral protein-specific nanobodies isolated (affinity<1 nM).
4. Neutralizing nanobodies obtained (IC_{50}<1 ug/ml; neutralize multiple strains).
5. High resolution nanobody/antigen complex structure solved (resolution<3A).
6. Engineered nanobody molecules with improved activity obtained (IC_{50}<0.1 ug/ml; neutralize more strains).
7. Positive therapeutic effect of nanobodies in virus-induced diseases achieved (no symptoms or weight loss at 10 mg/kg or lower dose).

<u>Criteria to be met for each activity before moving forward (Go/NoGo)</u>:

1. ELISA signal of post-immunization sera is at least 3 times higher than pre-immunization sera.
2. Nanobody phage library panning reach to at least 5 times enrichment.
3. Viral protein-specific nanobody with at least 1 nM affinity **<u>(if not achieved, No Go for Phase II)</u>**.
4. Nanobodies neutralize at IC_{50}<10 ug/ml and/or multiple strains (**<u>if not achieved, NoGo for Phase II)</u>**.
5. Resolve structure with high enough resolution to define nanobody epitopes.
6. Engineered nanobodies achieve better potency and breadth (IC_{50}<0.1 ug/ml; neutralize more strains).
7. Positive therapeutic effect achieved (no symptoms or weight loss at 10 mg/kg or lower dose).

E. Industry Expertise and Regulatory Considerations:

If new lead nanobodies engineered nanobodies are identified from **Research Project 5 (RP5),** collaboration with industry and regulatory experts in **Core D** and the **Scientific Advisory Board (SAB)** will be initiated. This collaboration will involve seeking their expertise in various areas such as the identification of correlates of protection, lead optimization, stability testing, and manufacturability testing of the newly discovered nanobodies. Nanobody candidates can be engineered as desired in collaboration with investigators from **Core D**. This may involve modifications such as altered N-glycans, Fc mutations to enhance effector functions and prolong half-life, and evaluation of multimeric formats. The engineered nanobodies will then be further evaluated to correlate their mechanisms of action with maximal efficacy using the rCedV model explored in the **RP3**, and potentially transitioned to testing in authentic NiV/HeV animal models under the supervision of **Core E**. Throughout this process, stability and manufacturability assessment will be conducted to ensure the identification of a lead prototype format for further development. The goal will be to identify single domain mAb prototypes suitable for long-term intramuscular prophylaxis and/or intravenous post-exposure prophylaxis and therapy. As monoclonal antibodies are regulated by The Center for Drug Evaluation and Research (CDER), a division of the U.S. Food and Drug Administration. Therefore, all activities related to the development and evaluation of the mAbs will be performed in accordance with regulatory expectations set forth by CDER.

References:

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Luby, S. P. & Gurley, E. S. Epidemiology of henipavirus disease in humans. *Curr Top Microbiol Immunol* **359**, 25-40, doi:10.1007/82_2012_207 (2012).

3 Kerber, R. *et al.* Research efforts to control highly pathogenic arenaviruses: a summary of the progress and gaps. *J Clin Virol* **64**, 120-127, doi:10.1016/j.jcv.2014.12.004 (2015).

4 Patterson, M., Grant, A. & Paessler, S. Epidemiology and pathogenesis of Bolivian hemorrhagic fever. *Curr Opin Virol* **5**, 82-90, doi:10.1016/j.coviro.2014.02.007 (2014).

5 Kummer, S. & Kranz, D. C. Henipaviruses-A constant threat to livestock and humans. *PLoS Negl Trop Dis* **16**, e0010157, doi:10.1371/journal.pntd.0010157 (2022).

6 Gurley, E. S. *et al.* Convergence of Humans, Bats, Trees, and Culture in Nipah Virus Transmission, Bangladesh. *Emerg Infect Dis* **23**, 1446-1453, doi:10.3201/eid2309.161922 (2017).

7 Halpin, K. *et al.* Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:10.4269/ajtmh.2011.10-0567 (2011).

8 Clayton, B. A. Nipah virus: transmission of a zoonotic paramyxovirus. *Curr Opin Virol* **22**, 97-104, doi:10.1016/j.coviro.2016.12.003 (2017).

9 Luby, S. P. *et al.* Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis* **15**, 1229-1235, doi:10.3201/eid1508.081237 (2009).

10 Kulkarni, D. D., Tosh, C., Venkatesh, G. & Senthil Kumar, D. Nipah virus infection: current scenario. *Indian J Virol* **24**, 398-408, doi:10.1007/s13337-013-0171-y (2013).

11 Hsu, V. P. *et al.* Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis* **10**, 2082-2087, doi:10.3201/eid1012.040701 (2004).

12 Soman Pillai, V., Krishna, G. & Valiya Veettil, M. Nipah Virus: Past Outbreaks and Future Containment. *Viruses* **12**, doi:10.3390/v12040465 (2020).

13 Sahay, R. R. *et al.* Experiential learnings from the Nipah virus outbreaks in Kerala towards containment of infectious public health emergencies in India. *Epidemiol Infect* **148**, e90, doi:10.1017/s0950268820000825 (2020).

14 Johnson, K. M., Kuns, M. L., Mackenzie, R. B., Webb, P. A. & Yunker, C. E. Isolation of Machupo virus from wild rodent Calomys callosus. *Am J Trop Med Hyg* **15**, 103-106, doi:10.4269/ajtmh.1966.15.103 (1966).

15 Aguilar, P. V. *et al.* Reemergence of Bolivian hemorrhagic fever, 2007-2008. *Emerg Infect Dis* **15**, 1526-1528, doi:10.3201/eid1509.090017 (2009).

16 Shaffer, J. G. *et al.* Lassa fever in post-conflict sierra leone. *PLoS Negl Trop Dis* **8**, e2748, doi:10.1371/journal.pntd.0002748 (2014).

17 Asogun, D. A. *et al.* Molecular diagnostics for lassa fever at Irrua specialist teaching hospital, Nigeria: lessons learnt from two years of laboratory operation. *PLoS Negl Trop Dis* **6**, e1839, doi:10.1371/journal.pntd.0001839 (2012).

18 Günther, S. & Lenz, O. Lassa Virus. *Critical Reviews in Clinical Laboratory Sciences* **41**, 339-390, doi:10.1080/10408360490497456 (2004).

19 Salazar-Bravo, J., Ruedas, L. A. & Yates, T. L. Mammalian reservoirs of arenaviruses. *Curr Top Microbiol Immunol* **262**, 25-63, doi:10.1007/978-3-642-56029-3_2 (2002).

20 Enria, D. A. & Barrera Oro, J. G. Junin virus vaccines. *Curr Top Microbiol Immunol* **263**, 239-261, doi:10.1007/978-3-642-56055-2_12 (2002).

21 Barrera Oro, J. G. & McKee, K. T., Jr. Toward a vaccine against Argentine hemorrhagic fever. *Bull Pan Am Health Organ* **25**, 118-126 (1991).

22 Hamers-Casterman, C. *et al.* Naturally occurring antibodies devoid of light chains. *Nature* **363**, 446-448, doi:10.1038/363446a0 (1993).

23 Muyldermans, S., Atarhouch, T., Saldanha, J., Barbosa, J. A. & Hamers, R. Sequence and structure of VH domain from naturally occurring camel heavy chain immunoglobulins lacking light chains. *Protein Eng* **7**, 1129-1135, doi:10.1093/protein/7.9.1129 (1994).

24 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annu Rev Biochem* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

25 Muyldermans, S. Applications of Nanobodies. *Annu Rev Anim Biosci* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

26 Kunz, P. *et al.* The structural basis of nanobody unfolding reversibility and thermoresistance. *Sci Rep* **8**, 7934, doi:10.1038/s41598-018-26338-z (2018).

27 Ewert, S., Cambillau, C., Conrath, K. & Plückthun, A. Biophysical properties of camelid V(HH) domains compared to those of human V(H)3 domains. *Biochemistry* **41**, 3628-3636, doi:10.1021/bi011239a (2002).

28 Omidfar, K., Rasaee, M. J., Kashanian, S., Paknejad, M. & Bathaie, Z. Studies of thermostability in Camelus bactrianus (Bactrian camel) single-domain antibody specific for the mutant epidermal-growth-factor receptor expressed by Pichia. *Biotechnol Appl Biochem* **46**, 41-49, doi:10.1042/ba20060104 (2007).

29 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

30 Schoof, M. *et al.* An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science* **370**, 1473-1479, doi:10.1126/science.abe3255 (2020).

31 Koenig, P. A. *et al.* Structure-guided multivalent nanobodies block SARS-CoV-2 infection and suppress mutational escape. *Science* **371**, doi:10.1126/science.abe6230 (2021).

32 Saelens, X. & Schepens, B. Single-domain antibodies make a difference. *Science* **371**, 681-682, doi:10.1126/science.abg2294 (2021).

33 Voss, J. E. Engineered single-domain antibodies tackle COVID variants. *Nature* **595**, 176-178, doi:10.1038/d41586-021-01721-5 (2021).

34 Bélanger, K. *et al.* Single-Domain Antibodies as Therapeutic and Imaging Agents for the Treatment of CNS Diseases. *Antibodies (Basel)* **8**, doi:10.3390/antib8020027 (2019).

35 Pothin, E., Lesuisse, D. & Lafaye, P. Brain Delivery of Single-Domain Antibodies: A Focus on VHH and VNAR. *Pharmaceutics* **12**, doi:10.3390/pharmaceutics12100937 (2020).

36 Ruiz-López, E. & Schuhmacher, A. J. Transportation of Single-Domain Antibodies through the Blood-Brain Barrier. *Biomolecules* **11**, doi:10.3390/biom11081131 (2021).

37 Wouters, Y., Jaspers, T., De Strooper, B. & Dewilde, M. Identification and in vivo characterization of a brain-penetrating nanobody. *Fluids Barriers CNS* **17**, 62, doi:10.1186/s12987-020-00226-z (2020).

38 Leitner, D. F. & Connor, J. R. Functional roles of transferrin in the brain. *Biochim Biophys Acta* **1820**, 393-402, doi:10.1016/j.bbagen.2011.10.016 (2012).

39 Johnsen, K. B. *et al.* Targeting transferrin receptors at the blood-brain barrier improves the uptake of immunoliposomes and subsequent cargo transport into the brain parenchyma. *Sci Rep* **7**, 10396, doi:10.1038/s41598-017-11220-1 (2017).

40 Zhang, X. A. *et al.* A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med* **387**, 470-472, doi:10.1056/NEJMc2202705 (2022).

41 Briese, T. *et al.* Genetic detection and characterization of Lujo virus, a new hemorrhagic fever-associated arenavirus from southern Africa. *PLoS Pathog* **5**, e1000455, doi:10.1371/journal.ppat.1000455 (2009).

42 Delgado, S. *et al.* Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. *PLoS Pathog* **4**, e1000047, doi:10.1371/journal.ppat.1000047 (2008).

43 Chan, Y. P. *et al.* Biochemical, conformational, and immunogenic analysis of soluble trimeric forms of henipavirus fusion glycoproteins. *J Virol* **86**, 11457-11471, doi:10.1128/jvi.01318-12 (2012).

44 Bradel-Tretheway, B. G., Liu, Q., Stone, J. A., McInally, S. & Aguilar, H. C. Novel Functions of Hendra Virus G N-Glycans and Comparisons to Nipah Virus. *J Virol* **89**, 7235-7247, doi:10.1128/jvi.00773-15 (2015).

45 Bonaparte, M. I. *et al.* Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A* **102**, 10652-10657, doi:10.1073/pnas.0504887102 (2005).

46 Negrete, O. A. *et al.* EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* **436**, 401-405, doi:10.1038/nature03838 (2005).

47 Negrete, O. A., Chu, D., Aguilar, H. C. & Lee, B. Single amino acid changes in the Nipah and Hendra virus attachment glycoproteins distinguish ephrinB2 from ephrinB3 usage. *J Virol* **81**, 10804-10814, doi:10.1128/jvi.00999-07 (2007).

48 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

49 Bradel-Tretheway, B. G. *et al.* Nipah and Hendra Virus Glycoproteins Induce Comparable Homologous but Distinct Heterologous Fusion Phenotypes. *J Virol* **93**, doi:10.1128/jvi.00577-19 (2019).

50 Xu, K. *et al.* Crystal Structure of the Pre-fusion Nipah Virus Fusion Glycoprotein Reveals a Novel Hexamer-of-Trimers Assembly. *PLoS Pathog* **11**, e1005322, doi:10.1371/journal.ppat.1005322 (2015).

51 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

52 Amaya, M. *et al.* A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses* **15**, doi:10.3390/v15051077 (2023).

53 Khetawat, D. & Broder, C. C. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol J* **7**, 312, doi:10.1186/1743-422x-7-312 (2010).

54 Li, S. *et al.* Acidic pH-Induced Conformations and LAMP1 Binding of the Lassa Virus Glycoprotein Spike. *PLoS Pathog* **12**, e1005418, doi:10.1371/journal.ppat.1005418 (2016).

55 Schlie, K. *et al.* Characterization of Lassa virus glycoprotein oligomerization and influence of cholesterol on virus replication. *J Virol* **84**, 983-992, doi:10.1128/jvi.02039-09 (2010).

56 Pontremoli, C., Forni, D. & Sironi, M. Arenavirus genomics: novel insights into viral diversity, origin, and evolution. *Curr Opin Virol* **34**, 18-28, doi:10.1016/j.coviro.2018.11.001 (2019).

57 Bederka, L. H., Bonhomme, C. J., Ling, E. L. & Buchmeier, M. J. Arenavirus stable signal peptide is the keystone subunit for glycoprotein complex organization. *mBio* **5**, e02063, doi:10.1128/mBio.02063-14 (2014).

58 Hastie, K. M. *et al.* Structural basis for antibody-mediated neutralization of Lassa virus. *Science* **356**, 923-928, doi:10.1126/science.aam7260 (2017).

59 Igonet, S. *et al.* X-ray structure of the arenavirus glycoprotein GP2 in its postfusion hairpin conformation. *Proc Natl Acad Sci U S A* **108**, 19967-19972, doi:10.1073/pnas.1108910108 (2011).

60 York, J. & Nunberg, J. H. Myristoylation of the Arenavirus Envelope Glycoprotein Stable Signal Peptide Is Critical for Membrane Fusion but Dispensable for Virion Morphogenesis. *J Virol* **90**, 8341-8350, doi:10.1128/jvi.01124-16 (2016).

61 Eichler, R. *et al.* Identification of Lassa virus glycoprotein signal peptide as a trans-acting maturation factor. *EMBO Rep* **4**, 1084-1088, doi:10.1038/sj.embor.embor7400002 (2003).

62 Katz, M. *et al.* Structure and receptor recognition by the Lassa virus spike complex. *Nature* **603**, 174-179, doi:10.1038/s41586-022-04429-2 (2022).

63 Baize, S. *et al.* Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. *J Virol* **83**, 5890-5903, doi:10.1128/jvi.01948-08 (2009).

64 Fisher-Hoch, S. P., Hutwagner, L., Brown, B. & McCormick, J. B. Effective vaccine for lassa fever. *J Virol* **74**, 6777-6783, doi:10.1128/jvi.74.15.6777-6783.2000 (2000).

65 Sommerstein, R. *et al.* Arenavirus Glycan Shield Promotes Neutralizing Antibody Evasion and Protracted Infection. *PLoS Pathog* **11**, e1005276, doi:10.1371/journal.ppat.1005276 (2015).

66 Warner, B. M., Safronetz, D. & Stein, D. R. Current research for a vaccine against Lassa hemorrhagic fever virus. *Drug Des Devel Ther* **12**, 2519-2527, doi:10.2147/dddt.S147276 (2018).

67 Lukashevich, I. S. & Pushko, P. Vaccine platforms to control Lassa fever. *Expert Rev Vaccines* **15**, 1135-1150, doi:10.1080/14760584.2016.1184575 (2016).

68 Robinson, J. E. *et al.* Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits. *Nat Commun* **7**, 11544, doi:10.1038/ncomms11544 (2016).

69 Gorman, J. *et al.* Prefusion-Stabilized Lassa Virus Trimer Identifies Neutralizing Nanobodies and Reveals an Apex-Situated Site of Vulnerability. *bioRxiv*, 2022.2004.2021.488985, doi:10.1101/2022.04.21.488985 (2022).

70 Abreu-Mota, T. *et al.* Non-neutralizing antibodies elicited by recombinant Lassa-Rabies vaccine are critical for protection against Lassa fever. *Nat Commun* **9**, 4223, doi:10.1038/s41467-018-06741-w (2018).

71 Brouwer, P. J. M. *et al.* Lassa virus glycoprotein nanoparticles elicit neutralizing antibody responses and protection. *Cell Host Microbe* **30**, 1759-1772.e1712, doi:10.1016/j.chom.2022.10.018 (2022).

72 Amanat, F. *et al.* Monoclonal Antibodies with Neutralizing Activity and Fc-Effector Functions against the Machupo Virus Glycoprotein. *J Virol* **94**, doi:10.1128/jvi.01741-19 (2020).

73 Xu, K. *et al.* Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

74 Xu, K. *et al.* New insights into the Hendra virus attachment and entry process from structures of the virus G glycoprotein and its complex with Ephrin-B2. *PLoS One* **7**, e48742, doi:10.1371/journal.pone.0048742 (2012).

75 Xu, K. *et al.* Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. *Proc Natl Acad Sci U S A* **105**, 9953-9958, doi:10.1073/pnas.0804797105 (2008).

76 Steffen, D. L., Xu, K., Nikolov, D. B. & Broder, C. C. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. *Viruses* **4**, 280-308, doi:10.3390/v4020280 (2012).

77 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

78 Burri, D. J., da Palma, J. R., Kunz, S. & Pasquato, A. Envelope glycoprotein of arenaviruses. *Viruses* **4**, 2162-2181, doi:10.3390/v4102162 (2012).

79 Koma, T. *et al.* Glycoprotein N-linked glycans play a critical role in arenavirus pathogenicity. *PLoS Pathog* **17**, e1009356, doi:10.1371/journal.ppat.1009356 (2021).

80 Pennington, H. N. & Lee, J. Lassa virus glycoprotein complex review: insights into its unique fusion machinery. *Biosci Rep* **42**, doi:10.1042/bsr20211930 (2022).

81 Brisse, M. E. & Ly, H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. *Front Immunol* **10**, 372, doi:10.3389/fimmu.2019.00372 (2019).

82 Shao, J., Liang, Y. & Ly, H. Human hemorrhagic Fever causing arenaviruses: molecular mechanisms contributing to virus virulence and disease pathogenesis. *Pathogens* **4**, 283-306, doi:10.3390/pathogens4020283 (2015).

83 Sanders, R. W. & Moore, J. P. Virus vaccines: proteins prefer prolines. *Cell Host Microbe* **29**, 327-333, doi:10.1016/j.chom.2021.02.002 (2021).

84 Li, H. *et al.* A cocktail of protective antibodies subverts the dense glycan shield of Lassa virus. *Sci Transl Med* **14**, eabq0991, doi:10.1126/scitranslmed.abq0991 (2022).

85 Bhowmik, A., Khan, R. & Ghosh, M. K. Blood brain barrier: a challenge for effectual therapy of brain tumors. *Biomed Res Int* **2015**, 320941, doi:10.1155/2015/320941 (2015).

86 Pardridge, W. M. Drug transport across the blood-brain barrier. *J Cereb Blood Flow Metab* **32**, 1959-1972, doi:10.1038/jcbfm.2012.126 (2012).

87 Pandit, R., Chen, L. & Götz, J. The blood-brain barrier: Physiology and strategies for drug delivery. *Adv Drug Deliv Rev* **165-166**, 1-14, doi:10.1016/j.addr.2019.11.009 (2020).

88 Wu, D. *et al.* The blood-brain barrier: structure, regulation, and drug delivery. *Signal Transduct Target Ther* **8**, 217, doi:10.1038/s41392-023-01481-w (2023).

89 Hersh, D. S. *et al.* Evolving Drug Delivery Strategies to Overcome the Blood Brain Barrier. *Curr Pharm Des* **22**, 1177-1193, doi:10.2174/1381612822666151221150733 (2016).

90 Pulgar, V. M. Transcytosis to Cross the Blood Brain Barrier, New Advancements and Challenges. *Front Neurosci* **12**, 1019, doi:10.3389/fnins.2018.01019 (2018).

91 Zhang, W. *et al.* Differential expression of receptors mediating receptor-mediated transcytosis (RMT) in brain microvessels, brain parenchyma and peripheral tissues of the mouse and the human. *Fluids Barriers CNS* **17**, 47, doi:10.1186/s12987-020-00209-0 (2020).

92 Edavettal, S. *et al.* Enhanced delivery of antibodies across the blood-brain barrier via TEMs with inherent receptor-mediated phagocytosis. *Med* **3**, 860-882.e815, doi:10.1016/j.medj.2022.09.007 (2022).

93 Abulrob, A., Sprong, H., Van Bergen en Henegouwen, P. & Stanimirovic, D. The blood-brain barrier transmigrating single domain antibody: mechanisms of transport and antigenic epitopes in human brain endothelial cells. *J Neurochem* **95**, 1201-1214, doi:10.1111/j.1471-4159.2005.03463.x (2005).

94 Rissiek, B., Koch-Nolte, F. & Magnus, T. Nanobodies as modulators of inflammation: potential applications for acute brain injury. *Front Cell Neurosci* **8**, 344, doi:10.3389/fncel.2014.00344 (2014).

95 Tsitokana, M. E., Lafon, P. A., Prézeau, L., Pin, J. P. & Rondard, P. Targeting the Brain with Single-Domain Antibodies: Greater Potential Than Stated So Far? *Int J Mol Sci* **24**, doi:10.3390/ijms24032632 (2023).

96 Chakraborty, S. *et al.* Langya virus, a newly identified Henipavirus in China - Zoonotic pathogen causing febrile illness in humans, and its health concerns: Current knowledge and counteracting strategies - Correspondence. *Int J Surg* **105**, 106882, doi:10.1016/j.ijsu.2022.106882 (2022).

97 Simulundu, E. *et al.* Lujo viral hemorrhagic fever: considering diagnostic capacity and preparedness in the wake of recent Ebola and Zika virus outbreaks. *Rev Med Virol* **26**, 446-454, doi:10.1002/rmv.1903 (2016).

98 Cohen-Dvashi, H., Kilimnik, I. & Diskin, R. Structural basis for receptor recognition by Lujo virus. *Nat Microbiol* **3**, 1153-1160, doi:10.1038/s41564-018-0224-5 (2018).

99 Loayza Mafayle, R. *et al.* Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. *N Engl J Med* **386**, 2283-2294, doi:10.1056/NEJMoa2110339 (2022).

100 Escalera-Antezana, J. P. *et al.* Clinical features of fatal cases of Chapare virus hemorrhagic fever originating from rural La Paz, Bolivia, 2019: A cluster analysis. *Travel Med Infect Dis* **36**, 101589, doi:10.1016/j.tmaid.2020.101589 (2020).

From: Xu, Kai on behalf of Xu, Kai <xu.4692@osu.edu>
To: Haller, Sherry
Cc: Lindsey Marie Hornsby; Jianliang Xu; Workman, Jeff; Geisbert, Thomas W.; CHRISTOPHER BRODER; jxu40@gsu.edu
Subject: Re: OSU/GSU RP5 Final Documents
Date: Monday, June 5, 2023 4:55:40 PM
Attachments: RP5_U19-UTMB_USU_Research Strategy.pdf
image001.png

Hi Sherry,

Here is the research strategy of RP5.

Thanks,
Kai

From: Haller, Sherry <shhaller@UTMB.EDU>
Date: Monday, June 5, 2023 at 1:39 PM
To: Xu, Kai <xu.4692@osu.edu>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Jianliang Xu <jianliang.xu@gmail.com>, Workman, Jeff <workman.45@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: RE: OSU/GSU RP5 Final Documents

Thanks, Kai. We do not need a narrative for your research project. This is only included in the overall component.

From: Xu, Kai <xu.4692@osu.edu>
Sent: Monday, June 5, 2023 12:08 PM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>; Jianliang Xu <jianliang.xu@gmail.com>; Workman, Jeff <workman.45@osu.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; jxu40@gsu.edu
Subject: Re: OSU/GSU RP5 Final Documents

Here is an update for resource sharing plan.

Do we need project narrative?

Thanks,
Kai

From: Xu, Kai <xu.4692@osu.edu>

Date: Monday, June 5, 2023 at 11:42 AM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Jianliang Xu <jianliang.xu@gmail.com>, Workman, Jeff <workman.45@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: Re: OSU/GSU RP5 Final Documents

Hi Sherry,

Please see attached documents from Jianliang and me.
Including:

1. Authentication of key resource (not sure if you received this last time)
2. Abstract (not sure if you received this last time)
3. LOS from me. (not sure if you received this last time)
4. MPI plan
5. Updated vertebrate animal
6. Updated specific aims

Research plan will be ready later today.

Thanks,
Kai

From: Xu, Kai <xu.4692@osu.edu>
Date: Thursday, June 1, 2023 at 3:31 AM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Lindsey Marie Hornsby <lhornsby@gsu.edu>, Jianliang Xu <jianliang.xu@gmail.com>, Workman, Jeff <workman.45@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: Re: OSU/GSU RP5 Final Documents

Hi Sherry,

Here are the requested documents.

Thanks,
Kai

From: Jianliang Xu <jianliang.xu@gmail.com>
Date: Thursday, June 1, 2023 at 12:20 AM
To: Haller, Sherry <shhaller@UTMB.EDU>
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Kai <xu.4692@osu.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, jxu40@gsu.edu <jxu40@gsu.edu>
Subject: Re: OSU/GSU RP5 Final Documents

Hi Sherry,

Thanks for the reminder. Attached please find my LOS for the application. Kai will be sending Abstract, Authentication of materials and his LOS shortly.

My GSU position starts today, June 1, 2023. I am ccing my GSU email **jxu40@gsu.edu** and I will communicate with the team from my GSU account from now on.

Best regards,
Jianliang

On Tue, May 30, 2023 at 1:31 PM Haller, Sherry <shhaller@utmb.edu> wrote:

> Hello Dr. Xu and Dr. Xu,
> This is a reminder of the documents that we still need to get from you both for RP5 on the UTMB/USUHS U19 application. Our administrative review is this coming Thursday, June 1, so we will need all of the items listed below in final form by **EOB tomorrow, Wednesday, May 31**.
>
> - Abstract- 30 lines, 1 for RP5
> - Authentication of Key Biological Materials- 1 for RP5
> - Letter of Support – If both PI's could provide letters o support for your role on this project, we can include them. This is optional, but strengthens the proposal.
>
> For the final application, please provide the following by **EOB Monday June 5** *(hard deadline!).*
>
> - Research Strategy – Please remember to include references for both items listed below from the RFA for your research project:
>
>   **Milestone Plan:** In a clearly labeled section titled "Project Milestones and Timelines" include a clear delineation of goals with measurable milestones, including detailed quantitative and qualitative criteria for Go/No-Go decision-making, and a timeline for the attainment of each goal and milestone and should be reflected in the Milestone Plan for the overall Program. This plan must include Go/No-Go criteria to be met by the end of Year 3 of the award for continuation to Phase II. Milestones must specify the outcome(s) for each activity. Milestones should be quantifiable and scientifically justified, and include the completion of major research study activities, for example, identification of protective epitopes, animal model development, vaccine or mAb candidate down-selection, identification of correlates of protection, validation of vaccine or mAb strategies for other family members, and analysis, sharing and publication of final data. Milestone criteria should not simply be a restatement of

the specific aims. Using a Gantt chart or equivalent tool, describe the associated timelines and identified outcomes for the research Center.

Industry Expertise and Regulatory Considerations: For projects proposing early vaccine development, describe how industry partners will be identified and incorporated into the proposed project including a timeline for inclusion. For projects proposing IND-enabling later stage vaccine development, NIAID requires Centers to include active participation of an industry partner to ensure access to vaccine technology platforms, expertise in manufacturing, clinical development, and regulatory pathways. Applicants should describe the role of this partner in the proposed project and/or team to facilitate discovery, candidate evaluation and/or product development. For the purpose of this FOA, "industry" is defined as a large or small, domestic or foreign, pharmaceutical, biotechnology, bioengineering, or chemical company, or a related non-profit entity.

Please let me know if you have any questions about these or have any concerns about getting them back to us by the deadlines.
Thank you,

Sherry L. Haller, PhD
Associate Director
Center for Biodefense and Emerging Infectious Diseases
The University of Texas Medical Branch
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E: shhaller@utmb.edu
utmb Health
Center for Biodefense &
Emerging Infectious Diseases

~~SPECIFIC AIMS~~

~~The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) - are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparednessThreats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches which can be adapted across members of each viral family in a **"plug-and-play"** manner. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a concerns tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GP co-delivered with LASV NP subunits or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed in or monoclonal antibody PREP strategies which can provide protective immunity against Old and New World arenaviruses or emerging nairoviruses. (2) Develop reverse genetics tools to evaluate emerging arenaviruses and nairoviruses for which no isolates are available. Specifically, we will:~~

~~**Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:**~~

~~**1.1 Subunit immunogens and MNP vaccination:** Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches. We will then utilize lessons learned to address related "test case" arenaviruses and nairoviruses to develop a subunit/MNP approach for an old world arenavirus (LUJV) a new world arenavirus (CHPV), and an emerging bat borne nairovirus, Kasokero virus. We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.~~

~~**1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments:** Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.~~

~~**Milestone 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:**~~

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~~We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.~~

RESEARCH STRATEGY:

~~1.~~ SIGNIFICANCE~~:~~

1.

~~The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.~~

T~~T~~he viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. ~~–~~ Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% ~~XX~~ and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] ~~(REF)~~ where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. ~~—~~ This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness [3] ~~(REF)~~. Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success [4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability [5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.

The Mammarenaviruses: All mammarenaviruses share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge[7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. [9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13], Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

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The Orthonairoviruses: Unique from other bunyaviruses, orthonairoviruses are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [20]. Like mammarenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

~~**Research Project 1(RP1)** proposes preclinical research that will support Investigational New Drug Application (IND) applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.~~

- ~~**Old Word Mammarenaviruses (OWAV):** RP1 will utilize **LASV as the prototype OWAV** due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with **XX** deaths across West Africa(REF). LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to over **XX** countries (REFS). Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) (REFS). Most of these vaccines utilize the LASV (glycoprotein) GP or the LASV GP + Nucleoprotein (NP) as the target immunogens in these vectors (REFs).~~
- ~~**New World Mamarenaviruses (NWAV):** A live attenuated vaccine for JUNV is currently in use in Argentina [2], but there is conflicting evidence of whether cross protection with other NWAV is possible [3, 4] and there are safety concerns related to reversion to pathogenic versions. This proposal will use **MACV and JUNV as the prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.~~
- ~~**Orthonairoviruses:** Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated **XXX** cases per year across Europe, Africa, and Asia. CCHFV outbreaks are often very large list some recent examples. A suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974, but has numerous safety concerns and limited demonstrated efficacy(**REFS**). Since then, a number of vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages(REF); however only an MVA-CCHFV vaccine has begun to advance towards clinical trials (REF).~~

~~Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e. the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the safety of live-attenuated vaccines and suggests more pathologically~~

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~~benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success (**REFS**). The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability(REFS). Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.~~

~~**Subunit vaccines** have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments [5]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from the each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.~~

~~Once a candidate subunit-patch vaccine approach is selected, this approach will use lessons learned to produce plug-and-play approach to develop vaccines against a representative human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.~~

- ~~In 2008, Lujo virus was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [6]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, underscoring the need for antiviral tools against this emerging threat.~~
- ~~First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [7, 8]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [8]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective, but the phylogenetic divergence of CHAPV of XX% from JUNV suggests it may not be.~~
- ~~In 1977, Kasokero virus (KASV), a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [9]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for *Ornithodoros faini* ticks to participate in enzootic maintenance of KASV [10]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised.~~

~~**Pre-exposure prophylaxis (PREP)**, and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high- risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis (REFS). The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will also perform pilot studies to examine the feasibility of PREP utilizing with a serum half-life extended mAb against JUNVs. Clinically validated Fc mutations can result in an improvement in serum~~

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~~half-life from 3-4 weeks to 2-4 months which can increase the instant barrier immunity from 30-45 days to 60+ days ([11-13] [14]refs).~~

~~The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.~~

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens:

The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of ~~the five~~the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective ~~vaccine(~~vaccine [9, 10, 31, 32]~~REFS)~~. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens:

CCHFV is a divergent virus with up to ~~seven~~ six genetic ~~lineages~~ clades~~(REFS)~~. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation [20]. Given the glycoprotein is a pivotal protein dictating viral entry ~~and immune~~and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on ~~(REF)~~. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface [20] ~~(REFs)~~. Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control mammarenaviruses:

For situations where a vaccine does not exist or is not appropriate (*e.g.* immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE [34, 35] and LA [36,37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

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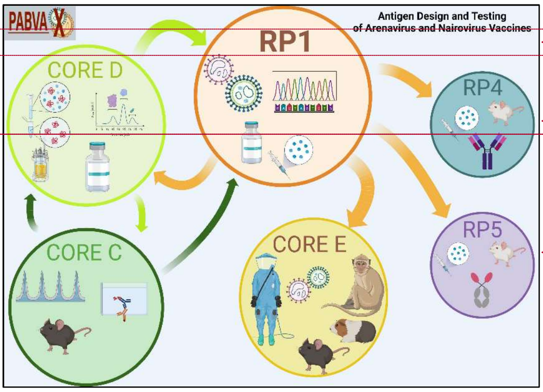
~~*Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses*~~

~~**3. Add blurb discussing unique access to field samples from across the world and established expertise to generate infectious clones from emerging arenaviruses and nairoviruses:**~~



~~3.~~ APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** and **orthonairovirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop **BSL4 research tools and animal models** for viruses that are otherwise not available to the research community.

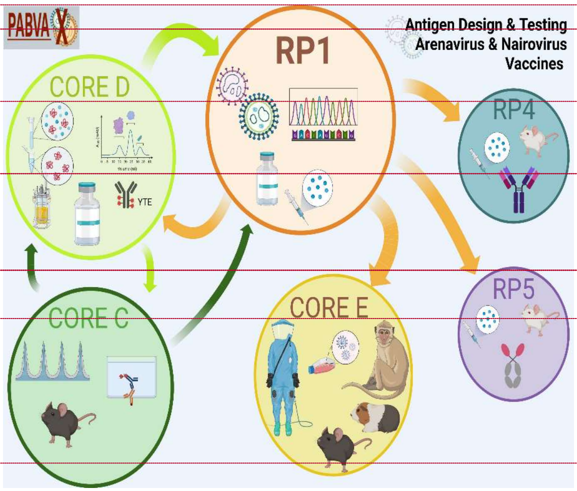


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed

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PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

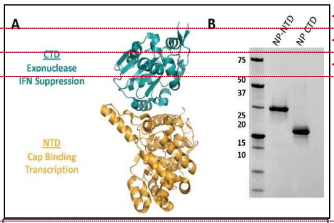

Figure ~~xx~~3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable ~~disulfide-linked~~disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and ~~-~~Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure ~~xx~~2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure ~~3~~2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure ~~xx~~2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

~~Our trimeric arenavirus GPs support efforts to isolate novel ultra-potent mAbs and to develop a second-generation arenavirus immunotherapeutic with reduced dosing requirements and broader reactivity compared to current LASV mAb candidates[25]. This significantly lowers the cost of goods while increasing ease of use for clinicians, both essential for a product used in developing nations where arenaviruses are prevalent.~~

LASV subunit vaccine protects guinea pigs against lethal challenge

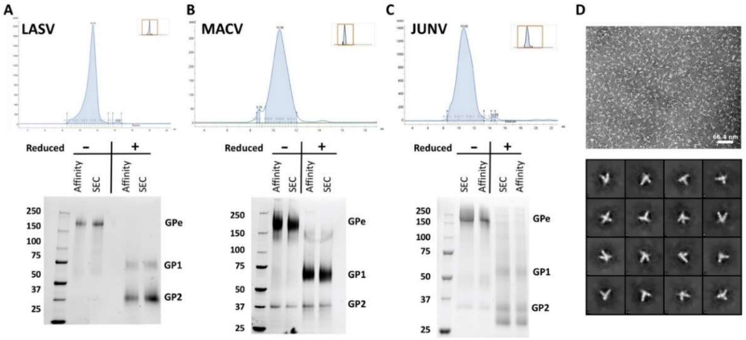

Figure ~~XX~~2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

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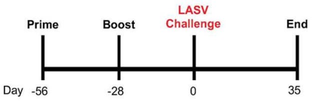
While classical vaccine approaches ~~have~~ largely focus~~ed~~ on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a ~~highly important~~ potent and complementary role in the long-term protection against pathogens. ~~Based on literature reports that~~ Understanding that T cell responses highly correlate with LASV recovery~~,~~ and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure ~~xx~~3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks.

We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3~~6~~)** to assess whether immune responses to GPe ~~only~~alone or~~,~~ ~~or~~ GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3~~6), and~~)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP ~~but~~ with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

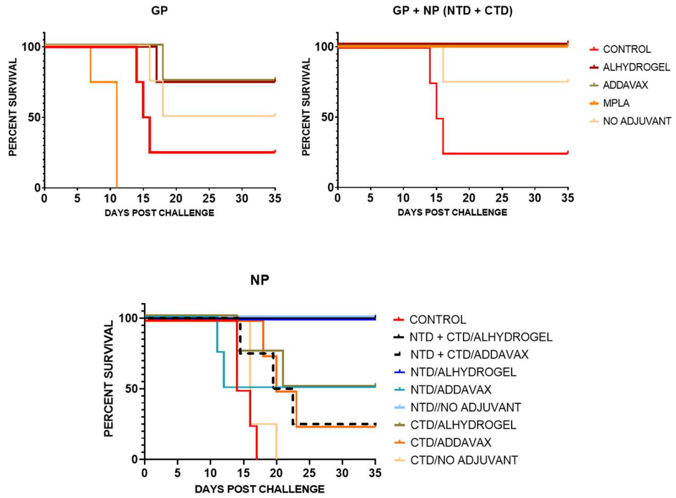


Figure ~~6~~3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 μg of GP, or 2x50 μg doses of GP + 2x25μg of NP-NTD + 2x25 μg of NP-CTD, or either 2x25 μg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

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Engineering glycoproteins antigens for ~~the~~ CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to ~~test~~ observe potent serum reactivity in CCHF survivors **(Figure ~~XX~~4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

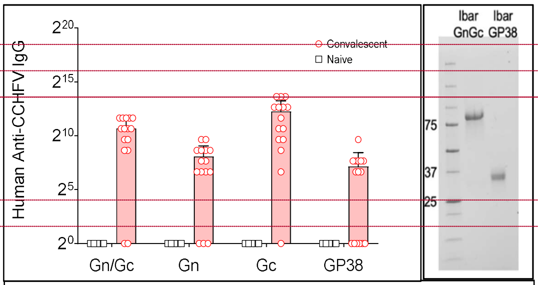

Figure ~~XX~~4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

~~*Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses*~~

~~***Rescue of recombinant viruses.*** An important goal of the PABVAX Center is to generate tools for pandemic preparedness. An important component of both RP1 and RP2 is to generate and recombinant viruses to fill gaps and provide tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with RP1 Core E will assist in the development and rescue of New World arenaviruses and the henipavirus Langya virus as detailed in the Research Strategies for RP1 and RP2.~~

~~**APPROACH**~~

~~***Overall Hypothesis:FILL THIS IN***~~

Specific Aim 1: Subunit immunogens and MNP vaccination ~~Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:~~

Rationale:

Scientific Approach: The choice of Mammarenavirus Prototype Pathogens is justified as follows:

- ~~*Old World Mammarenaviridae:* We propose LASV Josiah strain (Lineage 4) as the prototype pathogen due to the depth of available research data, availability of established rodent (**REF**) and nonhuman primate(NHP) models (**REFS**), frequency of exportation (**REFS**), and large number of annual cases throughout West Africa where the case fatality rate can be up to 60% in hospitalized cases (**REF**).~~
- ~~*New World Mammarenaviridae:* Due to the availability of a JUNV vaccine in the endemic region where JUNV circulates and the lack of cross protection against challenge with other NWAV, MACV, the causative agent of Bolivian hemorrhagic fever, will be used as the prototype pathogen for NWAV for studies outlined in Milestone 1.1. There are established guinea pig (**REF**) and NHP (**REF**) models for MACV. JUNV is the New World arenavirus with the largest knowledge base at present [**REF**]. For a pilot efficacy study outlined in Milestone 1.2, we will use JUNV Espindola Strain as a natural extension of previous published work demonstrating protective efficacy of the template PREP mAb (**REF**).~~
- ~~*Nairoviridae.* We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].~~

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RP1 proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Mammarenaviruses (OWAV)*; In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae (REFS). There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Mamarenaviruses (NWAV):* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[42] but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[43, 44]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[45]. For Phase 1, **RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola (Lineage XX)** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Orthonairoviruses*; In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[46]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[47]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[47]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[48]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert ~~[23]~~) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[49]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model [50] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38.

~~*Nairoviridae*. We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].~~

Once a candidate subunit-patch vaccine approach is selected, this RP1 will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative, “test case” human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case’s medical evacuation to South Africa[51]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal “test case” OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[52] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center’s **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[53, 54]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset

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demonstrating a clear need to specialized management and treatment[54]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

- In 1977, **Kasokero virus (KASV)**, a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [55]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini*, ticks to participate in enzootic maintenance of KASV[56]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[55]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[57]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families (REFS). An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs. ~~**Paragraph about subunit vaccines and PREP study.**~~

Milestone 1.1 Subunit immunogens and MNP vaccination

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Milestone ~~Milestone~~ 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in ~~table XX~~**Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in ~~Table XX~~Table 1. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E~~C~~** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[58] ~~(REFS)~~. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by ~~MNP Patch~~ **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table ~~: XX~~ **1:** ~~O~~Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table ~~XX~~ 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[59] ~~(REF)~~. Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[43, 44]. ~~(REFs).~~

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs. Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E~~C~~** as outlined in **Table ~~XX~~3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24
Control vaccine (VSV-LASV-GPC)	8	8	8	8
~~TOTALS~~	~~40~~	~~40~~	~~40~~	~~40~~

Table 3: ~~: XX o~~ Outline of PAN-LASV Vaccine Efficacy ~~S~~Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines

~~Similarly, , after down selecting the optimal adjuvant and vaccination route,~~ we will assess the potential ~~for our MACV subunit vaccines~~ to confer heterologous protection against three lineages of MACV ~~endemic to~~ genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core ~~C~~E** as outlined in **Table ~~XX~~4**. We will also utilize JUNV (Romero strain)[60] ~~(REF)~~ challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

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VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

~~Table: XX~~Table 4: ~~o~~Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table ~~XX~~5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.A~~1~~F.

VACCINE	LASV CHALLENGE
LASSA NP-NTD LASSA Gpe LASSA Gpe + NP-NTD	10 (TWO GROUPS OF 5)
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

~~Table: XX~~Table 5: ~~o~~Outline of NHP efficacy ~~studes~~studies for LASV Milestone 1.1.1.C~~B~~

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table ~~XX~~ 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[61] **~~(REF)~~.** ~~Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV,~~If Candid-1 confers protection in guinea pig study outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs [43, 44] **~~(REFs).~~**

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD MACV CHICAVA GPe MACV CHICAVA NP + GPe	10 (TWO GROUPS OF 5)
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

~~Table: XX~~Table 6: ~~o~~Outline of NHP efficacy studes for MACV Milestone

Milestone 1~~2~~.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:

Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[49]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
TOTALS	128		TOTALS	112

~~Table: XX~~Table 7: ~~o~~Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

	TOTALS	128	TOTALS	128

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times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

~~Table: XX~~Table 9: ~~o~~Outline of NHP efficacy studies for MACV Milestone

***Milestone* 1~~2~~.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice**: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
TOTALS	128		TOTALS	112

~~Table: XX~~Table 8: ~~o~~Outline of NHP efficacy studies for MACV Milestone

VACCINE	GUINEA PIG	VACCINE	LUJV
LUJV NP-NTD			(TWO GROUPS OF 5)
LUJV GPe + NP-NTD	8	LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	8	Control animals (NO VACCINE)	5
TOTALS	32	TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

Milestone

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

~~Table: XX~~Table 10: ~~o~~Outline of NHP efficacy studies for MACV Milestone

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2.1.2.C:~~—~~ Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

Milestone

~~2~~1.1.2.D:~~–~~ Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

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challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10)**

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LASSA NP-NTD	8
LASSA GPe	8
LASSA GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

Milestone

~~*2.1.2.E: Determination of protective efficacy against novel, emerging nairovirus using optimized subunit-patch vaccination in STAT-1KO mice and NHP*~~

Table: XX outline of NHP efficacy studes for MACV
Milestone 1.1.1.B

1.1.3~~1.F~~: Determination of cellular and humoral immune correlates associated with ~~protection~~protection.

Milestone 1.1.3.A~~1.F.a~~: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups:

We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard ~~whole blood~~ flow cytometric staining protocols will be used for surface and intracellular staining markers. ~~, b~~Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye ~~for 20 minutes at room temperature~~. ~~C~~Cells are then washed again and ~~,~~ surface stain cocktail is added to the tubes, incubated ~~for 20~~

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~~minutes~~, and washed. Cells are fixed ~~for 1 hour~~ with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed ~~in PBS~~. ~~Stained, fixed cells will then either be acquired directly.~~ Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: ~~–~~PBMC stimulations will be set up according to modifications of well established, previously published protocols.[62, 63] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37ºC for 1 hour. A protein transport inhibitor such as brefeldin A will then be added ~~at a concentration of 10mg/mL and~~ then incubated ~~at 37ºC for~~prior~~, until~~ to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37ºC. Stimulations to assess secreted analytes by multiplex-bead based assays ~~will be set up~~ in 96-well plates and supernatants ~~will be~~ will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye ~~for 20 minutes at room temperature~~, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated ~~for 20 minutes~~, and washed. Cells are incubated in BD Cytofix/Cytoperm solution ~~for 20 minutes~~ followed by a wash. ~~with BD Perm/Wash Buffer.~~ Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed ~~in PBS~~. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi~~4~~-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated ~~for 3 hours at room temperature, protected from light~~ and subsequently washed by pelleted centrifugation. ~~1mL of wash buffer will be added and beads will be pelleted by centrifugation at 200g for 5 minutes.~~ Wash buffer will be removed and discarded. Beads will be resuspended ~~in 300uL wash buffer~~ and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37ºC. Wells will be washed manually 3-times, followed by the addition of detection ~~antibody~~antibody, and incubated either overnight at 4ºC or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. ~~at room temperature, protected from light.~~ Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37ºC), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer~~**need model**~~ ~~ELISPOT Fluorescence Microplate Reader.~~

Data Analysis: We will ~~look at the~~examine differences in immune responses across groups between each timepoint and baseline. ~~Model assumptions will be checked for a one-way ANOVA to compare the three groups. we will run the analysis after checking model assumptions, if those are not met then we will adjust to a nonparametric model or transform the outcome appropriately. To look at the longitudinal aspect of the data, we will investigate measurements over time between groups, the interaction of group and time can be analyzed using covariance pattern models (or linear mixed models), where the correlation between observations measured over time can be handled more flexibly. As a more exploratory analysis we will also look at adjusting for possible confounding variables such as sex and age. For categorical variables, comparisons will be made using Chi-Square tests.~~ Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

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1.1.3.A:~~1.1.1.F.b~~: Systems serology Characterization of~~ ~~ Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups: ~~.~~

We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform ~~developed by Dr. Gunn in~~ with **Core D**[64-66] to define the Fc-mediated humoral antiviral functions associated with ~~vaccination~~vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, ~~CCHFV,LUJV~~CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens ~~utilized~~ used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages~~.~~, ~~W~~we will use a multiplexed analysis to determine ~~the~~ levels of antibodies against ~~the~~ viral antigens using ~~on~~ a MagPix xMAP instrument ~~as described~~.[65, 67] Specifically, each viral antigen will be coupled to coded magnetic beads ~~(Luminex)~~, mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM.~~ ~~ For quantitation of bound antibody, we will ~~make a standard~~ us~~e~~ing pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions:~~ ~~ We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.~~ at KGH.~~

ADCC: Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates ~~for 5 hours at 37C 5% CO_2,~~ and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses ~~were~~ will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction ~~was used~~ to determine statistical significance ~~between groups.~~. ~~A network analysis will be performed to identify significant antibody features. Associations between antibody features will be determined using nonparametric Spearman correlation coefficient. Statistically significant associations after Bonferroni correction for multiple comparisons (adjusted P < .05) were used to generate networks in Cytoscape (version 3.4.0).~~

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and ~~additional clinical information (ie~~clinical signs into the network analysis.~~; PES symptoms). These will be included in the network analysis.~~

***Milestone** **1.~~AIM 2~~2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing mammarenaviruses[68, 69] and orthonairovirus[70] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog but is still pathogenic in mice and humans (REFS). There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: ~~1.2~~ Pre-exposure Prophylaxis (PREP) using half-life extension antibody ~~treatments~~treatments.

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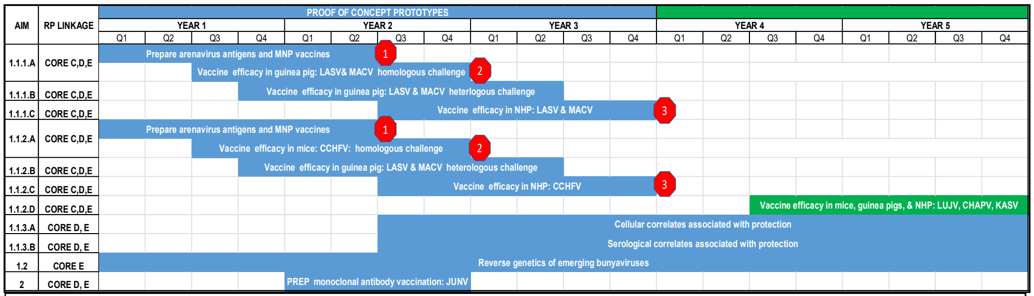
Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months(34-37).

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

Scientific Approach:

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine:~~.~~ In conjunction with ~~RP1~~ Cores D and ~~Core~~ E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb ~~with~~ whose potent therapeutic efficacy~~-~~[71, 72] ~~(guinea pig and NHP references)~~ we have previously described ~~by Abelson, Geisbert and Zeitlin~~ will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb~~(Table 4)~~. ~~The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (Bioqual, Inc.; Rockville, MDsee Core D) to select a lead candidate for testing in NHPs against Junin Romero challenge in Core E.~~ ~~For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference).~~ We will select t~~T~~he variant with the most favorable pharmacokinetic (described in **Core D**)~~s~~ ~~PK will be selected~~ for testing with ~~by RP1/~~**Core E.** Briefly, groups of 45 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV ~~(Espindola or Romero~~Espindola~~?)~~ delivered IV **(Table 11)**. For further details of the NHP testing, please see **Core E**. These data will be used by the Scientific Advisory ~~Committee~~ Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

Expected results, alternative ~~approaches and~~approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, ~~and~~ CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three “test case” viruses LUJV, CHAPV, and KASV in Phase II of the project.……. If ~~this~~ any~~is not the case any~~ of these Phase I vaccine projects have not met the “go” criteria, ~~three viruses by year 2~~ we will refocus ~~effects~~ efforts toward~~on~~ the remaining viruses ~~families~~ adjusting the requested budgets accordingly after ~~consulation~~consultation with NIAID and SAC.

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Please note: Discussion of expected results, other alternative ~~approaches~~approaches, and criteria for "go" or "no go" decisions for MILESTONES 1~~-4~~ and 2 are discussed as required in RFA-AI-20-023 in the ***Project Milestones and Timelines*** *section.*

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

References:

~~JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,~~

~~AIM 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:~~

~~Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines).~~ ~~We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC~~

~~*Please note: Discussion of expected results, other alternative approaches and criteria for "go" or "no go" decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*~~

~~JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,~~

~~Milestones-Timelines-GANT CHART~~

~~Summary statement impact~~

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1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Paweska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.

2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.

3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.

4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.

5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.

6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.

7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.

8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.

9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.

10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia I, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.

11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.

12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.
13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.
14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.
15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.
16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.
17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.
18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.
19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.
20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.
21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.
22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.
23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 20190523. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.
24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.
25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.
26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.
27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.
28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.
29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is

recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.

30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

43. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.

44. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.

45. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

46. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.

47. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.

48. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.

49. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.

50. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.

51. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.

52. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.

53. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.

54. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.

55. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.

56. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.

57. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.

58. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

59. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

60. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

61. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

62. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods. 2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

63. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

64. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

65. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

66. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

67. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

68. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

69. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

70. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

71. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

72. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

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RESEARCH STRATEGY:

1. SIGNIFICANCE

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness [3] . Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success [4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability [5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.

__The Mammarenaviruses__**:** All mammarenaviruses share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. [9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

__The Orthonairoviruses__: Unique from other bunyaviruses, orthonairoviruses are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [20]. Like mammarenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens: The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine [9, 10, 31, 32]. Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens: CCHFV is a divergent virus with up to six genetic clades. This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation [20]. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface [20] . Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control mammarenaviruses: For situations where a vaccine does not exist or is not appropriate (*e.g*. immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE [34, 35] and LA [3637] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

3. APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** and **orthonairovirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop **BSL4 research tools and animal models** for viruses that are otherwise not available to the research community.

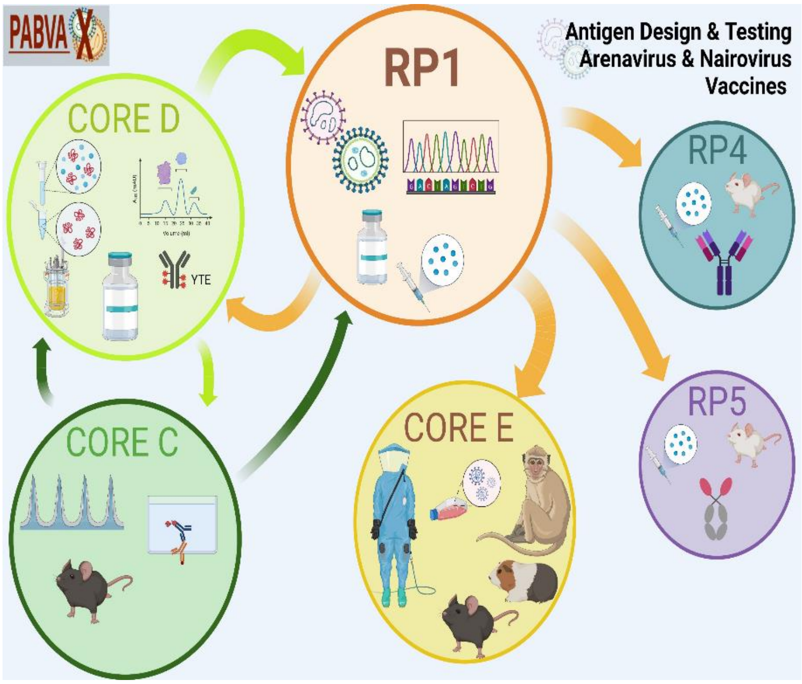


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed.

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE

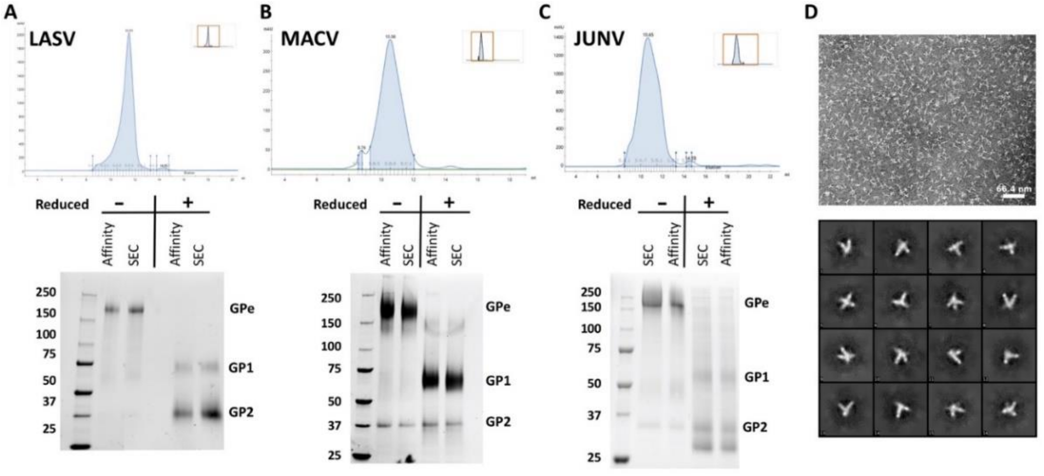


Figure 2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure 2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

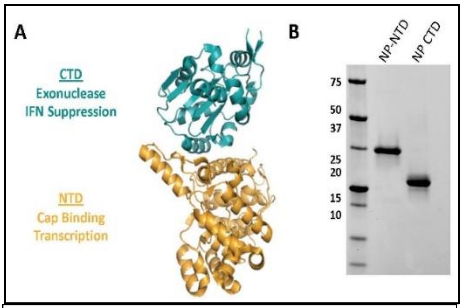
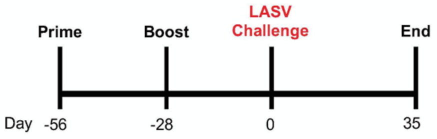


Figure 3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure 2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

<u>LASV subunit vaccine protects guinea pigs against lethal challenge</u>

While classical vaccine approaches largely focus on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a potent and complementary role in the long-term protection against pathogens. Understanding that T cell responses highly correlate with LASV recovery and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure 3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks. We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3)** to assess whether immune responses to GPe alone or GPe+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3)** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

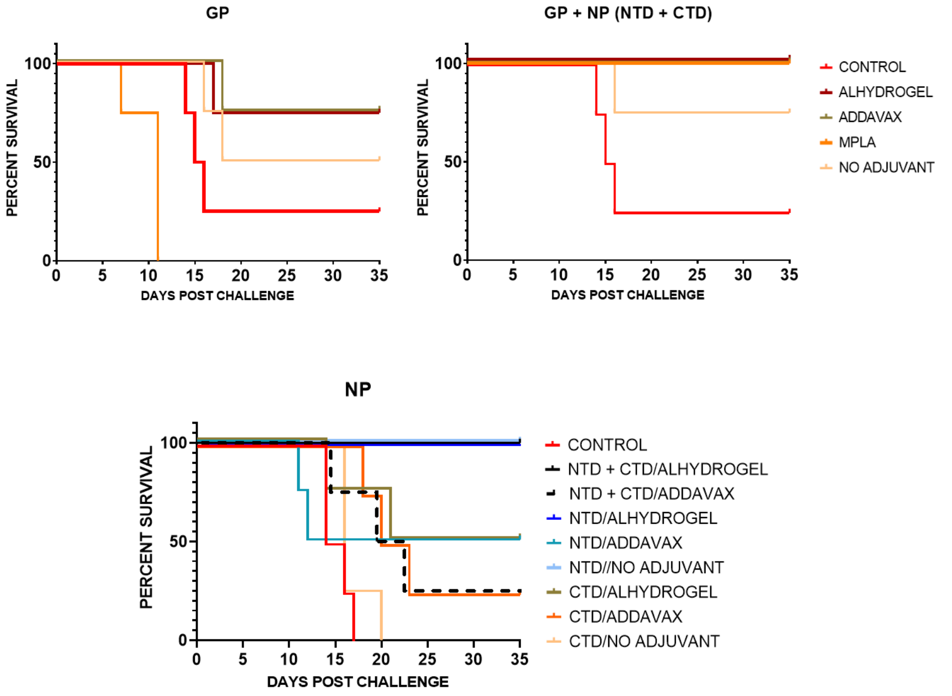


Figure 3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

Engineering glycoproteins antigens for CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to observe potent serum reactivity in CCHF survivors **(Figure 4).** We will use established protocols[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

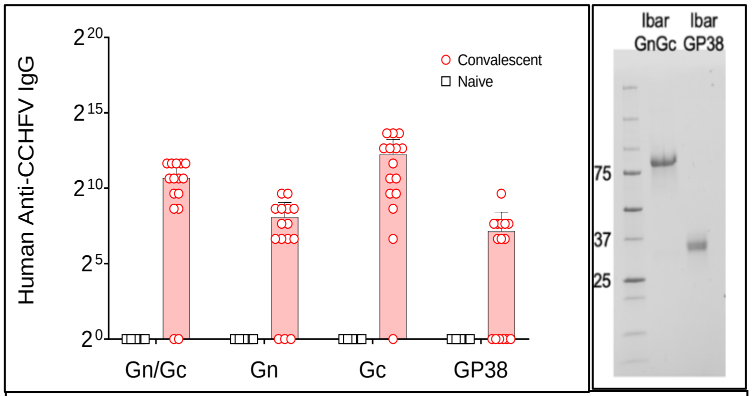


Figure 4: Engineered CCHFV proteins are reactive with serum from CCHFV survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

Specific Aim 1: Subunit immunogens and MNP vaccination Rationale:

Scientific Approach: The choice of Mammarenavirus Prototype Pathogens is justified as follows: **RP1** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- _Old Word Mammarenaviruses (OWAV):_ In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae (REFS). There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- _New World Mamarenaviruses (NWAV):_ A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [42], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [43, 44]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[45]. For Phase 1**, RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola (Lineage XX)** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- _Orthonairoviruses:_ In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[46]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[47]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying

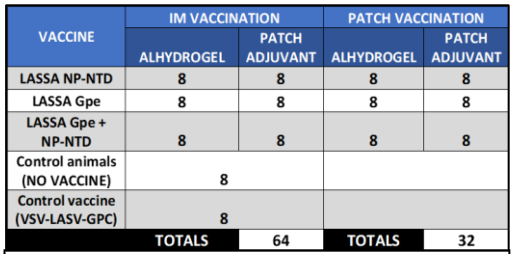
efficacy, advantages, and disadvantages[47]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[48]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[49]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model [50] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38.

Once a candidate subunit-patch vaccine approach is selected, this RP1 will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative, "test case" human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[51]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[52] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[53, 54]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[54]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [55]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV[56]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[55]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[57]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families (REFS). An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.

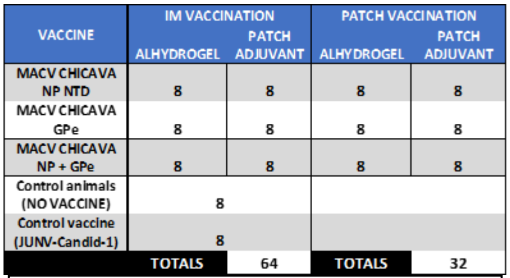


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
	TOTALS	64	TOTALS	32

Table 1: Outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in **Table 1** using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in **Table 1**. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core E** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[58]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

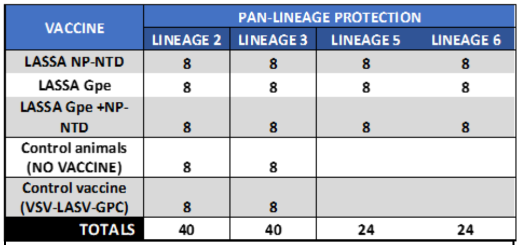


VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
	TOTALS	64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table 2** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain[59]**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[43, 44].

Milestone 1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs: Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core E** as outlined in **Table 3**. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.



VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: Outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines, we will assess the potential to confer heterologous protection against three lineages of MACV genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core E** as outlined in **Table 4.** We will also utilize JUNV (Romero strain)[60] challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

Table 4: Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

***Milestone* 1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table 5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.AF.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table 5: Outline of NHP efficacy studies for LASV Milestone 1.1.1.C

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[61]**.** If Candid-1 confers protection in guinea pig study outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs [43, 44]

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table 6: Outline of NHP efficacy studes for MACV Milestone 1.1.1.B

***Milestone* 1.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:** Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[49]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

Table 7: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone1.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
	TOTALS	128	TOTALS	112

Table 8: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table 9: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

Milestone 2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP: **RP1 and Core D** will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table 10: Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10)**

***Milestone* 1.1.3: Determination of cellular and humoral immune correlates associated with protection.**

Milestone 1.1.3.A: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups: We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard flow cytometric staining protocols will be used for surface and intracellular staining markers. Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye. Cells are then washed again and surface stain cocktail is added to the tubes, incubated, and washed. Cells are fixed with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed. Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: PBMC stimulations will be set up according to modifications of well established, previously published protocols.[62, 63] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37^{o}C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added then incubated prior to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37^{o}C. Stimulations to assess secreted analytes by multiplex-bead based assays in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated, and washed. Cells are incubated in BD Cytofix/Cytoperm solution followed by a wash. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated and subsequently washed by pelleted centrifugation. Wash buffer will be removed and discarded. Beads will be resuspended and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37^{o}C. Wells will be washed manually 3-times, followed by the addition of detection antibody, and incubated either overnight at 4^{o}C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37^{o}C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer.

Data Analysis: We will examine differences in immune responses across groups between each timepoint and baseline. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone 1.1.3.A:: Systems serology Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups*:* We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform with **Core D**[64-66] to define the Fc-mediated humoral antiviral functions associated with vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages, we will use a multiplexed analysis to determine levels of antibodies against viral antigens using a MagPix xMAP instrument .[65, 67] Specifically, each viral antigen will be coupled to coded magnetic beads , mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will use pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction to determine statistical significance.

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and clinical signs into the network analysis.

Milestone **1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing mammarenaviruses[68, 69] and orthonairovirus[70] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog but is still pathogenic in mice and humans (REFS). There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments.

Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the

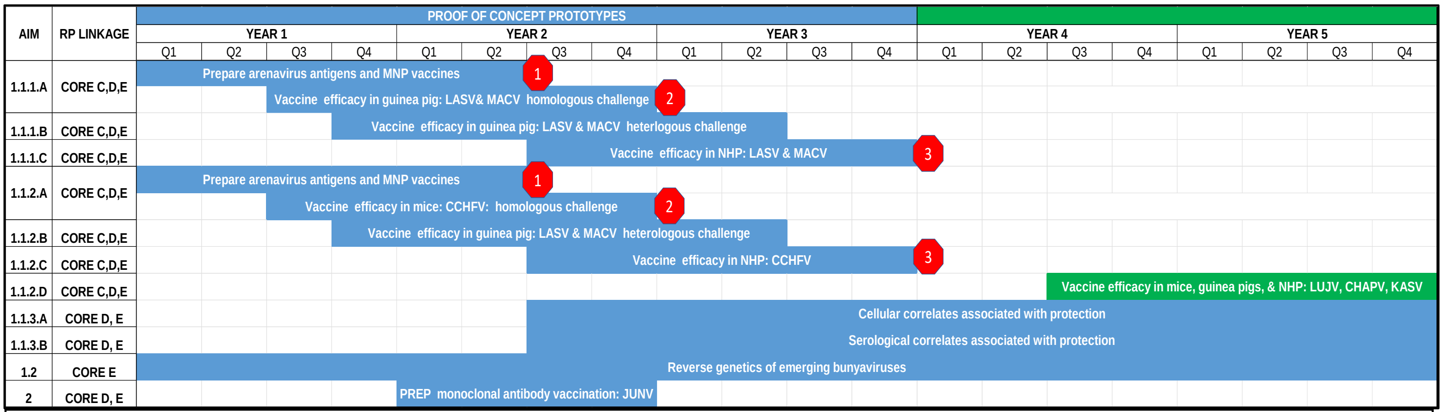
PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months([34-37]).

<u>Scientific Approach</u>: *A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine:* In conjunction with Cores D and E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb whose potent therapeutic efficacy[71, 72] we have previously described will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb. We will select the variant with the most favorable pharmacokinetic (described in **Core D**) for testing with **Core E.** Briefly, groups of 5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV Espindola delivered IV **(Table 11).** For further details of the NHP testing, please see **Core E**. These data will be used by the Scientific Advisory Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project. If any of the Phase I vaccine projects have not met the "go" criteria, we will refocus efforts toward the remaining viruses adjusting the requested budgets accordingly after consultation with NIAID and SAC. *Please note: Discussion of expected results, other alternative approaches, and criteria for "go" or "no go" decisions for MILESTONES 1 and 2 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***<u>Go</u>***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***<u>No-go</u>*** for respective vaccine component. (2) Protection in rodent models is a ***<u>Go</u>***. Lack of protection in rodents is a ***<u>No-go</u>***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***<u>No-go</u>*** for respective vaccine component.

Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

References:

1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.
2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.
5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.
6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.
8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.
9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.
10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia l, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.
11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.
12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.

13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.

16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.

17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.

18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.

19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.

20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.

21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.

22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.

23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 20190523. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.

24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.

25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.

26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.

27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.

28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.

29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.

30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

43. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.

44. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.

45. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

46. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.

47. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.

48. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.

49. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.

50. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.

51. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.

52. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.

53. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.

54. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.

55. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.

56. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.

57. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.

58. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

59. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

60. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

61. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

62. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods.

2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

63. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

64. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

65. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

66. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

67. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

68. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

69. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

70. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

71. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

72. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

~~SPECIFIC AIMS~~

~~The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparednessThreats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches which can be adapted across members of each viral family in a **"plug-and-play"** manner. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a concerns tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GP co-delivered with LASV NP subunits or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed in or monoclonal antibody PREP strategies which can provide protective immunity against Old and New World arenaviruses or emerging nairoviruses. (2) Develop reverse genetics tools to evaluate emerging arenaviruses and nairoviruses for which no isolates are available. Specifically, we will:~~

~~Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:~~

~~**1.1 Subunit immunogens and MNP vaccination:** Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches. We will then utilize lessons learned to address related "test case" arenaviruses and nairoviruses to develop a subunit/MNP approach for an old world arenavirus (LUJV) a new world arenavirus (CHPV), and an emerging bat borne nairovirus, Kasokero virus.We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.~~

~~**1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments:** Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.~~

~~Milestone 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:~~

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~~We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.~~

RESEARCH STRATEGY:

~~1.~~ SIGNIFICANCE~~:~~

1.

~~The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.~~

T~~T~~he viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1].~~–~~ Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% ~~XX~~ and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens [2] ~~(REF)~~ where six rodent borne arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens.~~—~~ This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness [3] ~~(REF)~~. Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e., the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the universal safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success [4]. The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability [5]. Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.

***The Mammarenaviruses*:** All mammarenaviruses share a common genomic organization consisting of two ambi-sense RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP)[6]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [7]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [8]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. [9-11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

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The Orthonairoviruses: Unique from other bunyaviruses, orthonairoviruses are transmitted primarily by ticks in nature. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[15], Issyk-Kul virus[16], Songling virus[17]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[18] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[19]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[20]. CCHFV is listed on the WHO priority pathogen list[2] and is also a NIH-NIAID category A pathogen[3]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[21] (REF). Ribivirin has been used to treat infections with limited success[22]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [20]. Like mammarenaviruses, CCHFV NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[20]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[20]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[23].

~~**Research Project 1(RP1)** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.~~

- ~~**Old Word Mammarenaviruses (OWAV):** RP1 will utilize **LASV as the prototype OWAV** due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with **XX** deaths across West Africa(REF). LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to over **XX** countries (REFS). Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) (REFS). Most of these vaccines utilize the LASV (glycoprotein) GP or the LASV GP + Nucleoprotein (NP) as the target immunogens in these vectors (REFs).~~
- ~~**New World Mamarenaviruses (NWAV):** A live attenuated vaccine for JUNV is currently in use in Argentina [2], but there is conflicting evidence of whether cross protection with other NWAV is possible [3, 4] and there are safety concerns related to reversion to pathogenic versions. This proposal will use **MACV and JUNV as the prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.~~
- ~~**Orthonairoviruses:** Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated **XXX** cases per year across Europe, Africa, and Asia. CCHFV outbreaks are often very large list some recent examples. A suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974, but has numerous safety concerns and limited demonstrated efficacy(**REFS**). Since then, a number of vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages(REF); however only an MVA-CCHFV vaccine has begun to advance towards clinical trials (REF).~~

~~Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e. the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the safety of live-attenuated vaccines and suggests more pathologically~~

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~~benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success (**REFS**). The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability(REFS). Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.~~

~~**Subunit vaccines** have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments [5]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from the each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.~~

~~Once a candidate subunit-patch vaccine approach is selected, this approach will use lessons learned to produce plug-and-play approach to develop vaccines against a representative human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.~~

- ~~In 2008, Lujo virus was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [6]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, underscoring the need for antiviral tools against this emerging threat.~~
- ~~First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [7, 8]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [8]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective, but the phylogenetic divergence of CHAPV of XX% from JUNV suggests it may not be.~~
- ~~In 1977, Kasokero virus (KASV), a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [9]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for *Ornithodoros faini* ticks to participate in enzootic maintenance of KASV [10]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised.~~

~~**Pre-exposure prophylaxis (PREP)**, and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high- risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis (REFS). The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will also perform pilot studies to examine the feasibility of PREP utilizing with a serum half-life extended mAb against JUNVs. Clinically validated Fc mutations can result in an improvement in serum~~

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~~half-life from 3-4 weeks to 2-4 months which can increase the instant barrier immunity from 30-45 days to 60+ days ([11-13] [14]refs).~~

~~The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.~~

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens:

The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[24] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[24]. Further, four of ~~the five~~the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[25-30]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[9]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model.~~ ~~ Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective ~~vaccine(~~vaccine [9, 10, 31, 32]~~REFS)~~.~~ ~~ Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens:

CCHFV is a divergent virus with up to ~~seven~~ six genetic ~~lineages~~ clades~~(REFS)~~. ~~ ~~This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages which can be less than 75% amino acid conservation [20].~~ ~~ Given the glycoprotein is a pivotal protein dictating viral entry ~~and immune~~and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on~~ (REF)~~. Conversely, NP is highly conserved but not exposed on the virus surface cellular surface [20] ~~(REFs)~~.~~ ~~ Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [33]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control mammarenaviruses:

For situations where a vaccine does not exist or is not appropriate (*e.g.* immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE [34, 35] and LA [36-37] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

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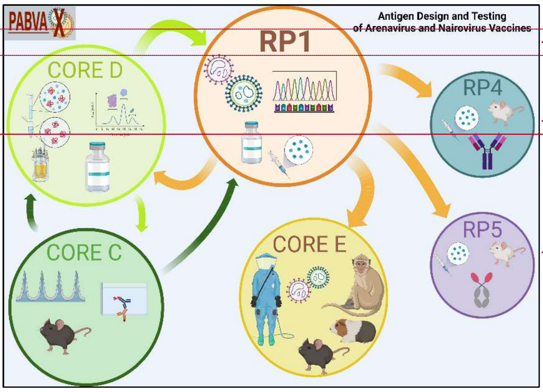
~~*Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses*~~

3. ~~Add blurb discussing unique access to field samples from across the world and established expertise to generate infectious clones from emerging arenaviruses and nairoviruses:~~



~~3.~~ APPROACH

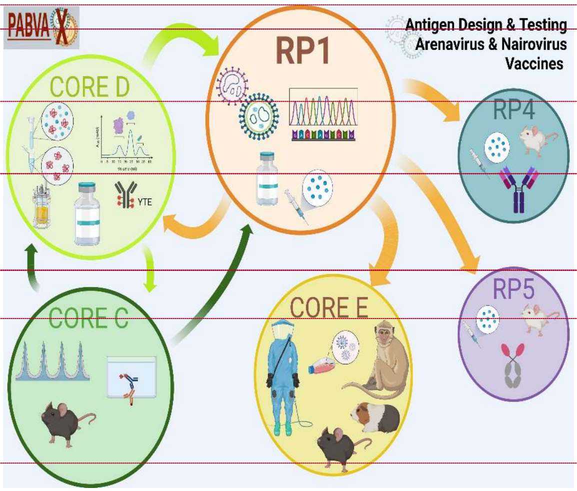


Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales (Figure 1).** In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope-based subunit vaccines allowing for broad cross-protection across **mammarenavirus** and **orthonairovirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C.** Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for mammarenaviruses and orthonairoviruses to **RP4** and **RP5** to produce broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop **BSL4 research tools and animal models** for viruses that are otherwise not available to the research community.

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed

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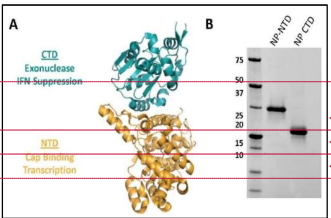

Figure ~~xx~~3. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable ~~disulfide-linked~~disulfide linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[8]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[8] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure ~~xx~~2).** Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure ~~3~~2).** Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure ~~xx~~2).** Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

~~Our trimeric arenavirus GPs support efforts to isolate novel ultra-potent mAbs and to develop a second-generation arenavirus immunotherapeutic with reduced dosing requirements and broader reactivity compared to current LASV mAb candidates[25]. This significantly lowers the cost of goods while increasing ease of use for clinicians, both essential for a product used in developing nations where arenaviruses are prevalent.~~

LASV subunit vaccine protects guinea pigs against lethal challenge

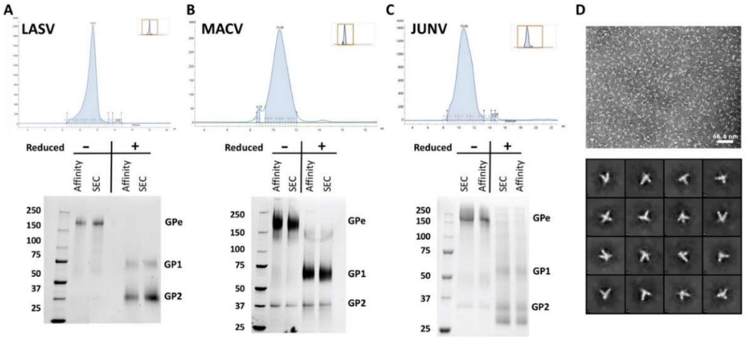

Figure ~~XX~~2. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

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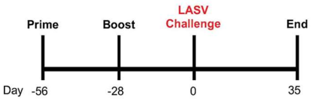
While classical vaccine approaches ~~have~~ largely focus~~ed~~ on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[25-30] suggests that the cellular response, mediated by T cells, can play a ~~highly important~~ potent and complementary role in the long-term protection against pathogens. ~~Based on literature reports that~~ Understanding that T cell responses highly correlate with LASV recovery~~,~~ and that LASV NP plays a significant role in inducing those responses[25, 38], we also generated LASV NP antigens. We also chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle **(Figure ~~xx~~3)** and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks.

We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 3~~6~~)** to assess whether immune responses to GPe ~~only~~alone or~~,~~ ~~or~~ GPe+NP ~~-~~would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking **(Figure 3~~6~~)~~, and)~~** and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP ~~but~~ with no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

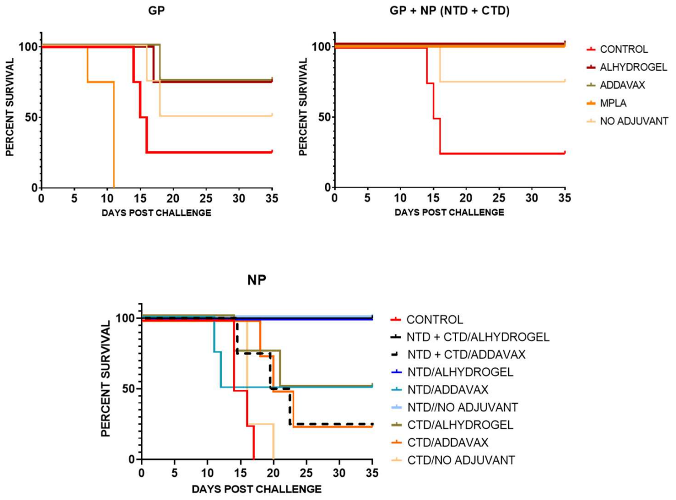


Figure ~~6~~3. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

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Engineering glycoproteins antigens for ~~the~~ CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of ~~—~~ soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to ~~test~~ observe potent serum reactivity in CCHF survivors **(Figure ~~XX~~4).** ~~——~~ We will use established protocols~~—~~[39] as a template to optimize expression of CCHFV and Kasokero NP proteins.

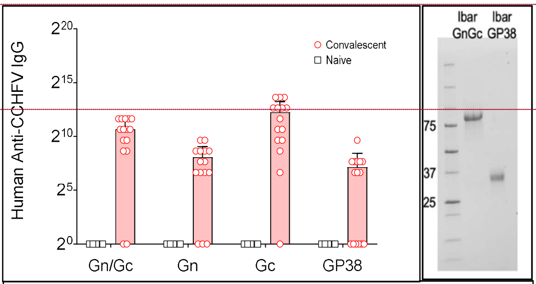

Figure ~~XX~~4: ~~–~~ ~~Engineered CCHFV proteins are reactive with serum from CCHFV~~ survivors (left bar graph). SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification (right electrophoresis gel).

~~*Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses*~~

~~***Rescue of recombinant viruses.*** An important goal of the PABVAX Center is to generate tools for pandemic preparedness. An important component of both RP1 and RP2 is to generate and recombinant viruses to fill gaps and provide tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with RP1 Core E will assist in the development and rescue of New World arenaviruses and the henipavirus Langya virus as detailed in the Research Strategies for RP1 and RP2.~~

~~**APPROACH**~~

~~***Overall Hypothesis:FILL THIS IN***~~

Specific Aim 1: Subunit immunogens and MNP vaccination ~~Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:~~

Rationale:

Scientific Approach: The choice of Mammarenavirus Prototype Pathogens is justified as follows:

- ~~*Old World Mammarenaviridae:* We propose LASV Josiah strain (Lineage 4) as the prototype pathogen due to the depth of available research data, availability of established rodent (**REF**) and nonhuman primate(NHP) models (**REFS**), frequency of exportation (**REFS**), and large number of annual cases throughout West Africa where the case fatality rate can be up to 60% in hospitalized cases (**REF**).~~
- ~~*New World Mammarenaviridae:* Due to the availability of a JUNV vaccine in the endemic region where JUNV circulates and the lack of cross protection against challenge with other NWAV, MACV, the causative agent of Bolivian hemorrhagic fever, will be used as the prototype pathogen for NWAV for studies outlined in Milestone 1.1. There are established guinea pig (**REF**) and NHP (**REF**) models for MACV. JUNV is the New World arenavirus with the largest knowledge base at present [**REF**]. For a pilot efficacy study outlined in Milestone 1.2, we will use JUNV Espindola Strain as a natural extension of previous published work demonstrating protective efficacy of the template PREP mAb (**REF**).~~
- ~~*Nairoviridae.* We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of~~

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~~the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].~~

RP1 proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Mammarenaviruses (OWAV)*: In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa[40]. LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to close to 40 times[41]. Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae (REFS). There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Mamarenaviruses (NWAV):* A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [42], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [43, 44]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations[45]. For Phase 1, **RP1** will use **MACV-Chicava Strain (Clade 2)** and **JUNV-Espindola (Lineage XX)** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Orthonairoviruses*: In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated 10,000-15000 cases across Europe, Africa, and Asia with an estimated 500 deaths per year[46]. A suckling mouse brain derived, inactivated whole virus vaccine for CCHFV has been approved for use only in Bulgaria but has numerous safety concerns and limited demonstrated efficacy[47]. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[47]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[48]. UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice[23] (Unpublished, Cross and Geisbert [23]) and CCHF-Hoti (Clade 4) and CCHF-Afghan-09(Clade V) in NHP[49]. Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model [50] and that human survivors **(Figure 4)** generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38.

~~*Nairoviridae*. We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].~~

Once a candidate subunit-patch vaccine approach is selected, this RP1 will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative, "test case" human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa[51]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach[52] and working with Core E, RP1 will work to establish a LUJV NHP model for use as the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60%[53, 54]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment[54]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [55]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini*, ticks to participate in enzootic maintenance of KASV[56]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible[55]. (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments[57]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families (REFS). An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.~~**Paragraph about subunit vaccines and PREP study.**~~

Milestone 1.1 Subunit immunogens and MNP vaccination

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VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-LASV-GPC)	8			
TOTALS		64	TOTALS	32

Table ~~: XX~~ 1: ~~O~~outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone ~~*Milestone*~~ ***1.1.1.A:*** ~~–~~ ***Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs:*** ~~–~~ Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in ~~table XX~~Table 1 using an intramuscular (IM) vaccination approach. ~~–~~ Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in ~~Table XX~~Table 1. ~~–~~ Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. ~~–~~ After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. ~~–~~ On day 56 post-immunization, animals will be transferred to BSL4 evaluation **Core** E~~C~~ and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group[58] ~~(REFS)~~. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. ~~–~~ Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by ~~MNP Patch~~ **CORE C**. ~~–~~ Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (JUNV-Candid-1)	8			
TOTALS		64	TOTALS	32

Table 2: Outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table** ~~XX~~ **2** using the identical experimental approach as outlined above. ~~–~~ The challenge virus will be the established MACV Chicava strain[59] ~~(REF)~~. Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV[43, 44]. ~~(REFs).~~

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (VSV-LASV-GPC)	8	8		
TOTALS	40	40	24	24

Table 3: ~~: XX o~~ Outline of PAN-LASV Vaccine Efficacy ~~S~~Schedule for Milestone 1.1.1.B

Control vaccine (VSV-LASV-GPC)	8	8	8	8
TOTALS	40	40	40	40

Milestone
1.1.1.B: ~~–~~ ***Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs:*** ~~.~~ ~~–~~ Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). ~~–~~ Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core** E~~C~~ as outlined in **Table** ~~XX~~3. ~~–~~ The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

~~———~~For our MACV subunit vaccines
~~Similarly, , after down selecting the optimal adjuvant and vaccination route,~~ we will assess the potential ~~for our MACV subunit vaccines~~ to confer heterologous protection against three lineages of MACV ~~endemic to~~ genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. ~~–~~ Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core** ~~C~~E as outlined in **Table** ~~XX~~**4**. We will also utilize JUNV (Romero strain)[60] ~~(REF)~~ challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

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VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8		
Control vaccine (JUNV-Candid-1)	8	8		
TOTALS	40	40	24	24

~~Table: XX~~Table 4: ~~o~~Outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

Milestone **1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals **(Table ~~XX~~5).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.3.A~~1~~F.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

~~Table: XX~~Table 5: ~~o~~Outline of NHP efficacy ~~studes~~studies for LASV Milestone 1.1.1.C~~B~~

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table ~~XX~~ 6** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain[61] ~~(REF)~~. ~~Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV,~~ If Candid-1 confers protection in guinea pig study outlined in 1.1.1.A we will confirm protection in NHPs if demonstrated in guinea pigs[43, 44] ~~(REFs).~~

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

~~Table: XX~~Table 6: ~~o~~Outline of NHP efficacy studes for MACV Milestone

Milestone 1~~2~~.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:

Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in **Table 7** using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in **Table 7.** Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti[49]. After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHFV-M)	8			
	TOTALS	128	TOTALS	112

~~Table: XX~~Table 7: ~~o~~Outline of NHP efficacy studies for MACV Milestone 1.1.1.B

	TOTALS	128	TOTALS	128

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times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

~~Table: XX~~Table 9: ~~o~~Outline of NHP efficacy studies for MACV Milestone

***Milestone* 1~~2~~.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice**: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(Uganda), 3 (Nigeria), 4 (Afghanistan) and 6(Greece) established in the **BSL4 evaluation Core C** as outlined in **Table 8.** The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8			
Control vaccine (VSV-CCHF-M)	8			
TOTALS	128		TOTALS	112

~~Table: XX~~Table 8: ~~o~~Outline of NHP efficacy studies for MACV Milestone

	GUINEA PIG		LUJV
LUJV NP-NTD			
LUJV GPe + NP-NTD	8	LUJV GPe + NP-NTD	(TWO GROUPS OF 5)
Control animals (NO VACCINE)	8	Control animals (NO VACCINE)	5
TOTALS	32	TOTALS	15

VACCINE	GUINEA PIG CHALLENGE CHAPV	VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	8	CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	8	CHAPV GPe	
CHAPV NP + GPe	8	CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	8	Control animals (NO VACCINE)	5
TOTALS	32	TOTALS	15

VIRUS	MOUSE CHALLENGE KASOKERO VIRUS	VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	8	KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	8	KASV NP	
KASV GnGc+NP	8	KASV GnGc +NP	
Control animals (NO VACCINE)	8	Control animals (NO VACCINE)	5
TOTALS	32	TOTALS	15

~~Table: XX~~Table 10: ~~O~~Oeutline of NHP efficacy studies for MACV Milestone

Milestone

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2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP**:** In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC, demonstrated effective in mice[23] and NHP (Unpublished, Cross and Geisbert) with 5 animals, and a control group of five unvaccinated animals **(Table 9).** Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

Milestone

~~2~~1.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP**:**

RP1 and Core D will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10). RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

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VIRUS	GUINEA PIG CHALLENGE
	LUJV
LASSA NP-NTD	8
LASSA GPe	8
LASSA GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively **(Table 10)**

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Milestone

~~*2.1.2.E: Determination of protective efficacy against novel, emerging nairovirus using optimized subunit-patch vaccination in STAT-1KO mice and NHP*~~

Table: XX outline of NHP efficacy studes for MACV
Milestone 1.1.1.B

1.1.3~~1.F~~: Determination of cellular and humoral immune correlates associated with ~~protection~~protection.

Milestone 1.1.3.A~~1.F.a~~: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups:

We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard ~~whole blood~~ flow cytometric staining protocols will be used for surface and intracellular staining markers. ~~, b~~Briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye ~~for 20 minutes at room temperature~~. ~~C~~Cells are then washed again and ~~,~~ surface stain cocktail is added to the tubes, incubated ~~for 20~~

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~~minutes~~, and washed. Cells are fixed ~~for 1 hour~~ with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed ~~in PBS~~. ~~Stained, fixed cells will then either be acquired directly.~~ Cell populations are then acquired and quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments: ~~-~~PBMC stimulations will be set up according to modifications of well established, previously published protocols.[62, 63] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldin A will then be added ~~at a concentration of 10mg/mL and~~ then incubated ~~at 37°C for~~prior ~~until~~ to flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays ~~will be set up~~ in 96-well plates and supernatants ~~will be~~ will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye ~~for 20 minutes at room temperature~~, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated ~~for 20 minutes~~, and washed. Cells are incubated in BD Cytofix/Cytoperm solution ~~for 20 minutes~~ followed by a wash. ~~with BD Perm/Wash Buffer.~~ Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed ~~in PBS~~. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a multi~~4~~-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated ~~for 3 hours at room temperature, protected from light~~ and subsequently washed by pelleted centrifugation. ~~1mL of wash buffer will be added and beads will be pelleted by centrifugation at 200g for 5 minutes.~~ Wash buffer will be removed and discarded. Beads will be resuspended ~~in 300uL wash buffer~~ and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection ~~antibody~~antibody, and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes. ~~at room temperature, protected from light.~~ Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer~~**need model**~~ ~~ELISPOT Fluorescence Microplate Reader.~~

Data Analysis: We will ~~look at the~~examine differences in immune responses across groups between each timepoint and baseline. ~~Model assumptions will be checked for a one-way ANOVA to compare the three groups. we will run the analysis after checking model assumptions, if those are not met then we will adjust to a nonparametric model or transform the outcome appropriately. To look at the longitudinal aspect of the data, we will investigate measurements over time between groups, the interaction of group and time can be analyzed using covariance pattern models (or linear mixed models), where the correlation between observations measured over time can be handled more flexibly. As a more exploratory analysis we will also look at adjusting for possible confounding variables such as sex and age. For categorical variables, comparisons will be made using Chi-Square tests.~~ Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

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1.1.3.A:~~1.1.1.F.b~~: Systems serology Characterization of~~–~~ Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups:

We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform ~~developed by Dr. Gunn in~~ with **Core D**[64-66] to define the Fc-mediated humoral antiviral functions associated with ~~vaccination~~vaccine protection. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, ~~CCHFV,LUJV~~CCHFV, LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens ~~utilized~~ used for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages~~.~~, ~~W~~we will use a multiplexed analysis to determine ~~the~~ levels of antibodies against ~~the~~ viral antigens using ~~on~~ a MagPix xMAP instrument ~~as described~~.[65, 67] Specifically, each viral antigen will be coupled to coded magnetic beads ~~(Luminex)~~, mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM.~~—~~ For quantitation of bound antibody, we will ~~make a standard~~ use~~ing~~ pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions:~~–~~ We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry.~~ at KGH.~~

ADCC**:** Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates ~~for 5 hours at 37C 5% CO_2,~~ and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses ~~were~~ will be performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction ~~was used~~ to determine statistical significance ~~between groups.~~. ~~A network analysis will be performed to identify significant antibody features. Associations between antibody features will be determined using nonparametric Spearman correlation coefficient. Statistically significant associations after Bonferroni correction for multiple comparisons (adjusted P < .05) were used to generate networks in Cytoscape (version 3.4.0).~~

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and ~~additional clinical information (ie~~clinical signs into the network analysis.~~; PES symptoms). These will be included in the network analysis.~~

***Milestone* 1.~~AIM 2~~2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:** We will use existing mammarenaviruses[68, 69] and orthonairovirus[70] reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog but is still pathogenic in mice and humans (REFS). There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

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Milestone 2: ~~1.2~~ Pre-exposure Prophylaxis (PREP) using half-life extension antibody ~~treatments~~treatments.

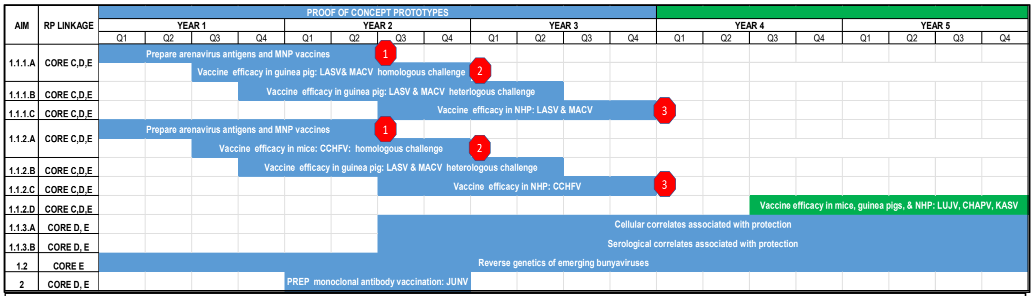
PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table 11: PREP schedule outline in Milestone 2

Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis. The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in improvement in serum half-life up 2-4 months(34-37).

***Scientific Approach*:**

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine: ~~-~~ In conjunction with ~~RP1~~ Cores D and ~~Core~~ E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb ~~with~~ whose potent therapeutic efficacy~~-~~[71, 72] ~~(guinea pig and NHP references)~~ we have previously described ~~by Abelson, Geisbert and Zeitlin~~ will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb~~(Table 4)~~. ~~The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (Bioqual, Inc.; Rockville, MD~~see Core D~~) to select a lead candidate~~



RP1 Gant Chart with Go/No Go Decision Points (red octagons). (1) Successful fabrication and validation of antigens and/or vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity and lack of antigenicity as determined in Core C (MNP only) is a ***No-go*** for respective vaccine component. (2) Protection in rodent models is a ***Go***. Lack of protection in rodents is a ***No-go***. (3) Protection in NHP models is a Go to project Phase II. Lack of protection is a ***No-go*** for respective vaccine component.

~~for testing in NHPs against Junin Romero challenge in~~ Core E. ~~For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference).~~ We will select t~~T~~he variant with the most favorable pharmacokinetic (described in **Core D**)~~s~~ ~~PK will be selected~~ for testing with ~~**by RP1/**~~**Core E.** Briefly, groups of ~~4~~5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV ~~(Espindola or Romero~~Espindola~~?)~~ delivered IV **(Table 11)**. For further details of the NHP testing, please see **Core E**. These data will be used by the Scientific Advisory ~~Committee~~ Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Expected results, alternative ~~approaches and~~approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, ~~and~~ CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three "test case" viruses LUJV, CHAPV, and KASV in Phase II of the project.……. If ~~this~~ any~~is not the case any~~ of the~~se~~ Phase I vaccine projects have not met the "go" criteria, ~~three viruses by year 2~~ we will refocus ~~effects~~

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efforts toward~~on~~ the remaining viruses ~~, families~~ adjusting the requested budgets accordingly after ~~consulation~~consultation with NIAID and SAC.

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Please note: Discussion of expected results, other alternative ~~approaches~~approaches, and criteria for “go” or “no go” decisions for MILESTONES 1~~-4~~ and 2 are discussed as required in RFA-AI-20-023 in the **Project Milestones and Timelines** *section.*

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Industry Expertise and Regulatory Considerations: Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP1** (Subunit-MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based Bunyavirus vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future “plug and play” pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

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~~**JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,**~~

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~~**AIM 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:**~~

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~~**Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines).** We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC~~

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~~*Please note: Discussion of expected results, other alternative approaches and criteria for “go” or “no go” decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*~~

~~**JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,**~~

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~~Summary statement impact~~

1. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Cháng C, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, Dèng F, Di Serio F, Digiaro M, Drebot MA, Duàn X, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hepojoki J, Hewson R, Hú Z, Hughes HR, Jonson MG, Junglen S, Klempa B, Klingström J, Kòu C, Laenen L, Lambert AJ, Langevin SA, Liu D, Lukashevich IS, Luò T, Lǚ C, Maes P, de Souza WM, Marklewitz M, Martelli GP, Matsuno K, Mielke-Ehret N, Minutolo M, Mirazimi A, Moming A, Mühlbach HP, Naidu R, Navarro B, Nunes MRT, Palacios G, Papa A, Pauvolid-Corrêa A, Pawęska JT, Qiáo J, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Shěn S, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Sū Z, Sūn S, Táng S, Turina M, Wáng B, Wáng C, Wáng H, Wáng J, Wèi T, Whitfield AE, Zerbini FM, Zhāng J, Zhāng L, Zhāng Y, Zhang YZ, Zhāng Y, Zhou X, Zhū L, Kuhn JH. Taxonomy of the order Bunyavirales: update 2019. Arch Virol. 2019;164(7):1949-65. Epub 20190507. doi: 10.1007/s00705-019-04253-6. PubMed PMID: 31065850; PMCID: PMC6641860.

2. World.Health.Organisation. List of Blueprint Priority Diseases 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.

3. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2023 [cited 2023 03/06]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.

4. US-FDA. Vaccines Licensed for Use in the United States 2023 [cited 2023 05/06]. Available from: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states.

5. Moyle PM, Toth I. Modern Subunit Vaccines: Development, Components, and Research Opportunities. ChemMedChem. 2013;8(3):360-76. doi: https://doi.org/10.1002/cmdc.201200487.

6. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.

7. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. doi: 10.1038/nm.4396.

8. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.

9. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.

10. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia I, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Sci Rep. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.

11. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C.

Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Sci Rep. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.

12. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLoS Med. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.

13. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

14. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLoS Negl Trop Dis. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

15. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.

16. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.

17. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.

18. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.

19. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.

20. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.

21. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral Res. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.

22. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infect Dis. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.

23. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 20190523. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.

24. Brisse ME, Ly H. Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors. Front Immunol. 2019;10:372. Epub 2019/03/29. doi: 10.3389/fimmu.2019.00372. PubMed PMID: 30918506; PMCID: PMC6424867.

25. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol. 2009;83(11):5890-903. Epub 2009/03/20. doi: 10.1128/JVI.01948-08. PubMed PMID: 19297492; PMCID: PMC2681932.

26. Fisher DG, Coppock GM, Lopez CB. Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination. Vaccine. 2018;36(28):4039-45. Epub 2018/06/05. doi: 10.1016/j.vaccine.2018.05.100. PubMed PMID: 29861183; PMCID: PMC6265594.

27. Meyer B, Ly H. Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses. J Virol. 2016;90(8):3810-8. Epub 2016/02/13. doi: 10.1128/JVI.03049-15. PubMed PMID: 26865707; PMCID: PMC4810556.

28. ter Meulen J, Badusche M, Kuhnt K, Doetze A, Satoguina J, Marti T, Loeliger C, Koulemou K, Koivogui L, Schmitz H, Fleischer B, Hoerauf A. Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. J Virol. 2000;74(5):2186-92. Epub 2000/02/09. doi: 10.1128/jvi.74.5.2186-2192.2000. PubMed PMID: 10666248; PMCID: PMC111699.

29. Meulen J, Badusche M, Satoguina J, Strecker T, Lenz O, Loeliger C, Sakho M, Koulemou K, Koivogui L, Hoerauf A. Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones. Virology. 2004;321(1):134-43. Epub 2004/03/23. doi: 10.1016/j.virol.2003.12.013. PubMed PMID: 15033572.

30. Goicochea MA, Zapata JC, Bryant J, Davis H, Salvato MS, Lukashevich IS. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. Vaccine. 2012;30(8):1445-52. Epub 2012/01/12. doi: 10.1016/j.vaccine.2011.12.134. PubMed PMID: 22234266; PMCID: PMC3562134.

31. Ibukun FI. Inter-Lineage Variation of Lassa Virus Glycoprotein Epitopes: A Challenge to Lassa Virus Vaccine Development. Viruses. 2020;12(4). Epub 20200331. doi: 10.3390/v12040386. PubMed PMID: 32244402; PMCID: PMC7232328.

32. Lukashevich IS. Advanced vaccine candidates for Lassa fever. Viruses. 2012;4(11):2514-57. Epub 20121029. doi: 10.3390/v4112514. PubMed PMID: 23202493; PMCID: PMC3509661.

33. Leventhal SS, Meade-White K, Rao D, Haddock E, Leung J, Scott D, Archer J, Randall S, Erasmus JH, Feldmann H, Hawman DW. Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge. EBioMedicine. 2022;82:104188. Epub 20220727. doi: 10.1016/j.ebiom.2022.104188. PubMed PMID: 35907368; PMCID: PMC9335360.

34. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults. Antimicrob Agents Chemother. 2013;57(12):6147-53. doi: 10.1128/AAC.01285-13. PubMed PMID: 24080653.

35. Rosenberg YJ, Lewis GK, Montefiori DC, LaBranche CC, Lewis MG, Urban LA, Lees JP, Mao L, Jiang X. Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques. PloS one. 2019;14(2):e0212649. Epub 20190220. doi: 10.1371/journal.pone.0212649. PubMed PMID: 30785963; PMCID: PMC6437720.

36. Rappazzo CG, Tse LV, Kaku CI, Wrapp D, Sakharkar M, Huang D, Deveau LM, Yockachonis TJ, Herbert AS, Battles MB, O'Brien CM, Brown ME, Geoghegan JC, Belk J, Peng L, Yang L, Hou Y, Scobey TD, Burton DR, Nemazee D, Dye JM, Voss JE, Gunn BM, McLellan JS, Baric RS, Gralinski LE, Walker LM. Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science. 2021;371(6531):823-9. Epub 20210125. doi: 10.1126/science.abf4830. PubMed PMID: 33495307; PMCID: PMC7963221.

37. Schmidt P, Gong J, Narayan K, Gupta D, Engler F, Li Y, Copans A, Campanaro E. Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults. Infect Dis Ther. 2023:1-13. Epub 20230425. doi: 10.1007/s40121-023-00794-1. PubMed PMID: 37185797; PMCID: PMC10127981.

38. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, Sidney J, Sette A, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Nichol ST, Spiropoulou CF. A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses. J Infect Dis. 2017;215(12):1862-72. doi: 10.1093/infdis/jix201. PubMed PMID: 28863472; PMCID: PMC5853890.

39. Carter SD, Surtees R, Walter CT, Ariza A, Bergeron É, Nichol ST, Hiscox JA, Edwards TA, Barr JN. Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein. J Virol. 2012;86(20):10914-23. Epub 20120808. doi: 10.1128/jvi.01555-12. PubMed PMID: 22875964; PMCID: PMC3457148.

40. Medugu N, Adegboro B, Babazhitsu M, Kadiri M, Abanida E. A review of the recent advances on Lassa fever with special reference to molecular epidemiology and progress in vaccine development. African Journal of Clinical and Experimental Microbiology. 2023;24(2):130-46.

41. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.

42. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

43. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.

44. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.

45. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

46. Control ECfDPa. Factsheet about Crimean-Congo haemorrhagic fever 2023 [cited 2023 05/06]. Available from: https://www.ecdc.europa.eu/en/crimean-congo-haemorrhagic-fever/facts/factsheet.

47. Dowall SD, Carroll MW, Hewson R. Development of vaccines against Crimean-Congo haemorrhagic fever virus. Vaccine. 2017;35(44):6015-23. doi: https://doi.org/10.1016/j.vaccine.2017.05.031.

48. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.

49. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 2020/08/14. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.

50. Mishra AK, Moyer CL, Abelson DM, Deer DJ, El Omari K, Duman R, Lobel L, Lutwama JJ, Dye JM, Wagner A, Chandran K, Cross RW, Geisbert TW, Zeitlin L, Bornholdt ZA, McLellan JS. Structure and Characterization of Crimean-Congo Hemorrhagic Fever Virus GP38. J Virol. 2020;94(8). Epub 20200331. doi: 10.1128/jvi.02005-19. PubMed PMID: 31996434; PMCID: PMC7108853.

51. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.

52. Bird BH, Dodd KA, Erickson BR, Albariño CG, Chakrabarti AK, McMullan LK, Bergeron E, Ströeher U, Cannon D, Martin B, Coleman-McCray JD, Nichol ST, Spiropoulou CF. Severe Hemorrhagic Fever in Strain 13/N Guinea Pigs Infected with Lujo Virus. PLoS Negl Trop Dis. 2012;6(8):e1801. doi: 10.1371/journal.pntd.0001801.

53. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.

54. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.

55. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.

56. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.

57. Balmert SC, Ghozloujeh ZG, Carey CD, Williams LH, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E, Falo LD, Jr. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. iScience. 2022;25(10):105045. Epub 20220830. doi: 10.1016/j.isci.2022.105045. PubMed PMID: 36062075; PMCID: PMC9425707.

58. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res. 2016; PMCID: PMC5032844.

59. Golden JW, Beitzel B, Ladner JT, Mucker EM, Kwilas SA, Palacios G, Hooper JW. An attenuated Machupo virus with a disrupted L-segment intergenic region protects guinea pigs against lethal Guanarito virus infection. Sci Rep. 2017;7(1):4679. doi: 10.1038/s41598-017-04889-x.

60. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63.

61. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of experimental Machupo virus infection, Chicava strain, in cynomolgus macaques (Macaca fascicularis) by intramuscular and aerosol exposure. Vet Pathol. 2015;52(1):26-37. Epub 20140702. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.

62. Meddows-Taylor S, Shalekoff S, Kuhn L, Gray GE, Tiemessen CT. Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome. J Virol Methods. 2007;144(1-2):115-21. Epub 2007/06/05. doi: 10.1016/j.jviromet.2007.04.004. PubMed PMID: 17543395; PMCID: PMC2292247.

63. Kagina BM, Mansoor N, Kpamegan EP, Penn-Nicholson A, Nemes E, Smit E, Gelderbloem S, Soares AP, Abel B, Keyser A, Sidibana M, Hughes JE, Kaplan G, Hussey GD, Hanekom WA, Scriba TJ. Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry. J Immunol Methods. 2015;417:22-33. Epub 2014/12/20. doi: 10.1016/j.jim.2014.12.003. PubMed PMID: 25523923; PMCID: PMC4339399.

64. Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, Schendel SL, Kim J, Cain C, Roy V, Suscovich TJ, Takada A, Halfmann PJ, Kawaoka Y, Pauthner MG, Momoh M, Goba A, Kanneh L, Andersen KG, Schieffelin JS, Grant D, Garry RF, Saphire EO, Bukreyev A, Alter G. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity. 2021;54(4):815-28.e5. Epub 2021/04/15. doi: 10.1016/j.immuni.2021.03.009. PubMed PMID: 33852832; PMCID: PMC8111768.

65. Gunn BM, Roy V, Karim MM, Hartnett JN, Suscovich TJ, Goba A, Momoh M, Sandi JD, Kanneh L, Andersen KG, Shaffer JG, Schieffelin JS, Garry RF, Grant DS, Alter G. Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses. J Infect Dis. 2020;221(1):156-61. Epub 2019/07/14. doi: 10.1093/infdis/jiz364. PubMed PMID: 31301137; PMCID: PMC7184900.

66. Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol. 2018;19(11):1169-78. Epub 2018/10/20. doi: 10.1038/s41590-018-0233-9. PubMed PMID: 30333617; PMCID: PMC6814399.

67. Brown EP, Dowell KG, Boesch AW, Normandin E, Mahan AE, Chu T, Barouch DH, Bailey-Kellogg C, Alter G, Ackerman ME. Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles. J Immunol Methods. 2017;443:33-44. Epub 20170203. doi: 10.1016/j.jim.2017.01.010. PubMed PMID: 28163018; PMCID: PMC5333794.

68. Martínez-Sobrido L, Paessler S, de la Torre JC. Lassa Virus Reverse Genetics. Methods Mol Biol. 2017;1602:185-204. doi: 10.1007/978-1-4939-6964-7_13. PubMed PMID: 28508222.

69. Patterson M, Seregin A, Huang C, Kolokoltsova O, Smith J, Miller M, Smith J, Yun N, Poussard A, Grant A, Tigabu B, Walker A, Paessler S. Rescue of a recombinant Machupo virus from cloned cDNAs and in vivo characterization in interferon (αβ/γ) receptor double knockout mice. J Virol. 2014;88(4):1914-23. Epub 20131127. doi: 10.1128/jvi.02925-13. PubMed PMID: 24284323; PMCID: PMC3911560.

70. Bergeron É, Zivcec M, Chakrabarti AK, Nichol ST, Albariño CG, Spiropoulou CF. Recovery of Recombinant Crimean Congo Hemorrhagic Fever Virus Reveals a Function for Non-structural Glycoproteins Cleavage by Furin. PLOS Pathogens. 2015;11(5):e1004879. doi: 10.1371/journal.ppat.1004879.

71. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci U S A. 2021;118(11). doi: 10.1073/pnas.2023332118. PubMed PMID: 33836604; PMCID: PMC7980402.

72. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci U S A. 2016;113(16):4458-63. Epub 2016/04/04. doi: 10.1073/pnas.1600996113. PubMed PMID: 27044104; PMCID: PMC4843420.

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SPECIFIC AIMS

The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparednessThreats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches which can be adapted across members of each viral family in a **"plug-and-play"** manner. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a concern tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GPe alone, co-delivered with LASV NP subunits, or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed against Old and New World arenaviruses or emerging nairoviruses. (2) Develop. in or monoclonal antibody PREP strategies which can provide protective immunity (2) Specifically, we will:

Specific Aim 1: Subunit immunogens and MNP vaccination. Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches.

- **Milestone 1.1.1.A-C: Development and optimization of patch vaccination approach employing mammarenavirus subunits in guinea pigs and non-human primates.**
- **Milestone 1.1.2.A-C: Development and optimization of patch vaccination approach employing orthonairvirus subunits; protection in STAT-1/KO mice and non-human primates.**
- **Milestone 1.1.2.D: Determination of protective efficacy against novel, emerging mammarenavirus and orthonairovirus "test case" viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP.**
- **Milestone 1.1.3. A-B: Determination of cellular and humoral immune correlates associated with protection.**
- **Milestone 1.2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates.**

Specific Aim 2: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments. Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.

SPECIFIC AIMS

The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to ~~XX~~ 60% and significant, long-term sequelae in survivors. These viruses are on the World Health Organization’s (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparednessThreats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches **which can be adapted across members of each viral family in a “plug-and-play” manner**. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a ~~concerns~~concern tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GPe alone, co-delivered with LASV NP subunits, or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed against Old and New World arenaviruses or emerging nairoviruses. (2) Develop. in or monoclonal antibody PREP strategies which can provide protective immunity ~~against Old and New World arenaviruses or emerging nairoviruses.~~ (2) ~~Develop reverse genetics tools to evaluate emerging arenaviruses and nairoviruses for which no isolates are available.~~ Specifically, we will:

~~Milestone~~ Specific Aim 1: ~~Develop novel arenavirus and nairovirus vaccination approaches:~~

~~1.1~~ **Subunit immunogens and MNP vaccination:** Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches. We will then utilize lessons learned to address related “test case” arenaviruses and nairoviruses to develop a subunit/MNP approach for an old world arenavirus (LUJV) a new world arenavirus (CHAP~~P~~V), and an emerging bat borne nairovirus, Kasokero ~~virus~~virus (KASV). We will also develop recombinant virus tools including ~~We will generate recombinant~~ infectious clones ~~(~~and reporter-gene versions~~)~~ of LASV, MACV, ~~and~~ and CCHFV ~~CCHFV isolates~~ for which isolates are not available using reverse genetics. Rescued viruses will be characterized in vitro using cell-based assays and~~. Recombinant viruses will be examined~~ *in vivo* *to develop new* ~~(~~ animal models ~~development)~~ within **Core E**. We will also develop an infectious clone recovery system for KASV for virulence determination studies.

Specific Aim 2~~1.2~~: Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments: Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.

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~~Milestone 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:~~

~~We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.~~

RESEARCH STRATEGY:

~~1.~~ SIGNIFICANCE~~:~~

1.

~~The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.~~

T~~T~~he viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens (REF) where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness (REF). Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e. the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success (**REFS**). The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability(REFS). Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.

***The Mammarenaviruses*~~;~~:** All mammarenaviruses share a common genomic organization consisting of two ambi-sense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP) [2]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP. Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [3]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [4]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV

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endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. To test these notions, investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccine approaches to test this idea and have demonstrated protection against lethal LASV-Josiah challenge using a stabilized prefusion, trimeric GPe, a highly conserved NP NTD epitope, or a combination of the two antigens using a lethal outbred guinea pig model. Despite the considerable molecular diversity in LASV strains, there is understood that there is some conservation in key antigenic sites within both the GPe and the NP, suggesting that a PAN-LASV vaccine may be possible[5-7]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[8], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[9] , Lineage III (Cross, Geisbert unpublished), Lineage V[10] and Lineage VI (Cross and Geisbert Unpublished).

The Orthonairoviruses: Unique from other bunyaviruses, orthonairovirues are transmitted primarily by ticks in nature (REF). While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[11], Issyk-Kul virus[12], Songling virus[13]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[14] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[15]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[16]. CCHFV is listed on the WHO priority pathogen list[17] and is also a NIH-NIAID category A pathogen[18]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[19] (REF). Ribivirin has been used to treat infections with limited success[20]. CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [16]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[16]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[16]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[21].

~~**Research Project 1(RP1)** proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.~~

- ~~**Old Word Mammarenaviruses (OWAV):** RP1 will utilize **LASV as the prototype OWAV** due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with **XX** deaths across West Africa(REF). LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to over **XX** countries (REFS). Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) (REFS). Most of these vaccines utilize the LASV (glycoprotein) GP or the LASV GP + Nucleoprotein (NP) as the target immunogens in these vectors (REFs).~~
- ~~**New World Mamarenaviruses (NWAV):** A live attenuated vaccine for JUNV is currently in use in Argentina [2], but there is conflicting evidence of whether cross protection with other NWAV is possible [3, 4] and there are safety concerns related to reversion to pathogenic versions. This proposal will use **MACV and JUNV as the prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.~~

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- ~~**Orthonairoviruses:** Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated **XXX** cases per year across Europe, Africa, and Asia. CCHFV outbreaks are often very large list some recent examples. A suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974, but has numerous safety concerns and limited demonstrated efficacy(**REFS**). Since then, a number of vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages(REF); however only an MVA-CCHFV vaccine has begun to advance towards clinical trials (REF).~~

~~Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e. the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success (**REFS**). The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability(REFS). Members of PABVAX, have developed protective protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.~~

~~**Subunit vaccines** have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments [5]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from the each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.~~

~~Once a candidate subunit-patch vaccine approach is selected, this approach will use lessons learned to produce plug-and-play approach to develop vaccines against a representative human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.~~

- ~~In 2008, Lujo virus was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [6]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, underscoring the need for antiviral tools against this emerging threat.~~
- ~~First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [7, 8]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [8]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective, but the phylogenetic divergence of CHAPV of XX% from JUNV suggests it may not be.~~
- ~~In 1977, Kasokero virus (KASV), a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [9]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick~~

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~~surveys have demonstrated the potential for *Ornithodoros faini* ticks to participate in enzootic maintenance of KASV [10]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised.~~

~~**Pre-exposure prophylaxis (PREP)** and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high- risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis (REFS). The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will also perform pilot studies to examine the feasibility of PREP utilizing with a serum half-life extended mAb against JUNVs. Clinically validated Fc mutations can result in an improvement in serum half-life from 3-4 weeks to 2-4 months which can increase the instant barrier immunity from 30-45 days to 60+ days ([11-13] [14]refs).~~

~~The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.~~

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens:

The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[22] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[22]. Further, four of ~~the five~~the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[23-28]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[5]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine(REFS). Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens:

CCHFV is a divergent virus with up to seven genetic lineages (REFS). This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on (REF). Conversely, NP is highly conserved but not exposed on the virus surface cellular surface (REFs). Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [29]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control mammarenaviruses:

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For situations where a vaccine does not exist or is not appropriate (*e.g.* immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE **[30, 31]** and LA **[32]**-**[33]** Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

~~***Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses***~~

3. ~~Add blurb discussing unique access to field samples from across the world and established expertise to generate infectious clones from emerging arenaviruses and nairoviruses:~~

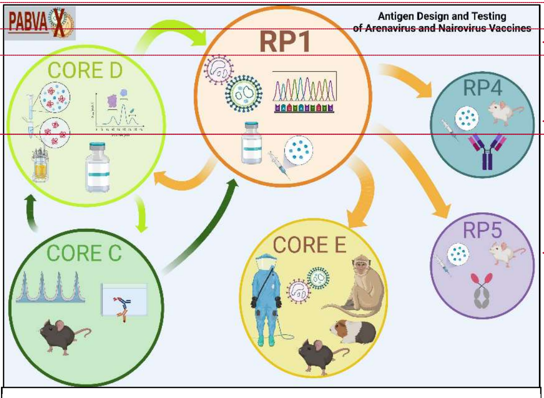

Figure 1: Overall Research Strategy for RP and demonstration of interactions with other RP and Cores of PABVAX

~~3.~~ APPROACH

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses. Collective expertise in virology, immunology, biological manufacturing, and vaccine innovation will be leveraged to develop cutting-edge vaccination approaches. **RP1** will work with several RPs and Cores in the center to advance two novel vaccination approaches targeting viral families within **Bunyavirales** (Figure **XX**). In collaboration with **Core D, RP1** will develop stabilized GP and NP epitope based subunit vaccines allowing for broad cross-protection across **mammarenavirus** and **orthonairovirus** species. This will be achieved by tailoring antigen design from prototype applicable to related members of these viral families. These optimized vaccines will be produced in **Core D** and evaluated for protective efficacy and determination of immune correlates associated with protection in animal models under maximum (BSL4) containment in collaboration with **Cores D and E** via intramuscular administration or Microneedle skin Patch (MNP). MNPs containing subunit vaccines manufactured in **Core D** and optimized for delivery efficiency in **Core C**.. Using optimized delivery methods developed in collaboration with **Cores C, D, and E, RP1** will provide stabilized GPe vaccines for mammarenaviruses and orthonairoviruses to **RP4** and **RP5** for the production of broadly reactive monoclonal antibodies or nanobodies, respectively. **RP1 and Cores D and E** will also collaborate on a pilot study to determine feasibility of PREP using half-life extended antibodies as an alternative to traditional vaccination. Lastly, **RP1** will work closely with **Core E** to develop infectious clones using reverse genetics to develop **BSL4 research tools and animal models** for viruses that are otherwise not available to the research community.

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

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Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide-linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[4]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[4] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization (**Figure xx**). Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV (**Figure 3**). Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes (**Figure xx**). Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

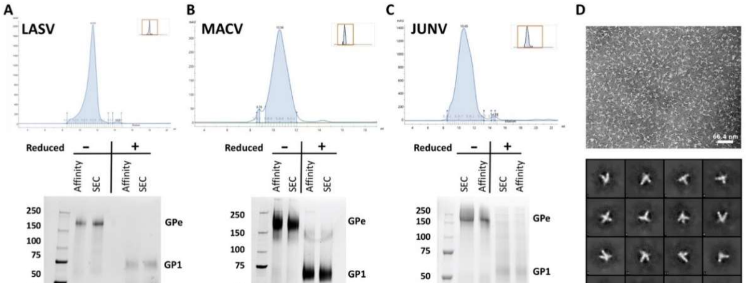

Figure XX. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

~~Our trimeric arenavirus GPs support efforts to isolate novel ultra-potent mAbs and to develop a second-generation arenavirus immunotherapeutic with reduced dosing requirements and broader reactivity compared to current LASV mAb candidates[25]. This significantly lowers the cost of goods while increasing ease of use for clinicians, both essential for a product used in developing nations where arenaviruses are prevalent.~~

LASV subunit vaccine protects guinea pigs against lethal challenge

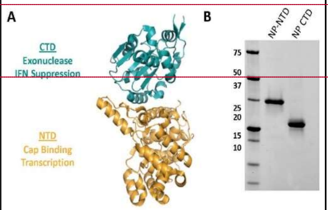

Figure xx. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for

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While classical vaccine approaches have largely focused on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[23-28] suggests that the cellular response, mediated by T cells, can play a highly important and complementary role in the long-term protection against pathogens. Based on literature reports that T cell responses highly correlate with LASV recovery, and that LASV NP plays a significant role in inducing those responses[23, 34], we also generated LASV NP antigens. We chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle (**Figure xx**) and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks.

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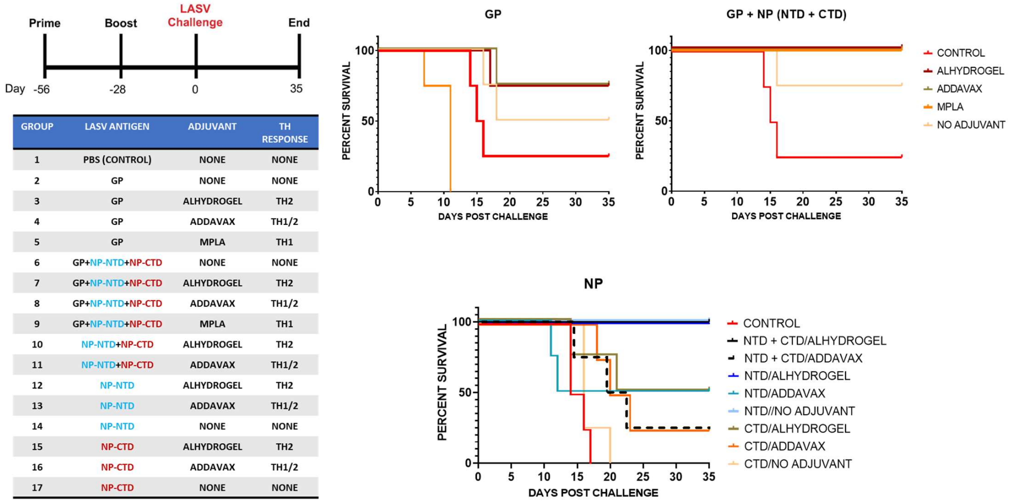
We then evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 6)** to assess whether immune responses to GP only, or GP+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking (**Figure 6**~~), and~~) and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP but no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

Figure 6. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

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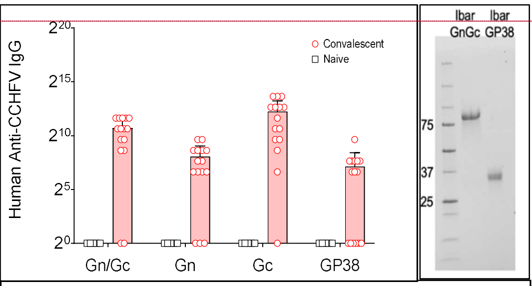

Figure XX: Engineered CCHFV proteins are reactive with serum from CCHFV survivors. SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification

Engineering glycoproteins antigens for ~~the~~ CCHFV: The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to test serum reactivity in CCHF survivors (Figure XX). We will use established protocols [35] as a template to optimize expression of CCHFV and Kasokero NP proteins.

~~*Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses*~~

~~***Rescue of recombinant viruses.*** An important goal of the PABVAX Center is to generate tools for pandemic preparedness. An important component of both RP1 and RP2 is to generate and recombinant viruses to fill gaps and provide tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with RP1 Core E will assist in the development and rescue of New World arenaviruses and the henipavirus Langya virus as detailed in the Research Strategies for RP1 and RP2.~~

APPROACH

~~***Overall Hypothesis:FILL THIS IN***~~

Specific Aim 1: Subunit immunogens and MNP vaccination ~~Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:~~

Rationale:

Overall rationale:

The choice of Mammarenavirus Prototype Pathogens is justified as follows:

- ~~*Old World Mammarenaviridae:* We propose LASV Josiah strain (Lineage 4) as the prototype pathogen due to the depth of available research data, availability of established rodent (**REF**) and nonhuman primate(NHP) models (**REFS**), frequency of exportation (**REFS**), and large number of annual cases throughout West Africa where the case fatality rate can be up to 60% in hospitalized cases (**REF**).~~
- ~~*New World Mammarenaviridae:* Due to the availability of a JUNV vaccine in the endemic region where JUNV circulates and the lack of cross protection against challenge with other NWAV, MACV, the causative agent of Bolivian hemorrhagic fever, will be used as the prototype pathogen for NWAV for studies outlined in Milestone 1.1. There are established guinea pig (**REF**) and NHP (**REF**) models for MACV. JUNV is the New World arenavirus with the largest knowledge base at present [**REF**]. For a pilot efficacy study outlined in Milestone 1.2, we will use JUNV Espindola Strain as a natural extension of previous published work demonstrating protective efficacy of the template PREP mAb (**REF**).~~
- ~~*Nairoviridae.* We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of~~

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~~the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].~~

RP1 proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- *Old Word Mammarenaviruses (OWAV)*: In Phase 1, **RP1** will utilize **LASV Josiah strain (Lineage 4)** as the prototype OWAV due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with an estimated 5000 deaths across West Africa(REF). LASV has is the most imported to non-endemic countries viral hemorrhagic fever (VHF) next to dengue virus and has been imported to over **XX** countries (REFS). Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae (REFS). There are no approved vaccines for LASV; however clinical trials are beginning to evaluate 5 vaccine candidates, 4 of which are live attenuated recombinant viruses and the fifth is a DNA based vaccine. This creates a vacuum for vaccine options for special populations such as the pregnant, the elderly, and the immunocomprimised. There are existing guinea pig and NHP animal models.
- *New World Mamarenaviruses (NWAV)*: A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [36], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [37, 38]. Further, there are safety concerns related to potential for reversion to pathogenic versions and the use of a live attenuated vaccine in immunodepressed populations (Ksiazek reference) For Phase 1, **RP1** will use **MACV-Chicava Strain (Clade 2) and JUNV-Espindola (Lineage XX)** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- *Orthonairoviruses*: In phase 1, **CCHFV-Hoti strain (Clade 4) will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated **XXX** cases per year across Europe, Africa, and Asia. A suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974, but has numerous safety concerns and limited demonstrated efficacy(**REFS**). Since then, a number of vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages (REF); however only an MVA-CCHFV vaccine has begun to advance towards clinical trials (REF). UTMB has established animal models to enable testing of countermeasures against CCHF-Hoti and 5/6 clades in mice (Cross and Geisbert Unpublished, REFS) and CCHF-Hoti (Clade 4) and CCHF- Afghan-09(Clade V) in NHP(REF). Investigators in RP1 and Cores D and E have also previously demonstrated the protective efficacy of a CCHFV GP38 monoclonal antibody in a mouse model (REF) and that human survivors (Cross, Abelson, Zeitlin, and Geisbert) generate potent humoral responses against it suggesting that this cleaved nonstructural glycoprotein may also be leveraged as a vaccine component to induce targeted antibodies against GP38.

~~*Nairoviridae*. We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].~~

Once a candidate subunit-patch vaccine approach is selected, this RP1 will use lessons learned to enable a **plug-and-play** approach to develop subunit vaccines against a representative, “test case” human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, **Lujo virus (LUJV)** was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case’s medical evacuation to South Africa [39]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal “test case” OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. UTMB maintains a Strain 13 guinea pig model which can support efficacy studies in rodents using previously described approach (REF) and working with Core E, RP1 will work to establish a LUJV NHP model for use as

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the test case. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

- First reported in 2003 in Bolivia, **Chapare virus (CHAPV)** is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [40, 41]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [41]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. UTMB has recently developed a uniformly lethal guinea pig model and a partially lethal infection model for CHAPV (unpublished, Cross and Geisbert). The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).
- In 1977, **Kasokero virus (KASV)**, a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [42]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini*, ticks to participate in enzootic maintenance of KASV [43]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. KASV has been demonstrated to be uniformly lethal in outbred mice suggesting a challenge model to determine vaccine efficacy is likely possible (REFS). (The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments [44]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from the each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Development of Recombinant Virus Tools: Rescue of recombinant viruses: An important objective of the PABVAX Center is to generate tools for pandemic preparedness. Investigators at UTMB have a long history of utilizing current molecular virology techniques to develop reverse genetics approaches to recover recombinant viruses for research and vaccine design for several different virus families (REFS). An additional goal of RP1 is to generate and recombinant viruses to fill gaps and provide research tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with Core E, RP1 develop and rescue of New World arenaviruses using existing reverse genetics approaches at UTMB or develop new ones to meet these needs.~~**Paragraph about subunit vaccines and PREP study.**~~

Milestone 1.1 Subunit immunogens and MNP vaccination

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VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-LASV-GPC)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Milestone ~~Milestone~~ 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in table XX using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in Table XX. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core** E~~C~~ and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group (REFS). After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (JUNV-Candid-1)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table XX** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain **(REF).** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV **(REFs).**

Milestone

1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs. Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core** E~~C~~ as outlined in Table XX. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (VSV-LASV-GPC)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

For our MACV subunit vaccines ~~Similarly, , after down selecting the optimal adjuvant and vaccination route,~~ we will assess the potential ~~for our MACV subunit vaccines~~ to confer heterologous protection against three lineages of MACV ~~endemic to~~ genetically distinct from the prototype strain (Chicava-Lineage 2) after down selecting the optimal adjuvant and vaccination rout. Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core** ~~C~~E as outlined in Table XX. We will also utilize JUNV (Romero strain)(**REF**) challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential

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VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (JUNV-Candid-1)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

Milestone **1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP:** 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals (Table XX). Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for LASV Milestone 1.1.1.B

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table XX** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain **(REF).** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will confirm protection in NHPs if demonstrated in guinea pigs **(REFs).**

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

Milestone

1~~2~~.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits:~~.~~

Here we will determine if our GPe + NP-NTD subunit vaccine approach can be deployed against CCHFV.We will compare the protective efficacy of two adjuvants coupled to our CCHFV GP and NP-NTD proteins developed and manufactured in collaboration with **Core D** either as individual subunit vaccines or combinations as outlined in table XX using an intramuscular (IM) vaccination approach. Eight 6-8 week STAT-1/KO mice (balanced sex) will be randomly assigned to groups as outlined in Table XX. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core E** and challenged with 100 pfu of a low passage CCHF-Hoti (REFS). After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHFV-M)	8		8	
	TOTALS	128	TOTALS	128

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

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assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHF-M)	8		8	
	TOTALS	128	TOTALS	128

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

Milestone
1~~2~~.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice: Using the adjuvant and vaccination route down selected in 1.1.2.A, we will assess the potential for our CCHFV subunit vaccines to confer heterologous protection against four lineages of CCHFV endemic to geographically distinct areas from the prototype strain (CCHF-Hoti). Employing optimized strain 13 or outbred, Hartley guinea pig models for Clade 1(country?), 3 (country?), 4 (country) and 6(country) established in the **BSL4 evaluation Core C** as outlined in Table XX. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.2.A above.

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VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

Milestone

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LUJV NP-NTD	8
LUJV GPe	8
LUJV GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP: In collaboration with **Core E**, 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-CCHFV-GPC (REF)) with 5 animals, and a control group of five unvaccinated animals (Table XX). Animals in vaccine groups 1 and 2 will be vaccinated using the down selected CCHFV antigens, adjuvants, and vaccination route identified in 1.1.2.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post-boost for boosted animals), all animals will be challenged with 5000 PFU of CCHFV-Hoti. The disease of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after CCHFV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

Milestone

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2.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses and nairovirus “test case” viruses using optimized subunit-patch vaccination in STAT-1/KO mice, guinea pigs and NHP

RP1 and Core D will produce optimized GPe and NP-NTD antigens to formulate vaccines for LUJV and CHAPV based off lessons learned from 1.1.1A-C. Protective efficacy studies then be carried out where **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively (Table XX). **RP1 and Core D** will also produce optimized KASV GP and NP-NTD antigens to formulate subunit/MNP vaccines based off lessons learned from 1.1.1A-C. Protective efficacy studies will then be carried **Core E** will vaccinate and challenge guinea pigs or NHP as outlined in 1.1.1A and 1.1.1C, respectively (Table XX)

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LASSA NP-NTD	8
LASSA GPe	8
LASSA GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

Milestone

~~*2.1.2.E: Determination of protective efficacy against novel, emerging nairovirus using optimized subunit-patch vaccination in STAT-1KO mice and NHP*~~

Table: XX outline of NHP efficacy studes for MACV
Milestone 1.1.1.B

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1.1.3~~1.F~~: Determination of cellular and humoral immune correlates associated with ~~protection~~protection.

Milestone 1.1.3.A~~1.F.a~~: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups.

We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard whole blood flow cytometric staining protocols will be used for surface and intracellular staining markers, briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye for 20 minutes at room temperature. Cells are then washed again, surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS. Stained, fixed cells will then either be acquired directly. Cell populations are then quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments:. PBMC stimulations will be set up according to modifications of well established, previously published protocols. [45, 46] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldinA will then be added at a concentration of 10mg/mL and incubated at 37°C for until flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays will be set up in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye for 20 minutes at room temperature, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are incubated in BD Cytofix/Cytoperm solution for 20 minutes followed by a wash with BD Perm/Wash Buffer. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a 4-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated for 3 hours at room temperature, protected from light. 1mL of wash buffer will be added and beads will be pelleted by centrifugation at 200g for 5 minutes. Wash buffer will be removed and discarded. Beads will be resuspended in 300uL wash buffer and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37°C. Wells will be washed manually 3-times, followed by the addition of detection antibody and incubated either overnight at 4°C or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes at room temperature, protected from light. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37°C), and spot forming units (SPU) will be read on an S6 Universal M2 ELISPOT analyzer~~**need model**~~ ~~ELISPOT Fluorescence Microplate Reader.~~

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Data Analysis: We will look at the differences in immune responses across groups between each timepoint and baseline. Model assumptions will be checked for a one-way ANOVA to compare the three groups. we will run the analysis after checking model assumptions, if those are not met then we will adjust to a nonparametric model or transform the outcome appropriately. To look at the longitudinal aspect of the data, we will investigate measurements over time between groups, the interaction of group and time can be analyzed using covariance pattern models (or linear mixed models), where the correlation between observations measured over time can be handled more flexibly. As a more exploratory analysis we will also look at adjusting for possible confounding variables such as sex and age. For categorical variables, comparisons will be made using Chi-Square tests. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

Milestone

1.1.3.A:~~1.1.1.F.b~~: Systems serology Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups.

We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform developed by Dr. Gunn in **Core D**[47-49] to define the Fc-mediated humoral antiviral functions associated with vaccination. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV,LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens utilized for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages. We will use a multiplexed analysis to determine the levels of antibodies against the viral antigens using on MagPix xMAP instrument as described.[48, 50] Specifically, each viral antigen will be coupled to coded magnetic beads (Luminex), mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will make a standard using pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry at KGH.

ADCC: Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates for 5 hours at 37C 5% CO_2, and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses were performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction was used to determine statistical significance between groups. A network analysis will be performed to identify significant antibody features. Associations between antibody features will be determined using nonparametric Spearman correlation coefficient. Statistically significant associations after Bonferroni correction for multiple comparisons (adjusted P < .05) were used to generate networks in Cytoscape (version 3.4.0).

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Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and ~~additional clinical information (ie~~clinical signs into the network analysis.~~; PES symptoms). These will be included in the network analysis.~~

***Milestone* 1.~~AIM~~ ~~2~~2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:**

We will use existing mammarenaviruses (REFS) and orthonairovirus (REFS) reverse genetics systems present at UTMB to recover new isolates of LASV, JUNV, MACV, CHAPV, or CCHFV from recent outbreaks if natural isolates are not otherwise available. In parallel, will also create reporter constructs to enable high throughput screening of antivirals with these new isolates. CCHFV GP38 protein is speculated to serve as a virulence factor as protective antibodies targeting this protein have been identified. KASV does not have an equivalent GP38 homolog but is still pathogenic in mice and humans (REFS). There is currently no reverse genetics system for KASV. We will develop an infectious clone system for KASV to create a reporter virus for screening antivirals, but also to build a tool for beginning to understand KASV pathogenesis through loss of function studies.

Milestone 2: ~~1.2~~ Pre-exposure Prophylaxis (PREP) using half-life extension antibody ~~treatments~~treatments.

Rationale: PREP and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis (REFS). The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will perform pilot studies to examine the feasibility of PREP with a serum half-life extended mAb against JUNV. Clinically validated Fc mutations can result in an improvement in serum half-life from 3-4 weeks to 2-4 months ([30-33]).

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

Table:XX

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine~~:~~ In conjunction with ~~RP1~~ Cores D and ~~Core~~ E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb ~~with~~ whose potent therapeutic efficacy [51, 52] ~~(guinea pig and NHP references)~~ we have previously described ~~by Abelson, Geisbert and Zeitlin~~ will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb~~(Table 4)~~. The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (~~Bioqual, Inc.; Rockville, MD~~see Core D) to select a lead candidate for testing in NHPs against Junin Romero challenge in Core E. ~~For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference).~~ We will select t~~T~~he variant with the most favorable pharmacokinetics ~~PK will be selected~~ for testing with ~~by RP1/~~Core E. Briefly, groups of ~~4~~5 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV ~~(Espindola or Romero~~Espindola~~?)~~ delivered IV (Table XX). For further details of the NHP testing, please see Core E. These data will be used by the Scientific Advisory ~~Committee~~ Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

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Expected results, alternative ~~approaches~~approaches, and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and ideally protective subunit-patch targeting LASV, MACV, ~~and~~ CCHFV and use lessons learned from this Phase I suite of studies to rapidly develop subunit-patch vaccines for three “test case” viruses LUJV, CHAPV, and KASV in Phase II of the project.~~…….~~ If ~~this~~ any~~is not the case any~~ of the~~se~~ Phase I vaccine projects have not met the “go” criteria, ~~three viruses by year 2~~ we will refocus ~~effects~~ efforts toward~~on~~ the remaining virus families adjusting the requested budgets accordingly after ~~consulation~~consultation with NIAID and SAC.

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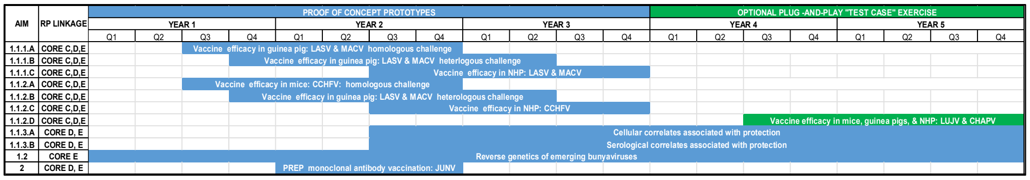

AIM	RP LINKAGE	PROOF OF CONCEPT PROTOTYPES												OPTIONAL PLUG -AND-PLAY "TEST CASE" EXERCISE							
		YEAR 1				YEAR 2				YEAR 3				YEAR 4				YEAR 5			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1.1.1.A	CORE C,D,E			Vaccine efficacy in guinea pig: LASV & MACV homologous challenge	🟦	🟦	🟦	🟦	🟦												
1.1.1.B	CORE C,D,E				Vaccine efficacy in guinea pig: LASV & MACV heterlogous challenge	🟦	🟦	🟦	🟦	🟦	🟦										
1.1.1.C	CORE C,D,E							Vaccine efficacy in NHP: LASV & MACV	🟦	🟦	🟦	🟦	🟦								
1.1.2.A	CORE C,D,E			Vaccine efficacy in mice: CCHFV: homologous challenge	🟦	🟦	🟦	🟦	🟦												
1.1.2.B	CORE C,D,E				Vaccine efficacy in guinea pig: LASV & MACV heterologous challenge	🟦	🟦	🟦	🟦	🟦	🟦										
1.1.2.C	CORE C,D,E							Vaccine efficacy in NHP: CCHFV	🟦	🟦	🟦	🟦	🟦								
1.1.2.D	CORE C,D,E															Vaccine efficacy in mice, guinea pigs, & NHP: LUJV & CHAPV	🟩	🟩	🟩	🟩	🟩
1.1.3.A	CORE D, E							Cellular correlates associated with protection	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
1.1.3.B	CORE D, E							Serological correlates associated with protection	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
1.2	CORE E	Reverse genetics of emerging bunyaviruses	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
2	CORE D, E					PREP monoclonal antibody vaccination: JUNV	🟦	🟦	🟦												

Please note: Discussion of expected results, other alternative approaches and criteria for “go” or “no go” decisions for MILESTONES 1~~-4~~ and 2 are discussed as required in RFA-AI-20-023 in the **Project Milestones and Timelines** *section.*

References:

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~~**AIM 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:**~~

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~~**Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines).** We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC~~

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~~*Please note: Discussion of expected results, other alternative approaches and criteria for “go” or “no go” decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*~~

~~**JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,**~~

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~~Milestones-Timelines-GANT CHART~~

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~~Summary statement impact~~

1. Abudurexiti, A., et al., *Taxonomy of the order Bunyavirales: update 2019.* Arch Virol, 2019. **164**(7): p. 1949-1965.
2. Hallam, S.J., et al., *Review of Mammarenavirus Biology and Replication.* Front Microbiol, 2018. **9**: p. 1751.
3. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nature Medicine, 2017. **23**(10): p. 1146-1149.
4. Hastie, K.M., et al., *Structural basis for antibody-mediated neutralization of Lassa virus.* Science, 2017. **356**(6341): p. 923-928.
5. Sullivan, B.M., et al., *High crossreactivity of human T cell responses between Lassa virus lineages.* PLoS Pathog, 2020. **16**(3): p. e1008352.
6. Heinrich, M.L., et al., *Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages.* Scientific Reports, 2020. **10**(1): p. 16030.
7. Ugwu, C., et al., *Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria.* Scientific Reports, 2022. **12**(1): p. 22330.
8. Geisbert, T.W., et al., *Development of a New Vaccine for the Prevention of Lassa Fever.* PLOS Medicine, 2005. **2**(6): p. e183.
9. Cross, R.W., et al., *A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever.* Cell Rep, 2022. **40**(3): p. 111094.
10. Safronetz, D., et al., *A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses.* PLOS Neglected Tropical Diseases, 2015. **9**(4): p. e0003736.
11. Burt, F.J., et al., *Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia.* Epidemiol Infect, 1996. **116**(3): p. 353-61.
12. Brinkmann, A., et al., *First detection of bat-borne Issyk-Kul virus in Europe.* Sci Rep, 2020. **10**(1): p. 22384.
13. Ma, J., et al., *Identification of a new orthonairovirus associated with human febrile illness in China.* Nat Med, 2021. **27**(3): p. 434-439.
14. Lorenzo Juanes, H.M., et al., *Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021.* Emerg Infect Dis, 2023. **29**(2): p. 252-259.
15. Grandi, G., et al., *First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden.* Ticks Tick Borne Dis, 2020. **11**(3): p. 101403.
16. Hawman, D.W. and H. Feldmann, *Crimean–Congo haemorrhagic fever virus.* Nature Reviews Microbiology, 2023.
17. World.Health.Organisation. *List of Blueprint Priority Diseases*. 2018 [cited 2018 November 14]; Available from: http://www.who.int/blueprint/priority-diseases/en/.
18. NIH-NIAID. *NIAID Emerging Infectious Diseases/ Pathogens*. 2023 [cited 2023 03/06]; Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
19. Keshtkar-Jahromi, M., et al., *Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies.* Antiviral Res, 2011. **90**(2): p. 85-92.
20. Soares-Weiser, K., et al., *Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis.* BMC Infectious Diseases, 2010. **10**(1): p. 207.
21. Rodriguez, S.E., et al., *Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever.* Sci Rep, 2019. **9**(1): p. 7755.
22. Brisse, M.E. and H. Ly, *Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors.* Front Immunol, 2019. **10**: p. 372.
23. Baize, S., et al., *Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys.* J Virol, 2009. **83**(11): p. 5890-903.
24. Fisher, D.G., G.M. Coppock, and C.B. Lopez, *Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination.* Vaccine, 2018. **36**(28): p. 4039-4045.
25. Meyer, B. and H. Ly, *Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses.* J Virol, 2016. **90**(8): p. 3810-3818.

26. ter Meulen, J., et al., *Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein.* J Virol, 2000. **74**(5): p. 2186-92.
27. Meulen, J., et al., *Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones.* Virology, 2004. **321**(1): p. 134-43.
28. Goicochea, M.A., et al., *Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model.* Vaccine, 2012. **30**(8): p. 1445-52.
29. Leventhal, S.S., et al., *Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge.* EBioMedicine, 2022. **82**: p. 104188.
30. Robbie, G.J., et al., *A Novel Investigational Fc-Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-Life in Healthy Adults.* Antimicrob Agents Chemother, 2013. **57**(12): p. 6147-53.
31. Rosenberg, Y.J., et al., *Introduction of the YTE mutation into the non-immunogenic HIV bnAb PGT121 induces anti-drug antibodies in macaques.* PLoS One, 2019. **14**(2): p. e0212649.
32. Rappazzo, C.G., et al., *Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody.* Science, 2021. **371**(6531): p. 823-829.
33. Schmidt, P., et al., *Safety, Pharmacokinetics, Serum Neutralizing Titers, and Immunogenicity of Adintrevimab, a Monoclonal Antibody Targeting SARS-CoV-2: A Randomized, Double-Blind, Placebo-Controlled, Phase 1 Dose-escalation Study in Healthy Adults.* Infect Dis Ther, 2023: p. 1-13.
34. McElroy, A.K., et al., *A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses.* J Infect Dis, 2017. **215**(12): p. 1862-1872.
35. Carter, S.D., et al., *Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein.* J Virol, 2012. **86**(20): p. 10914-23.
36. Saito, T., et al., *Vaccine Candidates against Arenavirus Infections.* Vaccines, 2023. **11**(3): p. 635.
37. Koma, T., et al., *Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic.* J Virol, 2016. **90**(3): p. 1290-7.
38. Jahrling P, T.R., Barrero O. . *Crossprotection against Machupo virus with Candid 1 Junin virus vaccine*. in *2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region*. 1988. Mar del Plata, Argentina.
39. Sewlall, N.H., et al., *Clinical features and patient management of Lujo hemorrhagic fever.* PLoS Negl Trop Dis, 2014. **8**(11): p. e3233.
40. Delgado, S., et al., *Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia.* PLOS Pathogens, 2008. **4**(4): p. e1000047.
41. Loayza Mafayle, R., et al., *Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019.* New England Journal of Medicine, 2022. **386**(24): p. 2283-2294.
42. Kalunda, M., et al., *Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda.* The American Journal of Tropical Medicine and Hygiene, 1986. **35**(2): p. 387-392.
43. Schuh, A.J., et al., *Human-Pathogenic Kasokero Virus in Field-Collected Ticks.* Emerg Infect Dis, 2020. **26**(12): p. 2944-2950.
44. Balmert, S.C., et al., *A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity.* iScience, 2022. **25**(10): p. 105045.
45. Meddows-Taylor, S., et al., *Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome.* J Virol Methods, 2007. **144**(1-2): p. 115-21.
46. Kagina, B.M., et al., *Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry.* J Immunol Methods, 2015. **417**: p. 22-33.
47. Gunn, B.M., et al., *A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus.* Immunity, 2021. **54**(4): p. 815-828.e5.
48. Gunn, B.M., et al., *Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses.* J Infect Dis, 2020. **221**(1): p. 156-161.
49. Saphire, E.O., et al., *Antibody-mediated protection against Ebola virus.* Nat Immunol, 2018. **19**(11): p. 1169-1178.
50. Brown, E.P., et al., *Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles.* J Immunol Methods, 2017. **443**: p. 33-44.
51. Zeitlin, L., et al., *Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody.* Proc Natl Acad Sci U S A, 2021. **118**(11).

52. Zeitlin, L., et al., *Monoclonal antibody therapy for Junin virus infection.* Proc Natl Acad Sci U S A, 2016. **113**(16): p. 4458-63.

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SPECIFIC AIMS

The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparednessThreats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches **which can be adapted across members of each viral family in a "plug-and-play" manner**. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a concerns tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GP co-delivered with LASV NP subunits or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed in or monoclonal antibody PREP strategies which can provide protective immunity against Old and New World arenaviruses or emerging nairoviruses. (2) Develop reverse genetics tools to evaluate emerging arenaviruses and nairoviruses for which no isolates are available. Specifically, we will:

Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:

1.1 Subunit immunogens and MNP vaccination: Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches. We will then utilize lessons learned to address related "test case" arenaviruses and nairoviruses to develop a subunit/MNP approach for an old world arenavirus (LUJV) a new world arenavirus (CHPV), and an emerging bat borne nairovirus, Kasokero virus.

1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments: Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.

Milestone 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:

We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.

RESEARCH STRATEGY:
1. SIGNIFICANCE:
The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens (REF) where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness (REF). Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Research Project 1(RP1) proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- **Old Word Mammarenaviruses (OWAV):** RP1 will utilize **LASV as the prototype OWAV** due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with **XX** deaths across West Africa(REF). LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to over **XX** countries (REFS). Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) (REFS). Most of these vaccines utilize the LASV (glycoprotein) GP or the LASV GP + Nucleoprotein (NP) as the target immunogens in these vectors (REFs).
- **New World Mamarenaviruses (NWAV):** A live attenuated vaccine for JUNV is currently in use in Argentina [2], but there is conflicting evidence of whether cross protection with other NWAV is possible [3, 4] and there are safety concerns related to reversion to pathogenic versions. This proposal will use **MACV and JUNV as the prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- **Orthonairoviruses:** Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated **XXX** cases per year across Europe, Africa, and Asia. CCHFV outbreaks are often very large list some recent examples. A suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974, but has numerous safety concerns and limited demonstrated efficacy(**REFS**). Since then, a number of vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages(REF); however only an MVA-CCHFV vaccine has begun to advance towards clinical trials (REF).

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e. the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success (**REFS**). The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability(REFS). Members of PABVAX, have developed protective

protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments [5]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from the each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Once a candidate subunit-patch vaccine approach is selected, this approach will use lessons learned to produce plug-and-play approach to develop vaccines against a representative human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, Lujo virus was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [6]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, underscoring the need for antiviral tools against this emerging threat.
- First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [7, 8]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [8]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective, but the phylogenetic divergence of CHAPV of XX% from JUNV suggests it may not be.
- In 1977, Kasokero virus (KASV), a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [9]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for *Ornithodoros faini* ticks to participate in enzootic maintenance of KASV [10]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised.

Pre-exposure prophylaxis (PREP) and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-~~-~~risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis (REFS). The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will ~~also~~ perform pilot studies to examine the feasibility of PREP ~~utilizing~~ with a serum half-life extended mAb against JUNV~~s~~. Clinically validated Fc mutations can result in an improvement in serum half-life from 3-4 weeks to 2-4 months ~~which can increase the instant barrier immunity from 30-45 days to 60+ days~~ ([44, 45][46] [47]~~refs~~).

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens

The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[11] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[11]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[12-17]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[18]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine(REFS). Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

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Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens

CCHFV is a divergent virus with up to seven genetic lineages (REFS). This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on (REF). Conversely, NP is highly conserved but not exposed on the virus surface cellular surface (REFs). Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [19]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control mammarenaviruses

For situations where a vaccine does not exist or is not appropriate (*e.g.* immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE [44, 45] and LA [46] [47] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

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Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses

Add blurb discussing unique access to field samples from across the world and established expertise to generate infectious clones from emerging arenaviruses and nairoviruses:

3. APPROACH

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

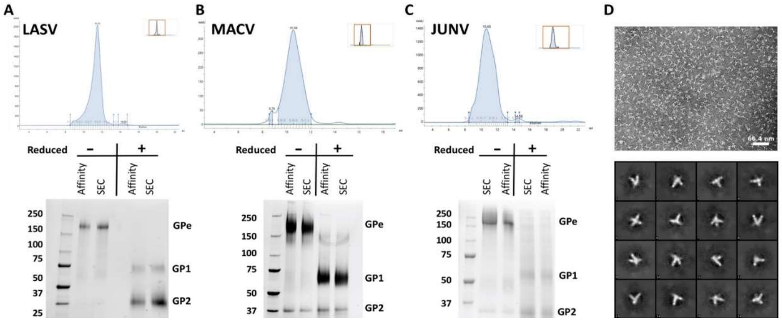


Figure XX. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide-linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[20]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[20] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure xx)**. Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 3)**. Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure xx)**. Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

Our trimeric arenavirus GPs support efforts to isolate novel ultra-potent mAbs and to develop a second-generation arenavirus immunotherapeutic with reduced dosing requirements and broader reactivity compared to current LASV mAb candidates[21]. This significantly lowers the cost of goods while increasing ease of use for clinicians, both essential for a product used in developing nations where arenaviruses are prevalent.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches have largely focused on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[12-17] suggests that the cellular response, mediated by T cells, can play a highly important and complementary role in the long-term protection against pathogens. Based on literature reports that T cell responses highly correlate with LASV recovery, and that LASV NP plays a significant role in inducing those responses[12, 22], we also generated LASV NP antigens. We chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle (**Figure xx**) and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks.

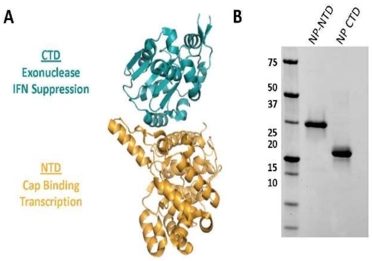
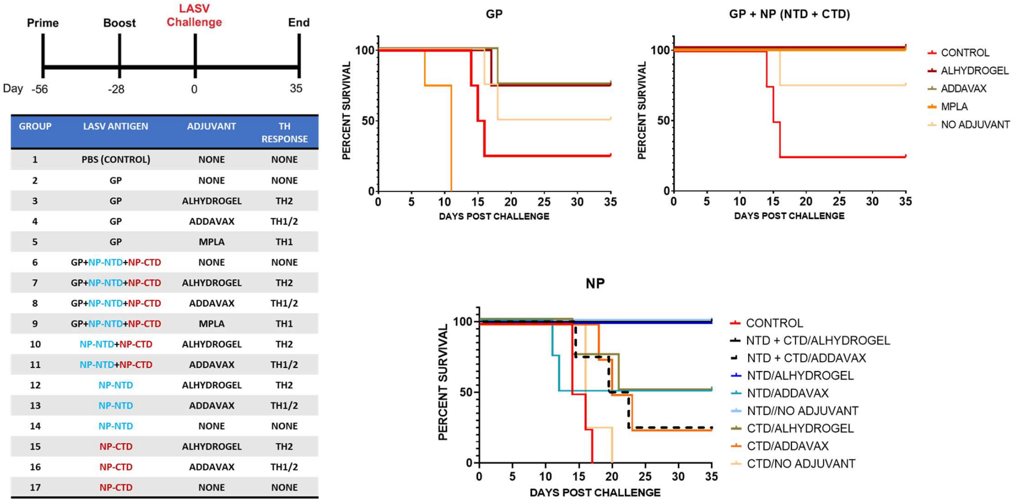


Figure xx. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B)



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

Figure 6. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

We evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed (**Figure 6**) to assess whether immune responses to GP only, or GP+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking (**Figure 6**), and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP but no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of

NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

*Engineering glycoproteins antigens for the CCHFV*The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to test serum reactivity in CCHF survivors (Figure XX). We will use established protocols [23] as a template to optimize expression of CCHFV and Kasokero NP proteins.

Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses

Rescue of recombinant viruses. An important goal of the PABVAX Center is to generate tools for pandemic preparedness. An important component of both RP1 and RP2 is to generate and recombinant viruses to fill gaps and provide tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with RP1 Core E will assist in the development and rescue of New World arenaviruses and the henipavirus Langya virus as detailed in the Research Strategies for RP1 and RP2.

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APPROACH

Overall Hypothesis:FILL THIS IN

Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:

Rationale: The choice of Mammarenavirus Prototype Pathogens is justified as follows:

- *Old World Mammarenaviridae:* We propose LASV Josiah strain (Lineage 4) as the prototype pathogen due to the depth of available research data, availability of established rodent (**REF**) and nonhuman primate(NHP) models (**REFS**), frequency of exportation (**REFS**), and large number of annual cases throughout West Africa where the case fatality rate can be up to 60% in hospitalized cases (**REF**).
- *New World Mammarenaviridae:* Due to the availability of a JUNV vaccine in the endemic region where JUNV circulates and the lack of cross protection against challenge with other NWAV, MACV, the causative agent of Bolivian hemorrhagic fever, will be used as the prototype pathogen for NWAV for studies outlined in Milestone 1.1. There are established guinea pig (**REF**) and NHP (**REF**) models for MACV. JUNV is the New World arenavirus with the largest knowledge base at present [**REF**]. For a pilot efficacy study outlined in Milestone 1.2, we will use JUNV Espindola Strain as a natural extension of previous published work demonstrating protective efficacy of the template PREP mAb (**REF**).
- *Nairoviridae*. We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].

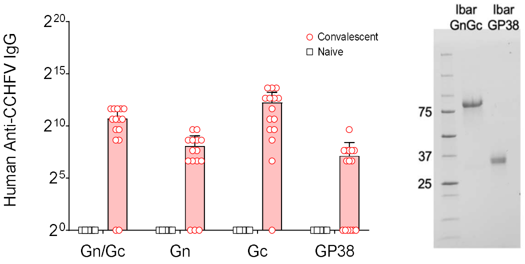


Figure XX: Engineered CCHFV proteins are reactive with serum from CCHFV survivors. SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification

Paragraph about subunit vaccines and PREP study.

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in table XX using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in Table XX. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core C** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group (REFS). After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-LASV-GPC)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of LASV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table XX** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain **(REF).** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV **(REFs).**

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (JUNV-Candid-1)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of MACV Vaccination Schedule for Milestone 1.1.1.A

1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs. Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core C** as outlined in Table XX. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (VSV-LASV-GPC)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (JUNV-Candid-1)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

Similarly, after down selecting the optimal adjuvant and vaccination route, we will assess the potential for our MACV subunit vaccines to confer heterologous protection against thre lineages of MACV endemic to genetically distinct from the prototype strain (Chicava-Lineage 2). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core C** as outlined in Table XX. We will also utilize JUNV (Romero strain)(**REF**) challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP: 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals (Table XX). Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

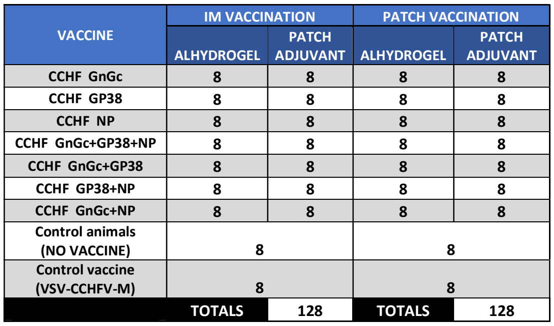
Table: XX outline of NHP efficacy studes for LASV Milestone 1.1.1.B

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

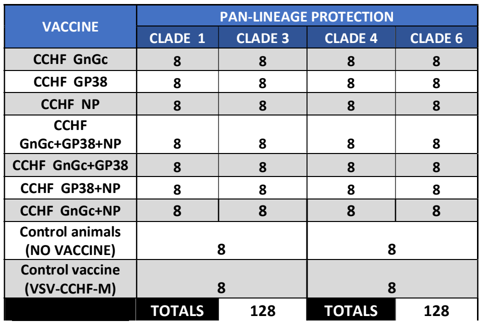
Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table XX** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain (**REF**)**.** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will confirm protection in NHPs if demonstrated in guinea pigs **(REFs).**

2.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits.



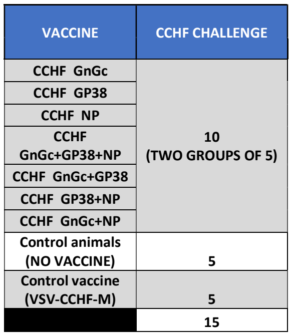
VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHFV-M)	8		8	
	TOTALS	128	TOTALS	128

2.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice



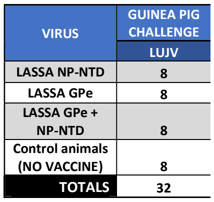
VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHF-M)	8		8	
	TOTALS	128	TOTALS	128

2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP

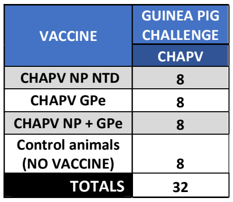


VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

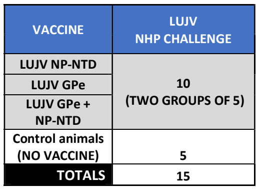
2.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses using optimized subunit-patch vaccination in guinea pigs and NHP



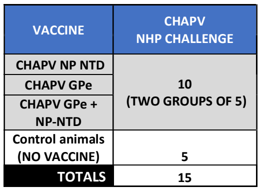
VIRUS	GUINEA PIG CHALLENGE
	LUJV
LASSA NP-NTD	8
LASSA GPe	8
LASSA GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32



VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

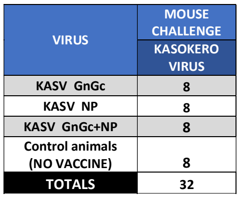


VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

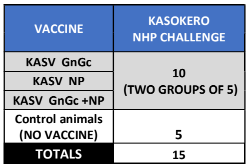


VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

2.1.2.E: Determination of protective efficacy against novel, emerging nairovirus using optimized subunit-patch vaccination in STAT-1KO mice and NHP



VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32



VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

1.1.1.F: Determination of cellular and humoral immune correlates associated with protection

1.1.1.F.a: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups.

We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard whole blood flow cytometric staining protocols will be used for surface and intracellular staining markers, briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye for 20 minutes at room temperature. Cells are then washed again, surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS.

Stained, fixed cells will then either be acquired directly. Cell populations are then quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments:. PBMC stimulations will be set up according to modifications of well established, previously published protocols. [24, 25] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37ºC for 1 hour. A protein transport inhibitor such as brefeldinA will then be added at a concentration of 10mg/mL and incubated at 37ºC for until flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37ºC. Stimulations to assess secreted analytes by multiplex-bead based assays will be set up in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye for 20 minutes at room temperature, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are incubated in BD Cytofix/Cytoperm solution for 20 minutes followed by a wash with BD Perm/Wash Buffer. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a 4-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated for 3 hours at room temperature, protected from light. 1mL of wash buffer will be added and beads will be pelleted by centrifugation at 200g for 5 minutes. Wash buffer will be removed and discarded. Beads will be resuspended in 300uL wash buffer and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37ºC. Wells will be washed manually 3-times, followed by the addition of detection antibody and incubated either overnight at 4ºC or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes at room temperature, protected from light. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37ºC), and spot forming units (SPU) will be read on an **need model** ELISPOT Fluorescence Microplate Reader.

Data Analysis: We will look at the differences in immune responses across groups between each timepoint and baseline. Model assumptions will be checked for a one-way ANOVA to compare the three groups. we will run the analysis after checking model assumptions, if those are not met then we will adjust to a nonparametric model or transform the outcome appropriately. To look at the longitudinal aspect of the data, we will investigate measurements over time between groups, the interaction of group and time can be analyzed using covariance pattern models (or linear mixed models), where the correlation between observations measured over time can be handled more flexibly. As a more exploratory analysis we will also look at adjusting for possible confounding variables such as sex and age. For categorical variables, comparisons will be made using Chi-Square tests. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

1.1.1.F.b: Systems serology ***Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups.***

We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform developed by Dr. Gunn in Core D[26-28] to define the Fc-mediated humoral antiviral functions associated with vaccination. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV,LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens utilized for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages. We will use a multiplexed analysis to determine the levels of antibodies against the viral antigens using on MagPix xMAP instrument as described.[27, 29] Specifically, each viral antigen will be coupled to coded magnetic beads (Luminex), mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will make a standard using pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry at KGH.

ADCC: Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates for 5 hours at 37C 5% CO_2, and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses were performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction was used to determine statistical significance between groups. A network analysis will be performed to identify significant antibody features. Associations between antibody features will be determined using nonparametric Spearman correlation coefficient. Statistically significant associations after Bonferroni correction for multiple comparisons (adjusted $P < .05$) were used to generate networks in Cytoscape (version 3.4.0).

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and additional clinical information (ie: PES symptoms). These will be included in the network analysis.

1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine. In conjunction with ~~RP1~~ Cores D and ~~Core~~ E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb ~~with~~ whose potent therapeutic efficacy [39, 43] ~~(guinea pig and NHP references)~~ we have previously described ~~by Abelson, Geisbert and Zeitlin~~ will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb~~(Table 4)~~. The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (~~Bioqual, Inc.; Rockville, MD~~see Core D) to select a lead candidate for testing in NHPs against Junin Romero challenge in Core E. ~~For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference).~~ We will select t~~T~~he variant with the most favorable pharmacokinetics ~~PK will be selected~~ for testing with ~~by **RP1/**~~**Core E**. Briefly, groups of 4 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV (~~Espindola or~~ Romero~~?~~) delivered IV. For further details of the NHP testing, please see **Core E**. These data will be used by the Scientific Advisory ~~Committee~~ Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Commented [CRW4]: From Larry, adapt from this.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC

Please note: Discussion of expected results, other alternative approaches and criteria for "go" or "no go" decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the **Project Milestones and Timelines** *section.*

JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,

AIM 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC

Please note: Discussion of expected results, other alternative approaches and criteria for "go" or "no go" *decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*

JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,

Milestones-Timelines-GANT CHART

Summary statement impact

1. Abudurexiti, A., et al., *Taxonomy of the order Bunyavirales: update 2019.* Arch Virol, 2019. **164**(7): p. 1949-1965.
2. Saito, T., et al., *Vaccine Candidates against Arenavirus Infections.* Vaccines, 2023. **11**(3): p. 635.
3. Koma, T., et al., *Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic.* J Virol, 2016. **90**(3): p. 1290-7.
4. Jahrling P, T.R., Barrero O. . *Crossprotection against Machupo virus with Candid 1 Junin virus vaccine*. in *2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region*. 1988. Mar del Plata, Argentina.
5. Balmert, S.C., et al., *A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity.* iScience, 2022. **25**(10): p. 105045.
6. Sewlall, N.H., et al., *Clinical features and patient management of Lujo hemorrhagic fever.* PLoS Negl Trop Dis, 2014. **8**(11): p. e3233.
7. Delgado, S., et al., *Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia.* PLOS Pathogens, 2008. **4**(4): p. e1000047.
8. Loayza Mafayle, R., et al., *Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019.* New England Journal of Medicine, 2022. **386**(24): p. 2283-2294.
9. Kalunda, M., et al., *Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda.* The American Journal of Tropical Medicine and Hygiene, 1986. **35**(2): p. 387-392.
10. Schuh, A.J., et al., *Human-Pathogenic Kasokero Virus in Field-Collected Ticks.* Emerg Infect Dis, 2020. **26**(12): p. 2944-2950.
11. Brisse, M.E. and H. Ly, *Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors.* Front Immunol, 2019. **10**: p. 372.
12. Baize, S., et al., *Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys.* J Virol, 2009. **83**(11): p. 5890-903.
13. Fisher, D.G., G.M. Coppock, and C.B. Lopez, *Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination.* Vaccine, 2018. **36**(28): p. 4039-4045.
14. Meyer, B. and H. Ly, *Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses.* J Virol, 2016. **90**(8): p. 3810-3818.
15. ter Meulen, J., et al., *Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein.* J Virol, 2000. **74**(5): p. 2186-92.

16. Meulen, J., et al., *Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones.* Virology, 2004. **321**(1): p. 134-43.
17. Goicochea, M.A., et al., *Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model.* Vaccine, 2012. **30**(8): p. 1445-52.
18. Sullivan, B.M., et al., *High crossreactivity of human T cell responses between Lassa virus lineages.* PLoS Pathog, 2020. **16**(3): p. e1008352.
19. Leventhal, S.S., et al., *Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge.* EBioMedicine, 2022. **82**: p. 104188.
20. Hastie, K.M., et al., *Structural basis for antibody-mediated neutralization of Lassa virus.* Science, 2017. **356**(6341): p. 923-928.
21. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nat Med, 2017. **23**(10): p. 1146-1149.
22. McElroy, A.K., et al., *A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses.* J Infect Dis, 2017. **215**(12): p. 1862-1872.
23. Carter, S.D., et al., *Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein.* J Virol, 2012. **86**(20): p. 10914-23.
24. Meddows-Taylor, S., et al., *Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome.* J Virol Methods, 2007. **144**(1-2): p. 115-21.
25. Kagina, B.M., et al., *Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry.* J Immunol Methods, 2015. **417**: p. 22-33.
26. Gunn, B.M., et al., *A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus.* Immunity, 2021. **54**(4): p. 815-828.e5.
27. Gunn, B.M., et al., *Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses.* J Infect Dis, 2020. **221**(1): p. 156-161.
28. Saphire, E.O., et al., *Antibody-mediated protection against Ebola virus.* Nat Immunol, 2018. **19**(11): p. 1169-1178.
29. Brown, E.P., et al., *Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles.* J Immunol Methods, 2017. **443**: p. 33-44.

From: Cross, Robert W. on behalf of Cross, Robert W. <rwcross@UTMB.EDU>
To: Geisbert, Thomas W.
Cc: CHRISTOPHER BRODER
Subject: RE: ReVAMPP Bunyavirales Project
Date: Monday, June 5, 2023 4:42:51 PM
Attachments: 05JUN23_RP1-BUNYAVIRALES VACCINES--RESEARCH STRATEGY_RWC_1534.docx
05JUN23_RP1-BUNYAVIRALES VACCINES--RESEARCH STRATEGY_RWC_1534.pdf

At the point in the grant writing where the document is making unintended changes on its own. Heres the fixed version with pdf in case things jump again

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Monday, June 5, 2023 3:30 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Some things appear to be moved around. There is a Figure on the PABVAX Figure. One thing on quick pass is that there are some areas of 12 point font below and if you change them to 11 point you are very close on 12 full pages.

***Milestone* 1.1.2.A:**

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines).

From: Cross, Robert W. <rwcross@UTMB.EDU>
Sent: Monday, June 5, 2023 3:02 PM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Larry Zeitlin <larry.zeitlin@mappbio.com>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
Subject: RE: ReVAMPP Bunyavirales Project

Attached is most recent version. I realized late I needed to add the industry section and add go/nogo to gant. Did a lot of editing/cutting to make those two fit.

If you have time, take a look. Working on abstract now.

From: Cross, Robert W.
Sent: Monday, June 5, 2023 1:14 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; 'Larry Zeitlin' <larry.zeitlin@mappbio.com>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; 'Dafna Abelson' <dafna.abelson@mappbio.com>
Cc: 'Gunn, Bronwyn Mei' <bronwyn.gunn@wsu.edu>; 'Antony Dimitrov' <antony.dimitrov.ctr@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>

Subject: RE: ReVAMPP Bunyavirales Project

Attached is the latest version. I still need to deal with the minor highlighted refs and table/figure descriptions but will do it tomorrow. There is a little room to mess around with how the milestones/objectives are presented, I just kept them in paragraph form for now but we can enumerate if it makes more sense to do so. Otherwise space is pretty tight until I can get the refs in and table/figure descriptions fixed.

I'm out of gas for now, welcome any feedback for refinement or if I left something important out.

Bob

From: Cross, Robert W.
Sent: Saturday, June 3, 2023 4:11 PM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Larry Zeitlin <larry.zeitlin@mappbio.com>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
Subject: RE: ReVAMPP Bunyavirales Project

I just talked with Tom to walk through some of RP1 and we have a plan to improve readability and flow. Aims will stay largely the same, but I will add more context and clarify a few items to help with readability and flow. I will send an updated if not complete version to the group by late tomorrow. Adding Court as a fresh set of eyes to help with review and refinement.

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Saturday, June 3, 2023 2:50 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>; Cross, Robert W. <rwcross@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Total free association here and I have been running a lot of things by Courtney this week to get a different opinion. So this below is really more along the lines of thinking about cross lineage and clade protection. And maybe some naivety on my part in the interactions with Core C. So I am guessing that antigens are made and then tested for immunogenicity by Core C and D and maybe Core C is involved earlier in an example below? I think for RP1 and RP2 in terms of recombinant viruses that for RP2 there is an obvious need to make Langya. For RP1 I think it is more of an alternate strategy if we cannot get authentic viruses and maybe as the RFA makes a point of tools for something like Chapare where there is no reverse genetics system we make one. So I am not sure that I would have making recombinant viruses a stand alone Aim for RP1 rather I wonder if it goes more in whatever Aim assesses protective efficacy where it is needed to make a new virus that pops up that we cannot get?

> Specific Aims:
>
> • Aim 1: Identification and characterization of conserved antigenic regions
> • Objective 1.1: Analyze genomes of arenaviruses and nairoviruses to identify conserved regions across multiple strains and species within each family.
> • Objective 1.2: Characterize the antigenicity of identified conserved regions using in silico methods and experimental assays (Core D).
>
> • Aim 2: Development of a multivalent vaccine candidate
> • Objective 2.1: Design and construct a multivalent vaccine formulation incorporating multiple conserved antigenic regions,
> covering a broad range of arenaviruses and nairoviruses (Core D).
> • Objective 2.2: Evaluate the immunogenicity and cross-reactivity of the multivalent vaccine candidate in preclinical models using MNP specifically assessing its ability to elicit protective immune responses against diverse arenavirus and nairovirus strains (Core E).
> • Objective 2.3: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates
>
> Aim 3: Identify correlates of protection and optimize vaccine formulation and delivery
> • Objective 3.1: Investigate the mechanisms of vaccine-induced protection against arenaviruses and nairoviruses, including humoral and cellular immune responses, to understand immune correlates of protection (Gunn).
> • Objective 3.2: Optimize MNP delivery systems and adjjuvants to enhance immunogenicity (Core C, E)?

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Saturday, June 3, 2023 10:25 AM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>; Cross, Robert W. <rwcross@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

I will try to take a look at this today. If not addressed here one theme that I think we need to get across are the problems that occurred with SARS-CoV-2 with the vaccines and treatments not offering broad coverage against different variants. So while on one hand being able to address that quickly with plug and play approaches is great on the other hand developing vaccines that can provide broad coverage from the beginning is even better. I think that is probably being addressed here by having the NP approach added as a comparator. One thing that I think may give our Center an advantage over some others is access to a more diverse group of viruses than other applications, e.g., for Lassa there are seven lineages and we have animal models that can cover a lot of those and even in the case of Machupo we can cover the most commonly used as well as the most diverse lineage VIII. For henipaviruses thanks to Chris we now have Hendra Redlands which is a beast as well as the HeVg2. Even where maybe we don't have established animal models we at least have viruses that cover most of the genetic diversity for the viruses in our Center so we can at least assess the immune response by ELISA and neut against wild type viruses.

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Friday, June 2, 2023 3:09 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

I added some language for the mAb as alternative to a vaccine sections…

From: Cross, Robert W. <rwcross@UTMB.EDU>
Date: Thursday, June 1, 2023 at 11:56 PM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Larry Zeitlin <larry.zeitlin@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Getting there, here is the update to now. I hope to finish filling out missing sections by tomorrow evening. Welcome any feedback

Bob

From: Cross, Robert W.
Sent: Monday, May 29, 2023 1:49 PM
To: Broder, Christopher <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Hi Chris,

Some thoughts…

On the PREP, this is just the one study being proposed as proof of concept. Will let Larry elaborate, but we will need to wordsmith to suggest that if successful this may be an avenue to look at with other viruses with the support of SAC and program.

On the cellular and humoral part. Space is a big issue here and I'm not sure we will need to describe in detail in every RP or core, but it needs to be outlined somewhere clearly and that will eat up some space. I put it in my RP since there was no room in the animal core and Larry's core didn't detail it too much either (so far). My version will likely need to be whittled down as well to make everything fit, so but not so much that the gist is lost. I wonder if we could find a small amount of space to describe the immunology work up I described will be carried out in the scientific cores (and in collaboration with RP1) as outlined in RP1? That said, we can maybe refer to methods used in RP1 for the core as well since that write-up is already having issues with space? Also note that I'm not doing any immunology on rodents since the patch core mentioned they would do rudimentary serology and ELISPOTs, all this advanced immunology work will be done with primates only due to lack of reagents.

Bob

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Monday, May 29, 2023 1:09 PM
To: Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Cross, Robert W. <rwcross@UTMB.EDU>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

hi all
RP2 is not looking anything like this post-vaccination subject analysis on the CD4/CD8 and cellular responses or the serological studies.

We were allowing the Upitt core to explore the mice with cellular responses as the pilot.
The plan for the MNP vaccines is to test efficacy in ferrets then proceed to AGMs.

Not sure what to do or how to proceed here! Are there analysis text sections
from what the animal core can or should propose here or outline here, as Bob laid
out? I dont have anything like this in hand,

also, i saw the comment on PREP
We dont have any plans for PREP for NiV/HeV included here either, our best candidates
are already in Mapp's pipeline, or in the CETR

chris

On Sun, May 28, 2023 at 3:35 PM Dafna Abelson <dafna.abelson@mappbio.com> wrote:

> Hey Bob
> Looks great! I added some language to use or lose in the Engineering Glycoproteins for CCHF section and the gel we used in the Core D preliminary results.
> Best,
> Dafna
>
> ---
>
> **From:** Cross, Robert W. <rwcross@UTMB.EDU>
> **Date:** Saturday, May 27, 2023 at 10:26 PM
> **To:** Larry Zeitlin <larry.zeitlin@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
> **Subject:** RE: ReVAMPP Bunyavirales Project
>
> A ways to go on the text, but studies mapped out fairly well at this point, working on filling in text for areas highlighted in blue. Sending now to give the group an idea on direction for aims and subaims
>
> Will work on fleshing out significance/innovation sections/and approach text tomorrow. Tables will be shrunk to fit, but followed Larry's color scheme to be consistent for now.
>
> - Bonnie I adapted the immunology support sections from our pilot grant to start with. Will very likely need to majorly consolidate, but better to have too much and refine to essentials once all the content is there.
>
> - Larry, I just included the pilot study for the PREP study assuming you and Tom already budgeted for it. You mentioned go-no-go, but I did not budget for any "go" studies, so maybe you mean PREP for henipaviruses?
>
> - Dafna and Larry, open to thoughts on the CCHF proteins for prelimary data or innovation. I had some serology which we did using your proteins on human survivors to demonstrate reactivity which I think is helpful. Open to other thoughts.
>
> - Tom, I added another group for each of the NHP studies to allow for 2 groups to be evaluated either with different doses or different vax formulations. Was thinking this might be ideal for grantsmanship to have at least another option. Thinking was that we would not use all 5 controls for all studies and may be able to make these up?
>
> Bob

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Wednesday, May 17, 2023 2:51 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Bob,

This is what is in Core D so far:

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine. In conjunction with RP1 and Core E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by Abelson, Geisbert and Zeitlin will be produced with YTE and LA Fc point mutations (Table 4). The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (Bioqual, Inc.; Rockville, MD) to select a lead candidate for testing in NHPs against Junin Romero challenge in Core E. For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference). The variant with the most favorable PK will be selected for testing by **RP1/Core E**. Briefly, groups of 4 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV (Espindola or Romero?) delivered IV. For further details of the NHP testing, please see **Core E**. These data will be used by the Advisory Committee to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes.

Feel free to tweak so it fits with what you are thinking about. I can also give you some innovation language if you'd like on this topic.

Best
LZ

From: Cross, Robert W. <rwcross@UTMB.EDU>
Date: Wednesday, May 17, 2023 at 3:47 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Gunn, Bronwyn

Mei <bronwyn.gunn@wsu.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

I'm trying to shoe horn the prophy into RP1, will ciruculate to the group as a whole shortly. If we add this PEP study into RP1, we will probably need to renaim RP1 to something like: **Novel Vaccine Approaches for Emerging Arenaviruses and Nairoviruses: (Cross, Abelson, Gunn)**

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Saturday, May 13, 2023 11:25 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Cross, Robert W. <rwcross@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Tom,

I like the idea of it as a sub-aim. Core D will refer to it (manufacturing the mAb as well as performing PK to select the best Fc mutant). Depending on how much you are mentioning about the protocols in the Animal Core, you could also mention it there (e.g. "For studies looking at long-acting mAbs as an alternative to a vaccine, NHPs will receive a single dose 1 month prior to challenge…).

I can also if preferred, describe the study in Core D rather than RP1. I'm certainly setting up the idea of it with the PK work…

Best
LZ

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Date: Saturday, May 13, 2023 at 12:15 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Larry Zeitlin <larry.zeitlin@mappbio.com>, Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Larry, Dafna

How do you want to handle the Junin NHP prophylaxis study? At the very least I think RP1 has to mention it somewhere. Does it go into RP1 as a subaim? I don't think we can say it is alternate strategy as that route is usually taken when something proposed fails. Does it get pitched as a subaim to derisk the project? Not quite sure here.

Many thanks!

Tom

From: Cross, Robert W. <rwcross@UTMB.EDU>
Sent: Saturday, May 13, 2023 11:10 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Great, thanks!

Yeah it was challenging to add a lot into the SA page. But we can refine to make it more inclusive of all points addressed. Definitely will stretch out in the Research strategy.

We can maybe add some reverse genetics, especially for missing MACV lineages…maybe CCHF for the same reason using the cassette approach. Need to think about it a little today, but this may work.

Do we need to add the JUNV prophy study into RP1? Or is that in the core?

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Saturday, May 13, 2023 9:38 AM
To: Cross, Robert W. <rwcross@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Thanks Bob! One thing that we need to think about as Chris outlined for the rescue of Langya in the RP2 Aims is are we going to tackle reverse genetics at all for arenaviruses and CCHF? If so will need to tie that into the Animal Core in terms of rescue. Other thing is tie into Mapp Core for the Junin NHP prophylaxis study in Year 1 as an alternative approach to vaccination. And then need to think about if we need an approach to tie into RP4 and RP5. All of this of course does not need to be on the Aims page and can be addressed in the 12 page Research Strategy. If you tackle reverse genetics it could be its own Aim along the lines of what Chris has in RP2.

From: Cross, Robert W. <rwcross@UTMB.EDU>
Sent: Friday, May 12, 2023 3:52 PM
To: Dafna Abelson <dafna.abelson@mappbio.com>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Here is the first attempt at the specific aims page. Kept it simple to start.

From: Dafna Abelson <dafna.abelson@mappbio.com>
Sent: Thursday, May 4, 2023 1:31 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>; Cross, Robert W. <rwcross@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Hi Bob

Here's a draft of an SBIR that Crystal wrote in case that's helpful. It only has data from the first Guinea pig study.

Best,

Dafna

From: Larry Zeitlin <larry.zeitlin@mappbio.com>

Date: Thursday, May 4, 2023 at 11:18 AM
To: Cross, Robert W. <rwcross@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Bob,

Please have at it and let us know how we can help.

Best

LZ

From: Cross, Robert W. <rwcross@UTMB.EDU>
Date: Thursday, May 4, 2023 at 2:07 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, Dafna Abelson <dafna.abelson@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: RE: ReVAMPP Bunyavirales Project

Agree, this SA page from Crystal is super helpful. Was going to start putting pen to paper to get a rough draft started for the Bunya RP. Unless you guys have already started?

Is there a format we need to follow for writing the proposal? I think we had 12 pages correct?

Assuming the standard NIH format?

- Significance
- Innovation
- Preliminary data
- Approach/SpecificAim 1-?
- Alternative strategies
- Milestones-Timelines
- Summary statement impact

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Tuesday, April 18, 2023 3:43 PM

To: Dafna Abelson <dafna.abelson@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Oh, and here is the specific aims page of Crystal's proposal from a few years ago…looks like it would fit pretty well

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From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Date: Tuesday, April 18, 2023 at 4:34 PM
To: Dafna Abelson <dafna.abelson@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Just a few thoughts:

1. It looks like we can have multiple prototypes per family if justified. I think lassa and junin would be great prototypes for old and new world arenas…seems easy to justify as we don't know if a single product could cover both and pursuing both could help to answer the question.
2. Might be good to confirm with program, but as I initially read it, it looks like if we start with CCHF we'd be expected in Y4-Y5 to expand to other nairos. Hantas may be a little easier – Jim has some mAbs and Mapp does as well, but those are encumbered a bit with Kartik's CETR so we'd have to have some conversations to make sure that would be kosher.
3. The RFA has a bit of mixed message on vaccines and mAbs. I do think they go well together and mAbs can be an alternative option to a vaccine.
4. Do the microneedle people have some experience transitioning to the clinic? I'm looking at this language: "Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine expertise in manufacturing, clinical development, and regulatory pathways." Mapp can fill that role for mAb vaccines, but not so well for traditional vaccines. It looks like we don't have to have them lined up yet, but might be nice to have a letter of support if needed. DVC is an option as is IBT.

From: Dafna Abelson <dafna.abelson@mappbio.com>
Date: Tuesday, April 18, 2023 at 4:15 PM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Cross, Robert W. <rwcross@UTMB.EDU>, Larry Zeitlin <larry.zeitlin@mappbio.com>
Subject: Re: ReVAMPP Bunyavirales Project

Hi Tom

We do have the Junin PBMCs you sent and I like the idea of including Junin in the proposal because we already have cell lines in house to express Machupo, Junin and Lassa GPs. I think we could hit the ground running on arenavirus antibody discovery.

I don't have strong feelings on Hanta vs CCHF. Larry?

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Date: Tuesday, April 18, 2023 at 12:53 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>, Larry Zeitlin <larry.zeitlin@mappbio.com>, Dafna Abelson <dafna.abelson@mappbio.com>
Subject: ReVAMPP Bunyavirales Project

All,

We need to think about the ReVAMPP Bunyavirales Project and what viruses and how to integrate with all of the other projects. I know it is tough to schedule a Zoom call so maybe we can kick this around on email a little. I think we all agree that the Mapp Lassa vaccine data is great. So maybe Lassa is the prototype even though we know CEPI is funding Lassa vaccines and it has a lot more attention than other things. But prototype with a plan to transition to other arenaviruses. So move the Lassa vaccine to the microneedle patch which ties in nicely with that Core. I guess we need to think about whether to have pan arenavirus vaccines or individual. I think Junin is a good choice as well. To that end I think we had sent PBMC from Junin Candid 1 vaccinees to Mapp right before COVID-19 hit. Do you still have those? If we go with Lassa do we have a plan for Lassa MAbs to support? Then for another family I think CCHF for Nairoviridae makes the most sense as we have already been working on it for MAbs as has Jim Crowe. We would need to sort out the vaccine angle that ties into the microneedle patch. So I guess then for arenaviruses we would have Lassa and maybe Junin as prototypes and for nairoviruses CCHFV. Where I get confused and am struggling is the transition in Years 4 and 5 that NIAID/NIH has to approve where I think the expectation is that you move from prototypes to more broad coverage. So do we say for example for Lassa since there are so many lineages that the prototype is lineage IV Josiah but in the out years we show that it protects against all lineages? Same thing for CCHF. Or is the expectation that you go beyond the prototype virus so for example from Lassa and Junin to also cover say Machupo, Guanarito, Chapare, etc.? Or is it better instead of CCHF say to start with Andes as the prototype and then move in the out years to Hantaan

> or vice versa? Thoughts?
>
> Tom

Research Strategy

Significance

The constant threat posed to global public health by emerging and reemerging zoonotic viruses emphasizes the importance for pandemic preparedness by developing countermeasures. The highly pathogenic Hendra virus (HeV) and Nipah virus (NiV) are the prototype members in the genus *Henipavirus* in the family Paramyxoviridae[1], and the selected prototypes chosen for **RP2**. HeV was first discovered in 1994, in Queensland, Australia where it was the causative agent of a severe and fatal respiratory disease among horses along with a human case fatality[1,2]. Whereas, in 1998-1999 in Malaysia and Singapore an outbreak of encephalitis in people and respiratory disease in pigs was eventually determined to be caused by a closely related virus - to NiV[3,4]. These viruses are capable of causing a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with fatality rates ranging from 40-100%[5,6]. As a result of this high lethality and lack of approved vaccines or antivirals, HeV and NiV are classified as Biosafety Level-4 (BSL-4) pathogens. NiV and henipaviral diseases are also included in the WHO's Blueprint List of Priority Pathogens[7], and NiV is among the Coalition for Epidemic Preparedness Innovations (CEPI) list of Priority Diseases needing urgent research and countermeasure development and are recognized as agents of pandemic threat potential[8,9]. HeV, though apparently restricted to Australia, has spilled over 66 times, most recently in 2022[10]. HeV has caused the death or euthanasia of >100 horses, 2 HeV positive and euthanized dogs, and 4 fatalities of 7 human cases[10,11]. Whereas NiV is present throughout a large area including South and Southeast Asia from the Philippines to western India. The frequent outbreaks of NiV in Bangladesh and India have high human case fatality rates 70-100%. NiV transmission in Bangladesh to humans has been linked to contaminated fruits or date palm sap, and person-to-person transmission[12-15]. In total, there have been >620 human cases of NiV infections >330 fatalities, the most recent in 2023[16]. The genus *Henipavirus* now includes nine other species; the four viral isolates of CedV, Gamak virus, Daeryong virus, and Langya virus (LayV)[17-19] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiang virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[20-23]. Pteropid fruit bats, known as flying foxes, are recognized natural reservoir hosts for NiV, HeV[24,25], CedV[18], GhV and AngV. One of these, CedV, has been shown to be nonpathogenic in several animals susceptible to HeV and NiV disease including mice, guinea pigs and ferrets[18,26] and African green monkeys (AGMs) (Geisbert and Broder, unpublished). The remaining six reported henipaviruses (HNVs) are likely of rodent origins. One of these six, the isolate LayV, is associated with nonfatal febrile illness in humans[19].

HNVs are enveloped, single-stranded, negative sense RNA viruses that share a similar genomic makeup. The viruses have two membrane anchored glycoproteins involved in entry: the attachment (G) glycoprotein that engages the host cell receptor and the fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F proteins are the major targets of neutralizing antibodies and the focus of all vaccine and therapeutic antibody strategies[27,28]. However, CedV is distinct from other HNVs, and the phosphoprotein (*P*) gene does not encode the interferon antagonist proteins V and W[18,29-31]. The nonpathogenic phenotype of CedV is most likely attributed to the lack of these nonstructural proteins. Based on genetic data, all identified HNVs, except CedV, can potentially express the V and W proteins. CedV is recognized as a BSL-2 restricted agent[32].

Innovation

There are multiple layers of innovation within **RP2**. **First**, the proposed development of henipavirus (HNV) subunit vaccine candidates using an innovative microneedle array patch (MNP) technology that may be suitable for the incorporation of immunogens derived from other undiscovered pathogenic HNVs or paramyxoviruses in the future. **Second**, the construction of a rLayV reverse genetics system will be highly valuable in the HNV toolbox to study putative differences in pathogenesis and vaccine design between HNVs of bat and rodent origin, and significant resource to the ReVAMPP network's objectives. **Third**, generation of BSL-2 surrogate platforms that are based on rCedV and VSV will allow for a wide-variety of *in vitro* studies to support infection and host response studies and to screen in a high throughput manner the neutralization activity of immune sera and monoclonal antibodies (mAbs) and nanobodies (nabs) without the requirement for high containment. **Fourth**, considering that the LayV and AngV F and G proteins are functionally fusion active, the generation of viable rCedV chimeras bearing either LayV or AngV F and G glycoproteins is feasible.

Approach

Scientific Premise

To date, the most extensively studied NiV/HeV vaccine is a soluble, recombinant, oligomeric form (dimers and/or tetramers) of the G glycoprotein of HeV (HeV-sG)[11,27,33], and has been demonstrated to confer complete protection against lethal challenge with NiV-M (Malaysia), NiV-B (Bangladesh) or HeV in multiple animal models[34-40]. It has most recently been shown to provide complete protection of AGMs against NiV-B challenge performed one week post-immunization (single dose)[41]. The HeV-sG vaccine is marketed by Zoetis, Inc., for equine use in Australia since 2012 and is the first commercialized vaccine against any BSL-4 pathogen. HeV-sG /Alum intramuscular formulation is currently in clinical trial as an anti-NiV vaccine. **RP2** proposes to enhance the formulation, immunogenicity, and delivery of recombinant subunit protein based HNV vaccines.

We have developed rCedVs as new tools to study HNV host cell interactions, including ephrin receptor use and infection tropism, and in the development of HNV countermeasures. Experiments with rCedV can be conducted in a BSL-2 containment setting. The rCedV reverse genetics system[42,43] allowed us to develop a rCedV chimeric platform, where the CedV F and G glycoproteins are replaced with that of either NiV-B or with HeV, generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV). We have successfully rescued and characterized the chimeric viruses with and without reporter genes[44]. The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G specific mAbs against these rCedV chimeras highly correlate with the potencies obtained for the same antibodies to neutralize authentic NiV-B and HeV by plaque reduction neutralization tests (PRNTs). The green fluorescent protein (GFP) reporter chimeras were employed to develop a rapid, high-throughput, and quantitative fluorescence reduction neutralization test (FRNT). The antibody potencies to neutralize the reporter gene containing chimeras in the FRNT highly corelated with the respective potencies obtained by PRNT[44]. The rCedV chimera assay has been employed in several studies and its high-throughput ability has also allowed for the conduct of NiV/HeV mAb synergy neutralization tests[45-47]. The rCedV chimeras have their most important application in an authentic HNV-based surrogate neutralization assay that is rapid, cost-effective, and can be utilized in BSL-2 containment. We believe that the rCedV reverse genetics system may be able to serve as a plug-and-play platform for other emerging or pathogenic HNVs. To accomplish this, **RP2** will develop a variety of rCedV variants with reporter genes that will serve as important tools for the PABVAX Center RPs and Cores, and also for the NIAID, ReVAMPP network for pandemic preparedness.

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed. rCedV laboratory manipulation guidelines and standard operating procedures under BSL-2 conditions were developed for the laboratory and staff, and all procedures were reviewed and approved by the Uniformed Services University Institutional Biosafety Committee in accordance with NIH guidelines. The rCedV tools described here have been transferred to multiple institutions and research organizations which have also reviewed and approved their use at BSL-2 containment.

Aim 1: Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV subunit vaccines.

Rationale and Preliminary Data. It is well documented that the HNV G and F glycoproteins are the primary targets for virus neutralizing antibodies[27,45,48-53]. As such, HeV-sG subunit has clinically advanced in the development of HeV and NiV vaccines. Building on this work, Aim 1 will compare the immunogenicity and efficacy of alternate NiV vaccine immunogens (NiV-sG_{tet}, NiV-sF_{stb}), HeV vaccine immunogen (HeV-sF_{stb}), and a bivalent HNV vaccine candidate (NiV-sF_{stb}+HeV-sG) along with the clinical subunit HeV-sG. All immunogens will be delivered as polyphasphazene (PPZ) adjuvanted MNP vaccines in three animal species (mice, ferrets, and non-human primates-NHPs (AGMs)). Immunogenicity will initially be performed in mice by **Core C**. The neutralizing potencies of the immunized mouse sera will then be evaluated by *in vitro* neutralization assays using rCedV-NiV-B-GFP and rCedV-HeV-GFP[44]. The superior neutralizing antibody inducing MNP vaccine type(s) will be evaluated by animal challenge studies ferrets (first-tier) challenged with NiV-B or HeV and the lead vaccine candidate(s) from those studies will then be evaluated in AGM (second-tier) challenge studies.

Adjuvants are included in vaccine designs to generate a robust and durable vaccine induced immune response. We will utilize a synthetic PPZ macromolecule as the adjuvant to complement the proposed NiV and HeV vaccine immunogens. The PPZ macromolecule has many distinctive properties that make it an attractive choice as an adjuvant; 1) water-soluble and biodegradable, 2) spontaneously self-assembles with antigens in aqueous solutions, 3) forms nanoassemblies that mimick the dimensions of a virus (60-100nm)[54,55] and 4) is completely compatible with MNPs[56-58], the delivery mechanism that will be employed for the HNV immunogens. The extensive clinical use and experimental evidence supports the safety and potency of PPZ in enhancing vaccine-induced protective immune responses[54-60]. In particular, the most commonly used PPZ adjuvant is the clinical stage *poly[di(carboxylato-phenoxy)phosphazene] (PCPP)* and its structural homologue *poly[di(carboxylato-ethylphenoxy) phosphazene] (PCEP)*, which, significantly boosts vaccine immunogenicity without the requirement for large amounts of antigen while providing long-lasting protection in live viral challenge studies[60-62]. MNPs are promising resources to achieve systemic effects by transdermal delivery. They are a minimally invasive, painless system, which can bypass the stratum corneum and overcome the potential drawbacks of subcutaneous injections[63]. (See **Core C** Research Plan).

Manufacturing and characterization of HeV-sG±PCEP MNPs. The technology for fabricating MNPs pioneered by Bediz et al.,[64] was developed and is described in detail in the **Core C** Research Plan). We have conducted preliminary studies with the dissolvable MNPs incorporating HeV-sG antigen with or without PPZ adjuvants including PCEP (HeV-sG±PCEP MNPs) in collaboration with the investigators of **Core C**. Spin-casting of carboxymethyl cellulose and trehalose was used to create MNPs incorporating HeV-sG AF555±PCEP into obelisk-shaped microneedles having the vaccine components at the tip region to maximize the vaccine delivery efficiency by locating the vaccine components at the skin-penetrating regions of the microneedles (**Figure 1a**). The HeV-sG±PCEP MNPs can have a pre-determined array size of 10x10mm (for mice studies) and 20x20mm or greater (for larger animal studies). Images of the HeV-sG AF555±PCEP MNPs before (**Figure 1a**) and after (**Figure 1b**) application (15-20 mins), confirm a nearly complete dissolution of the microneedles into the skin demonstrating that the vaccine was effectively deposited into the skin. To facilitate the characterization and to visualize skin penetration of the developed HeV-sG±PCEP MNPs, HeV-sG was fluorescently labeled with Alexa Fluor 555 dye, HeV-sG AF555. To test the intracutaneous delivery capacity, HeV-sG AF555±PCEP MNPs were applied to the abdomen skin of C57BL/6 mice for 15 min and then removed. The HeV-sG AF555±PCEP MNP-treated mice were imaged before and after *in vivo* skin applications by using an *in vivo* live animal fluorescent imaging system (IVIS Lumina XR) and the acquired images were post-processed using Living Image software (PerkinElmer). **Figures 1c** and **1d** further corroborate the microscopy analysis of these MNPs and show effective deposition of vaccine components to murine skin (**Figure 1c**: HeV-sG AF555 and **Figure 1d**: HeV-sG AF555+PCEP). Together, these data support the feasibility of our PPZ MNP vaccine platform for the translational development of skin-targeted HNV vaccines that could also be employed using recombinant protein immunogens (or even virus-like particles) from other viral agents from a wide-variety of virus families.

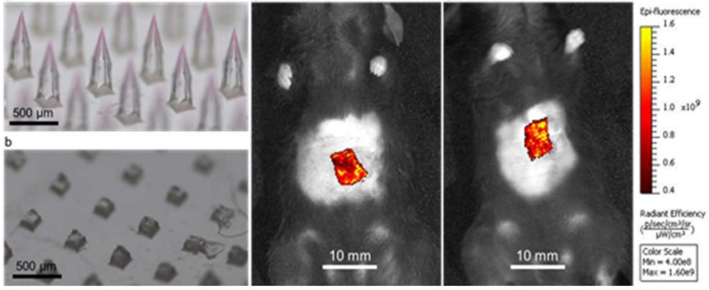


Figure 1: *In vivo* intracutaneous delivery efficacy of HeV-sG±PCEP MNPs in mice. An optical stereomicroscopy image of HeV-sG AF555+PCEP CMC/Trehalose microneedles before (**a**) and after (**b**) *in vivo* skin application. Administration of HeV-sG AF555 **(c)** and HeV-sG AF555+PCEP **(d)** to mouse skin *in vivo* with MNPs, captured by using a fluorescence *in vivo* imaging system (IVIS).

HeV-sG±PCEP MNPs with unlabeled HeV-sG were fabricated and the immunogenicity tested in C57BL/6 mice. Groups of 5 mice were immunized on Day 0 (D0) (prime) and D21 (boost) with HeV-sG±PCEP MNPs. Sera were collected on D35 and analyzed by ELISA for total binding antibodies (endpoint titers) (**Figure 2a**) and HNV neutralizing capacity of serum in the rCedV chimeras FRNT[44] (**Figure 2b**). These results show that MNPs are highly effective in inducing antigen-specific binding and neutralization antibodies, and improved with PCEP adjuvant. Additional data also demonstrate that the PPZ MNP platforms are superior to intramuscular injection of the current clinical Alum-formulated HeV-sG vaccine (**Core C; Figure 4a**). In addition, temperature stability was tested by storing the HeV-sG±PCEP MNPs at room temperature without refrigeration. Mice were immunized with fresh (group 1) and 30-day old MNP (group 2) by single dose immunization. Mice were bled on D14 post immunization and the

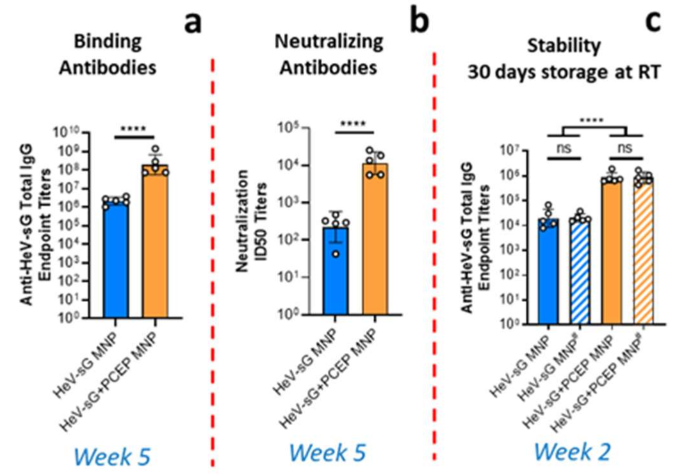


Figure 2: MNPs induced humoral responses and initial MNP stability data. Mice immunized prime/boost. MNPs applied to the abdominal skin of C57BL/6 mice 15 min, D0 (prime) and D21 (boost). Endpoint binding titers **(a)** ELISA. Neutralizing ID_{50} titers **(b)** by FRNT. Stability of MNPs **(c)** measured at D14 endpoint binding titers using a 30-day-old MNP compared to fresh MNP. Neutralizing ID_{50} titers with HeV-sG MNP (1:272) and HeV-sG+PCEP MNP (1:11,445) against rCedV-HeV-GFP.

endpoint titers of total IgG levels were determined by ELISA. **Figure 2c** suggests that MNP-embedded vaccine components retain their immunogenicity for at least a month without refrigeration with no statistically significant differences in the antibody binding titers between fresh and the 30-day-old MNPs.

Experimental Design.

Aim 1.1: Manufacturing and characterization of PPZ MNPs with HNV glycoprotein antigens. We have tested GLP-like HeV-sG antigen (above), manufactured by Catalent Pharma Solutions, and it is currently available. The remaining 3 immunogens (NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb}$) will be prepared by Genovac, Inc., and **Core C** will prepare MNPs with each of NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb}$, HeV-sG+NiV-sF$_{stb}$ and HeV-sG, all adjuvanted with PCEP. Each fabricated batch of PPZ MNPs will be tested *in vitro* for geometric integrity, mechanical integrity, vaccine loading accuracy and reproducibility, biological integrity, endotoxin content, vaccine stability, and *in vivo* skin delivery performance before the animal immunization studies. The specific quantitative release criteria are as follows: 1) geometric integrity and accuracy – >95% high-quality microneedles across a PPZ MNP vaccine and <10% deviation from the target geometric values; 2) structural integrity – factor of safety (FOS) > 2 (FOS is equal to the failure forces/penetration forces); 3) vaccine loading accuracy and reproducibility – 10% deviation from the mean of theoretical amounts and <10% standard deviation of the mean value; 4) biological integrity – no significant aggregation of adjuvant-antigen complexes and no significant loss of antigenicity ($p>0.05$); 5) endotoxin content – < 1 endotoxin unit per PPZ MNP vaccine; 6) thermostability – biological integrity at elevated temperatures; 7) skin delivery performance – <10% of remaining materials in terms volume and <15% of remaining materials in terms of vaccine after 20 min murine skin application *in vivo* and 20 min the RP-specific animal-skin application *ex vivo* (See **Core C** application for more details). Based on the preliminary data and experience with fabricating HeV-sG MNP, we expect the technology to be plug and play applicable with the new HNV immunogens and with the combination of two different HNV immunogens.

Aim 1.2: Immunogenicity assessment HeV/NiV antigen PPZ MNPs in mice. To evaluate the immunogenicity of PCEP MNP platform with NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb,}$ HeV-sG, and HeV-sG+NiV-sF$_{stb}$, murine immunization studies will be conducted (**Core C)**. The immunization strategy will be performed as single-dose (D0) and prime(D0)-boost(D21) with female C57BL/6 mice (n = 5 mice per group). A schematic of the immunization and bleeding schedule is shown in **Figure 3**. Naïve or unimmunized mice will serve as negative controls and mice immunized with the intramuscular injection of Alum-complexed antigens will serve as positive controls. Serum levels of antigen-specific antibodies (IgM, total IgG, IgG1, IgG2c, and IgA) will be assayed by ELISA. The sera will be tested for neutralization activity using the respective reporter rCedV chimeras – rCedV-HeV-GFP and rCedV-NiV-B-GFP in the FRNT assay[44].

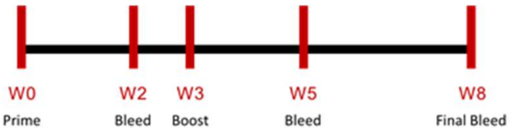


Figure 3. Immunization - bleeding schedule for mouse immunogenicity study.

Cellular immune responses will also be evaluated as described in the **Core C** Research Strategy. Briefly, mice will be sacrificed five days after the booster dose. Mouse blood PBMCs and cells from lymph nodes and spleen will be stimulated with a pool of antigenic peptides derived from the respective immunogen sequence(s). Antigen-specific polyfunctional cellular responses (IFN-γ, TNF-α, IL-2, IL-4, IL-17: CD4+ and CD8+ T-cell) will be determined by using multi-color flow cytometry and intracellular cytokine staining[65,66]. Studying the cellular responses for HNV immunogens has recently gained more interest and preliminary mouse studies have just begun using the HeV-sG vaccine subunit – antigenic peptides have been identified.

Aim 1.3: Evaluation of the immunogenicity and protection efficacy of PPZ MNP HNV vaccines in ferrets. The immunogenicity and efficacy of the lead PPZ MNP HNV vaccine candidates defined in Aim 1.2 will be tested in ferret challenge studies in Year 2 of the project (**Core E)**. Ferrets (n = 6 ferrets per group) will be immunized with the lead PPZ MNP HNV vaccine candidates via either single-dose (D0) or prime(D0)-boost(D21) immunization regimen. Unimmunized ferrets will serve as negative controls. (*Group 1*: Lead PPZ MNP HNV vaccine candidate 1 with the prime-boost regimen**.** *Group 2*: Lead PPZ MNP HNV vaccine candidate 2 with the prime-boost regimen. *Group 3*: Lead PPZ MNP HNV vaccine candidate 1 with the single-dose regimen. *Group 4*: Lead PPZ MNP HNV vaccine candidate 2 with the single-dose regimen. *Group 5*: Naïve/Unimmunized controls). The five groups will be challenged with a lethal dose (5000 TCID$_{50}$) of either NiV-B or HeV. Immunization, challenge and blood and swab sampling schedules are diagrammed in **Figure 4**. Vaccine-induced humoral responses in ferrets prior to virus challenge will be evaluated by **Core E** and **RP2**. All protected animals and survived controls will be euthanized on day 21 post-infection challenge (end of study) and lung and brain tissue along with swabs (oral and rectal) and blood samples will be collected. These will be tested for the presence of viral RNA by TaqMan PCR performed by using the primer sets and probes[35]. Virus isolation will only be attempted from specimens that were PCR positive for NiV-B or HeV genomes. Virus titration will be performed by plaque assay on Vero cells. The limit for detection will be 25 pfu/ml. The experimental details of the challenge studies in ferrets are described in **Core E**. End of study sera neutralization titers will also be measured by FRNT.

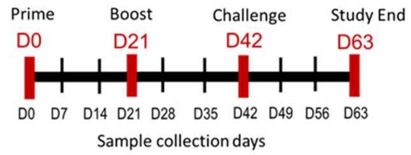


Figure 4. Expected schedule to immunize ferrets and AGMs in the planned efficacy studies. Blood and swabs, oral and rectal, samples will be taken weekly, on days 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63. Single dose groups do not receive boost immunization on D21.

Antigen-specific stimulation of cytokine production by ferret PBMCs will be assayed. The custom-synthesized antigen-specific Peptivators obtained from **Core C** will be compatible with these studies, but we acknowledge that there is a lack of ferret-specific reagents for comprehensive evaluation of cytokine production of peptivator-stimulated PBMCs. Unlike comprehensive studies in mice and AGMs, these studies will quantify the production of cytokines, for which ELISA kits are commercially available, such as ferret IL-17A, IFN-γ, TNF-α, and IL-2, 48 hr after stimulation (See, e.g.,[67]).

Aim 1.4: Immunogenicity and protection efficacy of the lead PPZ MNP HNV vaccine in AGMs. This challenge study will be in year 3 (**Core E**). The immunogen(s) and MNP vaccine type that provides the best cross-protection in the ferret studies will be tested in AGM NiV-B or HeV challenge studies. Fresh MNPs will be fabricated for this study. The study will be conducted by **Core E** and will entail four groups of AGMs (n = 3 AGMs per group). Immunizations with the lead PPZ MNP HNV vaccine candidate will be either as a single-dose (D0) or prime(D0)-boost(D21) regimen. Groups 1 and 2 will be immunized as a prime-boost strategy while groups 3 and 4 will be immunized with a single dose. Naïve unimmunized AGMs will serve as negative controls. The immunization, challenge and blood and swab sampling schedules will follow that as outlined in **Figure 4**. Groups 1 and 3 will be challenged with a lethal dose of NiV-B, while groups 2 and 4 will be challenged with a lethal dose of HeV (lethal dose = $1x10^5$ $TCID_{50}$). Sera from blood samples taken before the challenge will be tested by **RP2** to measure the serum neutralization titers using the same procedure as for mice and ferret sera. Antigen-specific total IgG levels will be determined by commercially available ELISA. Virus-specific neutralization activity against each of HeV and NiV-B will be tested by FRNT assay in the same way as proposed for mice and ferret sera samples. The AGM sera samples will also be tested for neutralization activity against authentic viruses (**Core E**) by using the BSL-4 PRNT assay. Antigen-specific stimulation of key cytokine production by AGM PBMCs, which will be obtained prior to live virus challenge studies, and will also be assessed as described in Aim 1.2.

Aim 1.5: Identifying the correlates of immunization with the lead PPZ MNP HNV vaccine across multiple species. Here we propose to determine the correlates of immunization with the lead PPZ MNP HNV vaccine candidate across multiple species – mice, ferrets, and AGMs. This aim will be inherently integrated with the Aims 1.2-1.4. During these experiments, 3hr post immunization, skin biopsies and serum samples will be obtained from mice, ferrets and AGMs. Prior to challenge studies in AGMs, serum and skin samples from the three different species (mice, ferrets, and AGMs) immunized with the lead PPZ MNP vaccine candidate will be assayed to quantify their cytokine levels (IL-6, TNF-α, IL-17A, IL-10, IL-1β, IL-6, IL-4, IL-5, TNF-α, IFN-α, IFN-β, IFN-γ, and IL-12p70). We note a limitation that while the multiplex assays are commercially available for mice and AGMs, we will have to use the cytokine-specific ELISAs for ferrets to determine only those cytokine levels for which ELISA kits are available, such as ferret IL-17A, IFN-γ, and TNF-α. We expect to identify within the obtained results correlates of immunization across the three species for the lead PPZ MNP vaccine candidate. We also expect that these correlates of immunization might be predictive for the vaccine protective efficacy.

Aim 1.6: Evaluate stability of the lead PPZ MNP HNV vaccine candidate. Here, we will test the thermostability of the lead PPZ MNP HNV vaccine candidates based on retention of their immunogenicity upon storage at room temperature and at 40°C for an extended period of time (1-, 3-, 6-, and 12-month storage). Mice (n = 5 mice per group) will be immunized with fresh and stored PPZ MNP HNV vaccines in a single-dose regimen and blood samples will be collected 2 weeks after immunization. To test the stability of PPZ MNP HNV vaccine candidates, serum levels of total IgG antibodies will be determined by **Core C** and the virus-specific neutralization capacity of these antibodies will be evaluated by the FRNT assay (**RP2**). Stability will be indicated by no statistically significant differences between binding antibody levels and neutralization titers induced by fresh PPZ MNP HNV vaccines compared to those elicited by stored PPZ MNP HNV vaccines. Additional stability testing will be initiated with the freshly manufactured MNP for the AGM challenge study.

Expected results, potential pitfalls and alternatives. Prior data on HNV vaccines support our expectations that we will be able to assess the potency and longevity of vaccine induced responses of lead PPZ MNP HNV vaccine candidates from the proposed studies. The HeV-sG immunogen has been tested as a HNV vaccine in multiple (2-dose prime-boost) prior studies and that protective pre-challenge ID_{50} neutralizing titers against NiV-

M can be as low as of 1:32 in the feline model[35,38]; that more than 1 year post-vaccination and pre-challenge neutralizing titers as low as 1:16 to 1:128 in the ferret model are protective against NiV-M[40]; as an equine vaccine, horses with pre-challenge HeV neutralizing titers ranging from 1:16 to 1:32 at 6 months post-vaccination are completely protected[36]; and in AGMs pre-challenge ID_{50} neutralizing titers ranging from 1:28 to 1:379 against NiV-M are completely protective[34]; and most recently, a HeV-sG/Alum formulation protects against NiV-B challenge in AGMs with pre-challenge ID_{50} titers ranging from only 1:10 to 1:160[41]. Almost without exception, complete protection from challenge in these studies also shows no gross or histologic evidence of infection in any of the vaccinated subjects at study completions, with all tissues examined negative for viral antigen, with no viral genome detected. We expect to obtain protection in AGMs with the lead PPZ MNP HNV vaccine candidate. From the stability study, we will determine the shelf-life of the lead PPZ MNP HNV vaccine candidate. We are confident that if the preliminary mouse immunogenicity studies reveal neutralizing antibody responses are robust, this will translate to protection in the planned challenge studies in ferrets. Indeed, preliminary data has shown robust neutralizing ID_{50} titers from the mouse immunizations. The non-adjuvanted HeV-sG MNP induced an ID_{50} titer of 1:272 and the adjuvanted form HeV-sG+PCEP MNP induced an ID_{50} titer of 1:11,445 against the HeV chimera; and an ID_{50} titer of 1:331 against the NiV chimera. There is a minimal risk of not finding any protecting immunogen (MNP vaccine type or immunogen combination). The PABVAX Center team is sufficiently experienced to handle and resolve any possible unforeseen issues and pitfalls.

<u>Aim 2:</u> Rescue Langya virus and construct LayV and AngV glycoprotein bearing reporter virus tools.

Rationale and Preliminary Data: Negative stain electron microscopy (EM) was performed on LayV F and G glycoproteins. The 2D and 3D classifications of LayV F (**Figure 5a**) and G (**Figure 5b**) revealed distinct trimer and tetramer assembly, respectively. In addition, using LayV-sF and LayV-sG, our previously generated mAbs to MojV F and G tested by ELISAs demonstrated that there was a dose dependent increase in LayV-sF (**Figure 5c**) and LayV-sG (**Figure 5d**) binding. This data forms the premise for rescuing a rCedV bearing LayV F and G glycoproteins (the sequences from LayV are likely correct). We will construct a full-length recombinant LayV sequence that encodes GFP. The LayV F and G coding sequences are the LayV isolate SDQD_H1801 (GenBank: OM101125.1); LayV F and LayV G; UUV47205.1 and UUV47206.1, respectively. The replication competent rCedV will be used. We will design, construct and rescue a new rCedV chimera that encodes the envelope glycoproteins (F and G glycoproteins) of LayV, i.e., a rCedV-LayV reporter virus encoding GFP. We will characterize it using cell-based infection tropism and growth kinetic assays. We have also utilized an established quantitative fusion assay based on a dual-split-luciferase reporter assay[42,43,68,69] to determine functionality of AngV envelope glycoproteins (F and G glycoproteins). Content mixing between F/G bearing effector cells and a

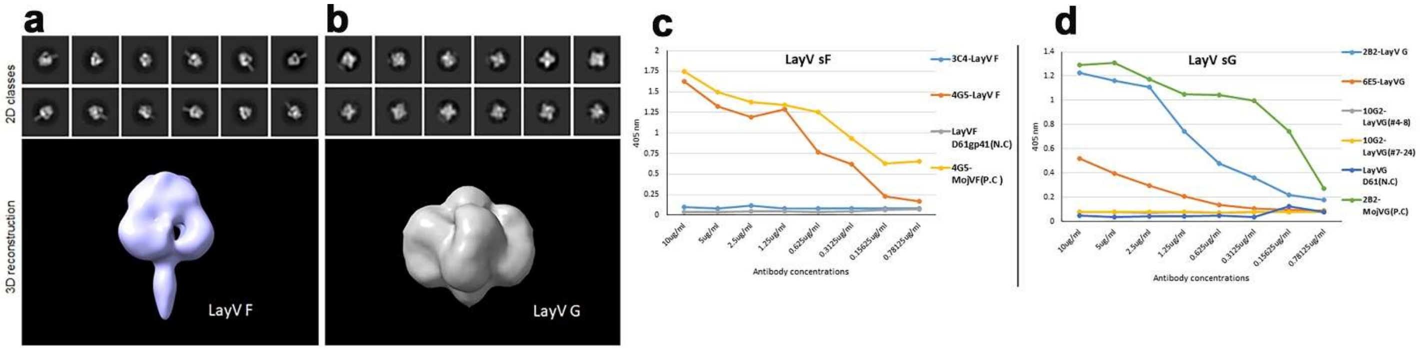


Figure 5. Negative stain EM analysis of LayV sF_{stb} and sG_{tet}. 2D and 3D classifications of LayV F trimers (**a**) and LayV G tetramers (**b**). ELISA cross-reactivity MojV F mAb (**c**, yellow) with LayV-sF and MojV G mAb (**d**, green) with LayV-sG.

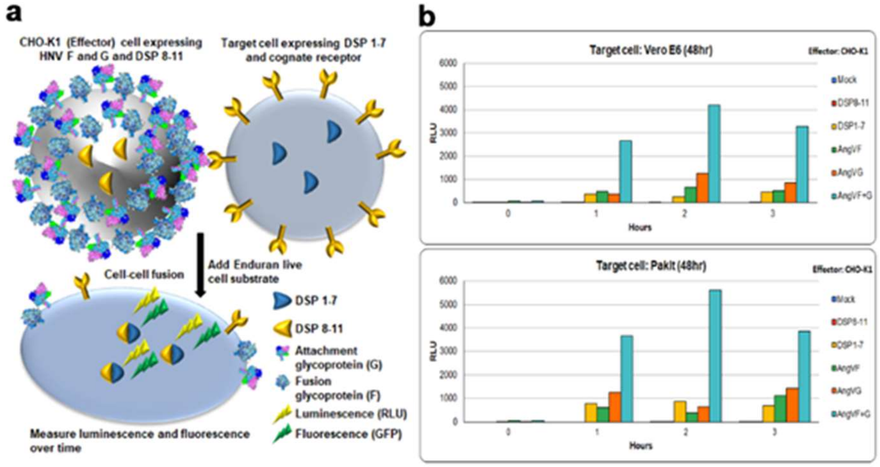


Figure 6. AngV F and G cell-cell fusion using a dual split luciferase. A schematic representation of the dual split-luc cell-cell fusion assay (**a**). Split-luc cell-cell fusion assay with AngV F and G plasmid constructs (**b**).

permissive target cell restores luciferase and a signal can be measured[43] (**Figure 6a**). In addition, we have already constructed AngV sG$_{tet}$ and sF proteins and their structural solutions (both pre-fusion and post-fusion sF and the tetrameric structure of sG$_{tet}$) have been made by the PL of **RP5**, and cell-fusion is observed in Vero E6 cells and the bat kidney cell line, PaKiT, indicating that full-length AngV F/G are functional (**Figure 6b**). Taken together, these data suggest that the rescue of reporter viruses with LayV and AngV F/G is feasible. We will characterize viruses in cell-based infection tropism and growth kinetics assays. Alternatively, we will also construct a set of reporter gene-encoding VSV-based (LayV and AngV F/G bearing) reporter viruses as previously done with Australian bat lyssavirus (ABLV) G glycoproteins[70,71] and authentic ABLV[72,73].

Experimental Design.

Aim 2.1: Design and construct a recombinant Langya virus reporter virus. Figure 7 diagrams each of the proposed virus constructs that will be generated. The genome design, length and position of the green fluorescent protein gene are shown, and the experimental plans are described below. LayV helper plasmids for the N, P, and L genes will be synthesized. To construct the rLayV-GFP antigenome plasmid, large gene fragments of LayV will be synthesized and sequentially cloned into pOLTV5$_{opt}$ (**Figure 7a**). Unique restriction sites will be chosen to facilitate ligation of the LayV DNA fragments and to ensure adherence to the genomic "rule of six". All cloning will be performed with *E.coli* Stbl2 cells. The fully sequenced plasmids will be transferred to **Core E**. Virus rescue and subsequent virus characterization by cell-based assays will be conducted in collaboration with **Core E**. In addition, rLayV will be examined by **Core E** in animal model development studies such including the hamster and/or ferret, both successful for the prototype HNVs.

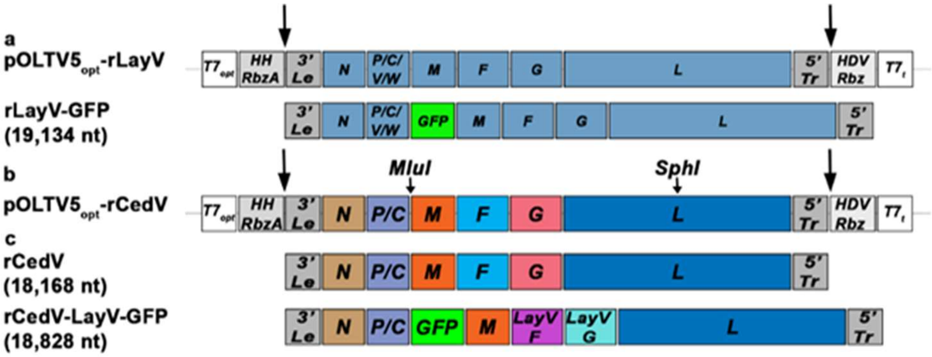


Figure 7. Schematic representation of recombinant reporter viruses. The pOLTV5$_{opt}$-rLayV plasmid (**a** top) and rLayV-GFP genome (**a** bottom). The arrows indicate regions of self-cleavage. The pOLTV5$_{opt}$-rCedV plasmid (**b**). The rLayV-GFP genome (**c** bottom). The long arrows indicate regions of self-cleavage. Shown are the unique restriction sites MluI and SphI that will be used to construct rCedV-LayV-GFP.

Aim 2.2: Construct, rescue and characterize a recombinant Cedar reporter virus encoding Langya virus envelope glycoproteins.

Generation of rCedV encoding LayV envelope glycoproteins. rCedV-LayV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the LayV F and G coding sequences. The CedV non-coding intergenic regions are retained to adhere to the genomic "rule of six". Unique restriction sites, will facilitate the insertion of the LayV F and G gene cassette into the genome clone (pOLTV5$_{opt}$-rCedV) (**Figure 7b**) generating rCedV-LayV-GFP (**Figure 7c**). All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-LayV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (HNV-CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. When maximal syncytia and GFP is observed (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[42-44,74].

***In vitro* characterization of rCedV-LayV-GFP**. The growth kinetics of rCedV-LayV-GFP compared to rCedV or rCedV-NiV/HeV chimeras will be assessed by infecting Vero cells with MOIs of 0.01, 0.1 and 1 and progeny virus recovered in 24hr intervals over a 72hr period and titered. In parallel experiments, infected cells will also be imaged for fluorescence and syncytia (HNV-CPE). **Figure 8** illustrates the syncytia (indicated by the yellow arrows) formed in Vero E6 cells infected with either rCedV-NiV-B-GFP or rCedV-HeV-GFP chimeras. The rCedV-LayV-GFP neutralization assays will be conducted by FRNT[44]. We have also already generated several murine mAbs to LayV F and G (3C4-LY and 4G5-LY to LayV F; and 2B2-LY and 6E5-LY to LayV F) and these will be used in neutralization assays. In addition, we will develop rabbit sera to LayV N, P and L proteins.

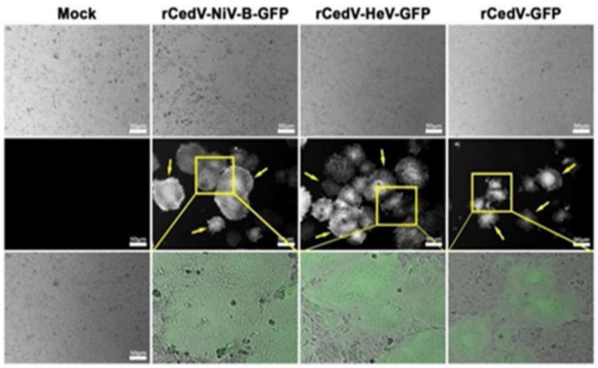


Figure 8. Syncytia induced by rCedV expressing NiV-B or HeV envelope glycoproteins. Vero E6 cells were uninfected (Mock) or infected with either rCedV-NiV-B-GFP, rCedV-HeV-GFP, or rCedV-GFP. All images are 24hrs post-infection. Zoomed-in fluorescence images (3rd row) are the yellow boxes. Arrows indicate giant multinucleated cells (syncytia). Representative images from three independent experiments are shown. Scale bar, 50 µm.

Aim 2.3: Construct, rescue and characterize recombinant reporter viruses encoding Angavokely virus envelope glycoproteins.

Generation of rCedV encoding AngV envelope glycoproteins. Figure 9 diagrams each of the proposed virus constructs that will be prepared. The genome designs, lengths and position of the *GFP* and *Luc* reporter genes are also shown. rCedV-AngV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the AngV F and G coding sequences. The AngV F and G coding sequences will be based on the GenBank: ON613535.1 sequence. The GenBank accession numbers for AngV F and AngV G are UVG43988.1 and UVG43989.1, respectively.

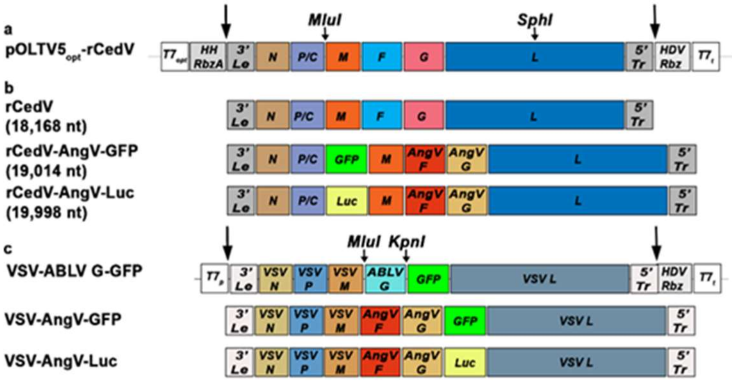


Figure 9. Schematic representation of the AngV chimeric reporter viruses. The pOLTV5$_{opt}$-rCedV plasmid (**a**). Shown are the unique restriction sites MluI and SphI that will be used to construct rCedV-AngV-GFP and rCedV-AngV-Luc. The rCedV-AngV-GFP and rCedV-AngV-Luc genome (**b**). Shown are the unique restriction sites MluI and KpnI that will be used to construct VSV-AngV-GFP and VSV-AngV-Luc (**c**). The long arrows indicate regions of self-cleavage.

CedV non-coding intergenic regions are retained to adhere to the genomic "rule of six". Unique restriction sites, will facilitate the insertion of the AngV F and G gene cassette into the rCedV antigenome plasmid (**Figure 9a**) generating rCedV-AngV-GFP (**Figure 9b**). The rCedV-AngV-Luc clone will be produced by swapping the reporter gene coding sequences. All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-AngV-GFP or pOLTV5$_{opt}$-rCedV-AngV-Luc antigenome plasmid. After 4 days cultures are examined for GFP expression and syncytia (HNV-CPE). BSR-T7/5 cells and supernatants from successful rescue are collected and passaged in Vero 76 cells (T-75 flasks) to prepare a virus stock. When maximal syncytia and GFP is observed (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[42-44,74].

Generation of a VSV encoding AngV envelope glycoproteins. We will also construct reporter gene encoding versions of a replication competent VSV encoding the AngV F and G glycoproteins (GFP and Luc) (**Figure 9c)**. A large gene cassette comprising VSV untranslated intergenic regions flanking the AngV F and G coding sequences will be synthesized. Unique restriction enzyme sites, will facilitate the insertion of the AngV F and G gene cassette into the VSV backbone plasmid to generate VSV-AngV-GFP (**Figure 9c**). The VSV-AngV-Luc clone will be produced by swapping the reporter gene coding sequences. Cloning procedures are performed in *E. coli* Stbl2 cells and all plasmid clones verified by sequencing. Rescue of VSV-AngV-GFP and VSV-AngV-Luc, will be performed in BSR-T7/5 cells, in 12-well plates, transfected with helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-AngV-GFP or VSV-AngV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus uses HEK293T cells (6-well plates) by inoculation with the supernatant from step 1. Typically, when GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above and the virus stocks stored at -80°C. Larger viral stocks are prepared in T-150 flasks in HEK293T cells, and harvested virus supernatants are purified by buffered sucrose cushion centrifugation and virus pellets resuspended in 10% sucrose/TNE buffer, and small volume single use aliquots stored at -80°C. All virus stocks are deep sequenced. Virus stocks (GFP) are titered (infectious units/ml (IU/ml)) by serial dilution on HEK293T cells in 96-well black walled plates. At 24hrs post-infection plates are fixed with 4% formaldehyde, washed 3x with diH$_2$0 and imaged (fluorescence) with a CTL S6 analyzer. Luciferase encoding VSV stocks are titered by plaque assay[75].

Characterization of the recombinant reporter viruses in cell-based infection assays. Replication kinetics of rCedV-based and VSV-based AngV F/G viruses are assayed by Vero cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in 24hr intervals and titered. rCedV-AngV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Similarly, VSV-AngV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G[71]. The rCedV-AngV-GFP and VSV-AngV-GFP assays will be conducted by FRNTs[44] and rCedV-AngV-Luc and VSV-AngV-Luc in luciferase assays[42].

Expected results, potential pitfalls and alternatives. We expect that rCedV-LayV-GFP and rCedV-AngV reporter viruses can be rescued. In addition, we expect that these chimeric viruses will induce syncytia and replicate to high titers. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. In the event that the rescue of rCedV chimeric viruses are not viable, multiple strategies may be examined. 1) test different ratios of helper plasmids and antigenome plasmid; 2) perform rescue in different cell lines; 3) construct alternate versions of the LayV and/or AngV F and G genes within the antigenome plasmids, such as, fuse the LayV and/or AngV F and G ectodomains with the transmembrane and/or cytoplasmic tail domains of the corresponding CedV F and G elements which may facilitate virus particle formation and membrane fusion activity.

<u>Aim 3:</u> Evaluate HNV-specific antibodies and nanobodies developed by the Center's RPs and Core D.

Rationale and Preliminary Data. The rCedV chimeric system we established replaces the need for authentic NiV-B and HeV in neutralization assays. Neutralization values of well-characterized NiV/HeV cross-reactive neutralizing mAbs highly correlated between rCedV-NiV-B-GFP and rCedV-HeV-GFP and authentic NiV-B and HeV at BSL-4 when tested by conventional PRNT[44]. The Pearson correlation analysis between the rCedV chimeras BSL-2 PRNT and the NiV-B or HeV BSL-4 PRNT is shown in **Figure 10a**. We further developed the rCedV-NiV-B-GFP and rCedV-HeV-GFP neutralization assay into a high-throughput and quantitative assay that is based on the reduction of GFP foci, a FRNT[44]. The Pearson correlation analysis between the chimeras PRNT and FRNT is shown in **Figure 10b**. Taken together, these data demonstrate that rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeric viruses are an ideal set of suitable surrogate viruses for conducting a rapid FRNT-based assay for assessing NiV and HeV antibody neutralization.

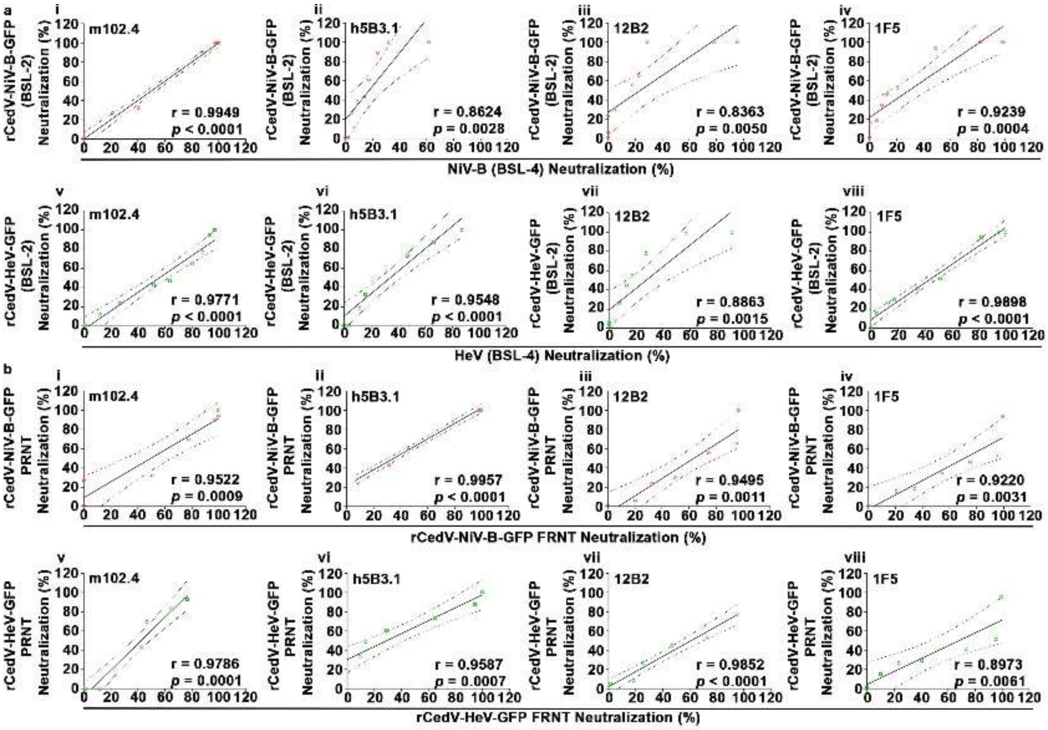


Figure 10. Pearson correlation analysis of rCedV chimeras. Pearson correlation analysis of PRNT neutralization (%) values of rCedV-NiV-B-GFP versus NiV-B (**a**, **i-iv**) and rCedV-HeV-GFP versus HeV (**a**, **v-viii**). Pearson correlation analysis from PRNTs (y-axes) and FRNTs (x-axes) of rCedV-NiV-B-GFP (**b**, **i-iv**) and rCedV-HeV-GFP (**b, v-viii**). Neutralization with mAbs m102.4, h5B3.1, 12B2 or 1F5 are shown. The Pearson correlation coefficient 'r,' p-value (two-tailed), linear regression line (solid lines), and 95% confidence intervals (dashed lines).

Experimental Design. Here, using the new tools (rCedV-LayV-GFP, rCedV-AngV-GFP, rCedV-AngV-Luc, VSV-AngV-GFP and VSV-AngV-Luc) we will assess neutralizing activity, of HNV specific mAbs and/or sera from HNV immunized mice that will be generated by **Core D**. The FRNT is high throughput and performed in 96-well plates to establish a 7-point dose response. Serially diluted immune sera or mAbs are mixed with either rCedV-NiV-B-GFP or rCedV-HeV-GFP for 2hrs. The virus-mAb or virus-sera mixtures are then added in triplicate to confluent pre-seeded Vero 76 cells in black-walled clear bottom 96-well plates and incubated for an additional 24hrs. Plates are then fixed and imaged and fluorescent foci counted using the CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) will be determined as the mAb concentration or serum dilution at which there was a 50% reduction in fluorescent foci versus untreated control wells. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9.

Expected results, potential pitfalls and alternatives. We do not anticipate any unexpected limitations in assessing the neutralization potential of antibodies and sera generated from other research projects and/or cores as we have successfully demonstrated that the rCedV-GFP chimeric FRNT platform can serve as a surrogate BSL-2 neutralization assay that is high throughput with a fast turnaround time.

Aim 4: Validate PPZ-MNP platform with another paramyxovirus-specific antigen in Phase 2.

At completion of Phase I we will have i) a procedure to fabricate PPZ adjuvanted MNP using sG, sF or a combination of both viral glycoproteins; ii) generated rLayV with an associated animal model in collaboration with **Core E** with reporter virus construct (rCedV-LayV-GFP); and iii) generated reporter viruses AngV (rCedV-AngV-GFP, rCedV-AngV-Luc, VSV-AngV-GFP and VSV-AngV-Luc). Here, in Phase II we propose to test the hypothesis that the PPZ-MNP vaccine platform technology can be adapted and transferred as a ‘plug and play’ strategy for other paramyxoviruses, namely the divergent HNVs LayV and AngV. The major goal rests on the rescue of LayV and establishing an infection/disease model of this most recently discovered pathogenic, zoonotic, HNV. Nevertheless, if rescue of rLayV with an associated animal model is not achieved, we will still be able to test the MNP approach as proof-of-concept to the extent of generating robust immune responses in animals and testing neutralizing antibody responses using both LayV and AngV reporter virus systems.

PPZ-MNP manufacture. The fabrication of the MNPs by **Core C** will be prepared for both the F and G viral glycoproteins: LayV-sG, LayV-sF, AngV-sG and AngV-sF depending on the data and outcomes, and vaccine down-selection in Phase I we may also include a combination of sG and sF. Vaccine antigens will have been prepared by **Core D**. It may be important to evaluate both the attachment and fusion glycoprotein immunogens for LayV and AngV in Phase II. Although the preponderance of data to date for the pathogenic prototype HNVs (NiV/HeV) indicates that the attachment glycoprotein (G) alone can serve as an effective vaccine immunogen, this is presently unknown for these two divergent HNVs, so test both sF and sG for these HNVs is needed.

Immunogenicity. As in Aim 1, groups of 5 mice will be used for each immunogen(s) and a control of nonimmunized subjects. Sera will be drawn as scheduled in **Figure 3**. ELISA titers of sera to vaccine immunogens will be evaluated by **Core C** and sera will be evaluated for virus neutralization with newly developed reporter viruses. Cellular immune responses will be assessed as described in Aim 1.

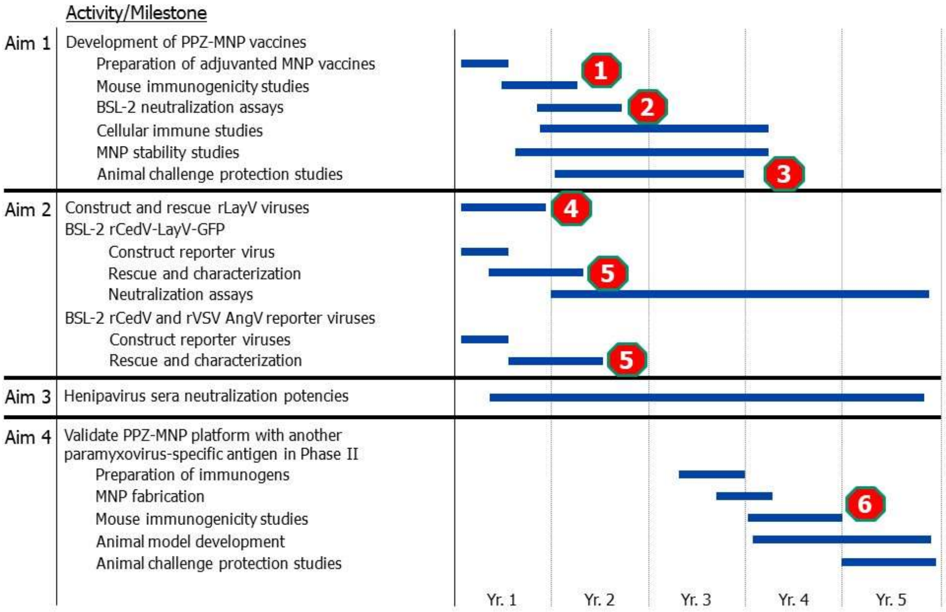
Animal challenge studies. The experimental design for these studies will be adjusted once the animal model has been developed. The basic design will follow those for ferrets and AGMs described in Aim 1. The immunogen will be chosen based on the results from the mouse sera neutralization tests.

Expected results, potential pitfalls and alternatives. We expect that LayV / AngV glycoproteins MNPs fabrication will be possible. Mouse immunization studies with MNPs are likely to be uncomplicated and we will be able to assess immune responses. These data will determine whether the sG or sF is the immunogen of choice for best virus neutralization antibody induction. If rescue of rLayV and establishing an animal model are not met, the ability to measure inhibition of virus in cell-based assays, using either the new reporter viruses or a cell-cell fusion surrogate assays will be done. The mouse immunogenicity studies, together with the virus neutralization assays will provide a foundation for potential anti-LayV and anti-AngV MNP vaccine approaches.

Project Milestones, Timelines, and Go/No-go decisions. The **RP2** Aims, milestones, and timeline with activities having periodic assessments by Center leaders, SAB, and NIAID staff as shown in the Gannt Chart.

Aim 1: Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV subunit vaccines

Milestone 1: Adjuvanted MNP vaccines are successfully prepared: In year 1, the MNP vaccine fabrication by **Core C** will commence and formulation of recombinant antigens with PCEP adjuvant in the context of MNPs will be assessed. We are confident that this stage of the research plan will be achievable because we have already had success in MNP fabrication with the HeV-sG formulated with two different PPZ adjuvants (PCEP and PCPP) and the preliminary mouse immunization and cellular immune responses and induction of NiV/HeV cross-neutralizing antibody titers was excellent, with ID_{50} titers significantly greater than previously measured pre-challenge ID_{50} titers that provided complete protection across several prior studies in cats, ferrets, horses, and AGMs (preliminary data). We expect that the formulation of the remaining 3 prototype HNV immunogens (NiV-sG_{tet}, NiV-sF_{stb}, HeV-sF_{stb} (and the combination test: HeV-sG+NiV-sFstb)) adjuvanted with PCEP and applied to the MNPs will be fully achievable. Analysis will also include antigen stability studies by re-release of sG_{tet} and sF_{stb} immunogens and recognition by select conformation dependent mAbs. A go/no-go decision may be made pending the results of MNP fabrication and antigen analysis of each MNP vaccine type, poor fabrication or loss of antigen integrity will trigger termination of those individual vaccines prior to immunogenicity studies.



Gannt chart outlining project milestones (left). Solid lines represent periods of activity to achieve milestones. ***Go/No-go*** decision points are indicated by red octagons as follows:

(1) Successful fabrication of vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity is a ***No-go*** for the respective individual vaccine MNP.

(2) An SNT <10 is a ***No-go*** for the tested immunogen. The highest SNT >10 is a ***Go*** for the respective immunogen.

(3) Successful protection is a ***Go*** to the project Phase II. No protection is a ***No-go*** for the MNP vaccine platform.

(4) Successful rescue of rLayV is a ***Go*** to the development animal models in Aim 4. Otherwise is a ***No-go*** for the animal models development and the challenge protection studies in Aim 4.

(5) Successful rescue of a reporter virus is a ***Go*** for neutralization assays development. Inability to rescue a reporter virus is a ***No-go*** to that reporter virus neutralization assay development.

(6) Successful fabrication of vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity is a ***No-go*** for the respective individual vaccine MNP.

<u>Milestone 2: High immunogenicity and best in class immunogen(s) identified</u>: **Core C** will conduct immunogenicity studies in mice as detailed in the preliminary data and experimental plan and the Core research plan; multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction, virus-neutralization, and polyfunctional T-cell responses by in vitro stimulation). As sera are made available from the mice MNP vaccine cohorts, we will assess the NiV/HeV-neutralizing antibody activities of each of the MNP vaccine formulations of the prototype HNV immunogens (NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb}$, HeV-sG, and HeV-sG+NiV-sF$_{stb}$). The criteria to judge success and go/no-go decisions will be levels of cross-neutralizing antibody responses (neutralization of both HeV and NiV-B) induced by each vaccine type. We expect to be able to down-select to 'best-in-class' vaccine as a single antigen or perhaps the combination antigen MNP vaccine formulation at this stage prior to progressing to the first-round ferret challenge models.

<u>Milestone 3: MNP vaccination protection in animal challenge studies:</u> Here, the determination of whether MNP vaccination affords protection in animal model challenge experiments will be made. For the NiV and HeV prototypes the animal models (ferret and AGM) are extremely well-developed and the PABVAX Center leadership and staff have extensive experience in this area, and outcomes at this stage should be readily assessable. If we are successful in developing and validating the PPZ adjuvanted MNP recombinant protein vaccine approach for the HNV prototypes (NiV/HeV) and demonstrate protection in the planned homologous and heterologous HNV challenge studies, this will be expected to be a 'go' decision point in year 3, and the proposed Specific Aim 4 tasks (below) will continue in preparation for Phase II of the PABVAX Center. If in the unlikely event that the MNP vaccine approach fails (no-go) then further consultation with industry and regulatory expertise within **Core D** and partners and the **SAB** will be geared towards using the previously identified 'best-in-class' immunogens with alternative vaccine platforms (IM formulations; virus vectored such as a VSV technology held by the PABVAX Center PI and RP1 PI at UTMB; or mRNA, Moderna Inc., has provided the Center a letter of support).

<u>Aim 2: Rescue Langya virus and construct LayV and AngV glycoprotein bearing reporter virus tools.</u>

<u>Milestone 1: Successful rescue of recombinant Langya virus.</u> Since the genetic data on LayV is derived from an actual human virus isolate, it suggests that rescue of rLayV will be possible. **RP2** staff have extensive experience in HNV reverse genetics with rCedV (and VSV) with unique newly developed reagents. Virus rescue is in collaboration with **Core E**, followed by virus characterization in cell-based assays. **Core E** will then initiate small animal model development studies such as the hamster and/or ferret, both successful for the prototype HNVs. If rescue and animal modeling is successful, it will be very significant advancement for this pandemic preparedness initiative and objectives, and the virus, reagents, tools and models supplied to the ReVAMPP network. If rescue is not possible a no-go decision is obvious for animal model development. However, even if a LayV isolate remains unavailable by other means, we can still test the MNP approach with LayV and also our alternative divergent HNV prototype (AngV) by characterizing G and/or F vaccine-induced neutralizing antibodies using alternative reporter viruses (Milestone 2 below) or cell-based (cell-cell fusion assays; **Figure 6B**).

Milestone 2: Construction of rCedV and rVSV reporter viruses encoding LayV and AngV F/G glycoproteins: Here, we have reasonable confidence that one or both alternate reporter virus platforms will be possible. Successful rescue of these reporter viruses will enable the determination of immune sera neutralization titers and is a 'go' for the development of the neutralization assays. An unsuccessful rescue of either of the LayV or AngV reporters will be a no-go for those assay developments. If neither reporter virus is rescued we will proceed to cell-cell fusion assays development to study the immune sera neutralization titers. We have already successfully generated recombinant sF_{stb} and sG_{tet} glycoproteins from AngV and LayV based on available sequence data (AngV, **RP2** in collaboration with **Dr. Cara Brook,** The University of Chicago, and letter of support included in the PABVAX Overall component; and LayV, in collaboration with **RP5** leaders and **Genovac, Inc.**). In our prior experience, success in making soluble and secreted oligomeric forms of virus glycoproteins strongly indicates that genetic sequences are correct which should also translate into functional proteins. Rescued viruses will be characterized in various cell-based assays. These new virus tools will be used in collaboration with **RP4**, **RP5** and **Core D**, in assessing newly developed mAbs and nabs against LayV and AngV, and also vaccine-induced neutralizing antibody responses in PABVAX Center activities in Phase II.

Aim 3: Is also shown which is the collaborative support provided by **RP2** in assessing specific antibody development activities to HNVs by **RP4** and **RP5** is also included.

Aim 4: Validate PPZ-MNP platform with another paramyxovirus-specific antigen in Phase 2. At this stage one or both of the divergent HNV prototypes (LayV and/or AngV) would be selected and the milestones for progress would include MNP vaccine manufacture; mouse immunogenicity studies; and virus challenge protection. These milestones will be similar as described for Specific Aim 1. We have obtained preliminary structural characterizations of sF_{stb} and sG_{tet} glycoproteins from AngV and LayV (native-like oligomeric and stabilized glycoproteins) in collaboration with **RP5** leaders, and have also determined (preliminary data) that the LayV sF_{stb} and sG_{tet} glycoproteins are recognized by 3 previously generated mAbs to MojV F/G from our lab (**RP2**). As discussed above, the primary HNV target of **RP2** is LayV, and the alternate is AngV, but the go/no-go decisions on the virus target will depend on the progress and outcomes of the earlier studies. Also, as discussed above, even in the absence of rLayV rescue or its availability from another source and its characterization in animal models by **Core E**; or the possible isolation of AngV and its work-up *in vivo* and pathogenesis studies by Core E; we expect to still be able to assess and validate the recombinant subunit protein MNP vaccine approach with our already prepared immunogens and the other virus tools we plan on developing.

Industry Expertise and Regulatory Considerations

Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP2** (MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based HNV vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

If new neutralizing mAbs to the F glycoprotein of NiV/HeV (cross-reactive) or to LayV or AngV or nabs or bi-specific nabs that show antiviral activities to NiV/HeV, from the collaborations of **RP2, RP3, RP4** and **RP5** are found, we will seek industry and regulatory expertise in **Core D** and the **SAB** in areas such as; identification of correlates of protection, lead optimization, stability and manufacturability testing. Antibodies may be engineered (such as altered N-glycans, Fc mutations for effector functions and extended half-life, multimeric formats) by **Core D** and further evaluated to correlate mechanisms of action with maximal efficacy by **Core E** (authentic HNVs) and **RP3** (rCedV chimera model). This work will identify lead prototypes to transition to early process development. Further details on antibody development, milestones, and go/no-go decisions are discussed in the relevant RPs and **Cores D** and **E**.

References cited

1 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33 (1995).

2 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

3 Chua, K. B. Nipah virus outbreak in Malaysia. *J Clin Virol* **26**, 265-275 (2003).

4 Chua, K. B. *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**, 1257-1259 (1999). https://doi.org:10.1016/S0140-6736(99)04299-3 [doi]

5 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res* **100**, 8-13 (2013). https://doi.org:10.1016/j.antiviral.2013.06.012

6 Geisbert, T. W., Feldmann, H. & Broder, C. C. in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 153-177 (Springer, Berlin, Heidelberg, 2012).

7 Mehand, M. S., Al-Shorbaji, F., Millett, P. & Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral Res* **159**, 63-67 (2018).

8 *Priority Diseases 2021*, <https://cepi.net/research_dev/priority-diseases/> (2021).

9 Brende, B. *et al.* CEPI-a new global R&D organisation for epidemic preparedness and response. *Lancet* **389**, 233-235 (2017). https://doi.org:10.1016/s0140-6736(17)30131-9

10 Anonymous. Hendra virus - Australia (02): (QL) horse. Report No. 20220710.8704331, (International Society for Infectious Diseases, 2022).

11 Broder, C. C., Weir, D. L. & Reid, P. A. Hendra virus and Nipah virus animal vaccines. *Vaccine* **34**, 3525-3534 (2016). https://doi.org:10.1016/j.vaccine.2016.03.075

12 Homaira, N. *et al.* Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect* **138**, 1630-1636 (2010). https://doi.org:10.1017/S0950268810000695 [doi]

13 Homaira, N. *et al.* Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One* **5**, e13570 (2010). https://doi.org:10.1371/journal.pone.0013570 [doi]

14 Luby, S. P. *et al.* Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis* **15**, 1229-1235 (2009).

15 Rahman, M. A. *et al.* Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector borne and zoonotic diseases (Larchmont, N.Y.)* **12**, 65-72 (2012). https://doi.org:10.1089/vbz.2011.0656

16 Anonymous. Nipah virus - Bangladesh (05). Report No. 20230208.8708217, (International Society for Infectious Diseases, 2023).

17 Lee, S. H. *et al.* Discovery and Genetic Characterization of Novel Paramyxoviruses Related to the Genus Henipavirus in Crocidura Species in the Republic of Korea. *Viruses* **13** (2021). https://doi.org:10.3390/v13102020

18 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836 (2012). https://doi.org:10.1371/journal.ppat.1002836

19 Zhang, X. A. *et al.* A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med* **387**, 470-472 (2022). https://doi.org:10.1056/NEJMc2202705

20 Drexler, J. F. *et al.* Henipavirus RNA in African bats. *PLoS One* **4**, e6367 (2009). https://doi.org:10.1371/journal.pone.0006367 [doi]

21 Madera, S. *et al.* Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol* **96**, e0092122 (2022). https://doi.org:10.1128/jvi.00921-22

22 Vanmechelen, B. *et al.* The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol* **8**, veac061 (2022).

23 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066 (2014). https://doi.org:10.3201/eid2006.131022

24 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses and public health* **60**, 69-83 (2013). https://doi.org:10.1111/j.1863-2378.2012.01501.x

25 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951 (2011). https://doi.org:10.4269/ajtmh.2011.10-0567 [doi]

26 Schountz, T. *et al.* Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **11** (2019). https://doi.org:10.3390/v11030291

27 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473 (2020). https://doi.org:10.1146/annurev-virology-021920-113833

28 Broder, C. C. *et al.* in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 197-223 (Springer, Berlin, Heidelberg, 2012).

29 Basler, C. F. in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 123-152 (Springer, Berlin, Heidelberg, 2012).

30 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral Res* **124**, 69-76 (2015). https://doi.org:10.1016/j.antiviral.2015.09.017

31 Marsh, G. A. & Wang, L. F. Hendra and Nipah viruses: why are they so deadly? *Curr Opin Virol* **2**, 242-247 (2012). https://doi.org:10.1016/j.coviro.2012.03.006

32 NIH, C. a. (ed U.S. Department of Health and Human Services) 248-250 (US Government Printing Office, Washington, 2020).

33 Bossart, K. N. *et al.* Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol* **79**, 6690-6702 (2005).

34 Bossart, K. N. *et al.* A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Science translational medicine* **4**, 146ra107 (2012). https://doi.org:10.1126/scitranslmed.3004241

35 McEachern, J. A. *et al.* A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* **26**, 3842-3852 (2008). https://doi.org:10.1016/j.vaccine.2008.05.016

36 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379 (2014). https://doi.org:10.3201/eid2003.131159

37 Mire, C. E. *et al.* A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol* **88**, 4624-4631 (2014). https://doi.org:10.1128/jvi.00005-14

38 Mungall, B. A. *et al.* Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol* **80**, 12293-12302 (2006).

39 Pallister, J. *et al.* A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine* **29**, 5623-5630 (2011). https://doi.org:10.1016/j.vaccine.2011.06.015

40 Pallister, J. A. *et al.* Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J* **10**, 237 (2013). https://doi.org:10.1186/1743-422x-10-237

41 Geisbert, T. W. *et al.* A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. *NPJ Vaccines* **6**, 23 (2021).

42 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084 (2021). https://doi.org:10.1016/j.antiviral.2021.105084

43 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018). https://doi.org:10.1186/s12985-018-0964-0

44 Amaya, M. *et al.* A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses* **15** (2023). https://doi.org:10.3390/v15051077

45 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021). https://doi.org:10.1016/j.celrep.2021.109628

46 Wang, Z. *et al.* Architecture and antigenicity of the Nipah virus attachment glycoprotein. *Science* **375**, 1373-1378 (2022). https://doi.org:10.1126/science.abm5561

47 Wang, Z. *et al.* Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. *Proc Natl Acad Sci U S A* **119**, e2122769119 (2022). https://doi.org:10.1073/pnas.2122769119

48 Avanzato, V. A. *et al.* A structural basis for antibody-mediated neutralization of Nipah virus reveals a site of vulnerability at the fusion glycoprotein apex. *Proc Natl Acad Sci U S A* **116**, 25057-25067 (2019). https://doi.org:10.1073/pnas.1912503116

49 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987 (2019). https://doi.org:10.1038/s41594-019-0308-9

50 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021). https://doi.org:10.1038/s41594-021-00584-8

51 Dong, J. *et al.* Potent Henipavirus Neutralization by Antibodies Recognizing Diverse Sites on Hendra and Nipah Virus Receptor Binding Protein. *Cell* **183**, 1536-1550 e1517 (2020). https://doi.org:10.1016/j.cell.2020.11.023

52 Mire, C. E. *et al.* A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis* **221**, S471-S479 (2020). https://doi.org:10.1093/infdis/jiz515

53 Playford, E. G. *et al.* Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis* (2020). https://doi.org:10.1016/s1473-3099(19)30634-6

54 Andrianov, A. K., Marin, A. & Fuerst, T. R. Molecular-Level Interactions of Polyphosphazene Immunoadjuvants and Their Potential Role in Antigen Presentation and Cell Stimulation. *Biomacromolecules* **17**, 3732-3742 (2016). https://doi.org:10.1021/acs.biomac.6b01251

55 Andrianov, A. K. *et al.* In Vivo and In Vitro Potency of Polyphosphazene Immunoadjuvants with Hepatitis C Virus Antigen and the Role of Their Supramolecular Assembly. *Mol Pharm* **18**, 726-734 (2021). https://doi.org:10.1021/acs.molpharmaceut.0c00487

56 Andrianov, A. K. & Langer, R. Polyphosphazene immunoadjuvants: Historical perspective and recent advances. *J Control Release* **329**, 299-315 (2021). https://doi.org:10.1016/j.jconrel.2020.12.001

57 Chand, D. J., Magiri, R. B., Wilson, H. L. & Mutwiri, G. K. Polyphosphazenes as Adjuvants for Animal Vaccines and Other Medical Applications. *Front Bioeng Biotechnol* **9**, 625482 (2021). https://doi.org:10.3389/fbioe.2021.625482

58 Eng, N. F. *et al.* The potential of polyphosphazenes for delivery of vaccine antigens and immunotherapeutic agents. *Curr Drug Deliv* **7**, 13-20 (2010). https://doi.org:10.2174/156720110790396481

59 Andrianov, A. K. *et al.* Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A* **106**, 18936-18941 (2009). https://doi.org:10.1073/pnas.0908842106

60 Valencia, S. M. *et al.* Improvement of RG1-VLP vaccine performance in BALB/c mice by substitution of alhydrogel with the next generation polyphosphazene adjuvant PCEP. *Hum Vaccin Immunother* **17**, 2748-2761 (2021). https://doi.org:10.1080/21645515.2021.1875763

61 Magiri, R. *et al.* Intradermal immunization with inactivated swine influenza virus and adjuvant polydi(sodium carboxylatoethylphenoxy)phosphazene (PCEP) induced humoral and cell-mediated immunity and reduced lung viral titres in pigs. *Vaccine* **36**, 1606-1613 (2018). https://doi.org:10.1016/j.vaccine.2018.02.026

62 Magiri, R. B., Lai, K. J., Mutwiri, G. K. & Wilson, H. L. Experimental PCEP-Adjuvanted Swine Influenza H1N1 Vaccine Induced Strong Immune Responses but Did Not Protect Piglets against Heterologous H3N2 Virus Challenge. *Vaccines (Basel)* **8** (2020). https://doi.org:10.3390/vaccines8020235

63 Guillot, A. J. *et al.* Microneedle-Based Delivery: An Overview of Current Applications and Trends. *Pharmaceutics* **12** (2020). https://doi.org:10.3390/pharmaceutics12060569

64 Bediz, B. *et al.* Dissolvable microneedle arrays for intradermal delivery of biologics: fabrication and application. *Pharm Res* **31**, 117-135 (2014). https://doi.org:10.1007/s11095-013-1137-x

65 Al-Attiyah, R., El-Shazly, A. & Mustafa, A. S. Comparative analysis of spontaneous and mycobacterial antigen-induced secretion of Th1, Th2 and pro-inflammatory cytokines by peripheral blood mononuclear cells of tuberculosis patients. *Scand J Immunol* **75**, 623-632 (2012). https://doi.org:10.1111/j.1365-3083.2012.02692.x

66 Balmert, S. C. *et al.* A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. *iScience* **25**, 105045 (2022). https://doi.org:10.1016/j.isci.2022.105045

67 van de Ven, K., de Heij, F., van Dijken, H., Ferreira, J. A. & de Jonge, J. Systemic and respiratory T-cells induced by seasonal H1N1 influenza protect against pandemic H2N2 in ferrets. *Commun Biol* **3**, 564 (2020). https://doi.org:10.1038/s42003-020-01278-5

68 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715 (2019). https://doi.org:10.1073/pnas.1911773116

69 Navaratnarajah, C. K., Rosemarie, Q. & Cattaneo, R. A Structurally Unresolved Head Segment of Defined Length Favors Proper Measles Virus Hemagglutinin Tetramerization and Efficient Membrane Fusion Triggering. *J Virol* **90**, 68-75 (2016). https://doi.org:10.1128/JVI.02253-15

70 Weir, D. L., Laing, E. D., Smith, I. L., Wang, L. F. & Broder, C. C. Host cell virus entry mediated by Australian bat lyssavirus G envelope glycoprotein occurs through a clathrin-mediated endocytic pathway that requires actin and Rab5. *Virol J* **11**, 40 (2014). https://doi.org:10.1186/1743-422X-11-40

71 Weir, D. L., Smith, I. L., Bossart, K. N., Wang, L. F. & Broder, C. C. Host cell tropism mediated by Australian bat lyssavirus envelope glycoproteins. *Virology* **444**, 21-30 (2013). https://doi.org:10.1016/j.virol.2013.06.016

72 Laing, E. D. *et al.* Enhanced Autophagy Contributes to Reduced Viral Infection in Black Flying Fox Cells. *Viruses* **11** (2019). https://doi.org:10.3390/v11030260

73 Mastraccio, K. E. *et al.* Establishment of a longitudinal pre-clinical model of lyssavirus infection. *J Virol Methods* **281**, 113882 (2020). https://doi.org:10.1016/j.jviromet.2020.113882

74 Weingartl, H. M. *et al.* Recombinant nipah virus vaccines protect pigs against challenge. *J Virol* **80**, 7929-7938 (2006). https://doi.org:10.1128/JVI.00263-06

75 Abdelmageed, A. A. & Ferran, M. C. The Propagation, Quantification, and Storage of Vesicular Stomatitis Virus. *Curr Protoc Microbiol* **58**, e110 (2020). https://doi.org:10.1002/cpmc.110

Research Strategy

Significance

The constant threat posed to global public health by emerging and reemerging zoonotic viruses emphasizes the importance for pandemic preparedness by developing countermeasures. The highly pathogenic Hendra virus (HeV) and Nipah virus (NiV) are the prototype members in the genus *Henipavirus* in the family Paramyxoviridae[1], and the selected prototypes chosen for **RP2**. HeV was first discovered in 1994, in Queensland, Australia where it was the causative agent of a severe and fatal respiratory disease among horses along with a human case fatality[1,2]. Whereas, in 1998-1999 in Malaysia and Singapore an outbreak of encephalitis in people and respiratory disease in pigs was eventually determined to be caused by a closely related virus - to NiV[3,4]. These viruses are capable of causing a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with fatality rates ranging from 40-100%[5,6]. As a result of this high lethality and lack of approved vaccines or antivirals, HeV and NiV are classified as Biosafety Level-4 (BSL-4) pathogens. NiV and henipaviral diseases are also included in the WHO's Blueprint List of Priority Pathogens[7], and NiV is among the Coalition for Epidemic Preparedness Innovations (CEPI) list of Priority Diseases needing urgent research and countermeasure development and are recognized as agents of pandemic threat potential[8,9]. HeV, though apparently restricted to Australia, has spilled over 66 times, most recently in 2022[10]. HeV has caused the death or euthanasia of >100 horses, 2 HeV positive and euthanized dogs, and 4 fatalities of 7 human cases[10,11]. Whereas NiV is present throughout a large area including South and Southeast Asia from the Philippines to western India. The frequent outbreaks of NiV in Bangladesh and India have high human case fatality rates 70-100%. NiV transmission in Bangladesh to humans has been linked to contaminated fruits or date palm sap, and person-to-person transmission[12-15]. In total, there have been >620 human cases of NiV infections >330 fatalities, the most recent in 2023[16]. The genus *Henipavirus* now includes nine other species; the four viral isolates of CedV, Gamak virus, Daeryong virus, and Langya virus (LayV)[17-19] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiang virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[20-23]. Pteropid fruit bats, known as flying foxes, are recognized natural reservoir hosts for NiV, HeV[24,25], CedV[18], GhV and AngV. One of these, CedV, has been shown to be nonpathogenic in several animals susceptible to HeV and NiV disease including mice, guinea pigs and ferrets[18,26] and African green monkeys (AGMs) (Geisbert and Broder, unpublished). The remaining six reported henipaviruses (HNVs) are likely of rodent origins. One of these six, the isolate LayV, is associated with nonfatal febrile illness in humans[19].

HNVs are enveloped, single-stranded, negative sense RNA viruses that share a similar genomic makeup. The viruses have two membrane anchored glycoproteins involved in entry: the attachment (G) glycoprotein that engages the host cell receptor and the fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F proteins are the major targets of neutralizing antibodies and the focus of all vaccine and therapeutic antibody strategies[27,28]. However, CedV is distinct from other HNVs, and the phosphoprotein (*P*) gene does not encode the interferon antagonist proteins V and W[18,29-31]. The nonpathogenic phenotype of CedV is most likely attributed to the lack of these nonstructural proteins. Based on genetic data, all identified HNVs, except CedV, can potentially express the V and W proteins. CedV is recognized as a BSL-2 restricted agent[32].

Innovation

There are multiple layers of innovation within **RP2**. **First**, the proposed development of henipavirus (HNV) subunit vaccine candidates using an innovative microneedle array patch (MNP) technology that may be suitable for the incorporation of immunogens derived from other undiscovered pathogenic HNVs or paramyxoviruses in the future. **Second**, the construction of a rLayV reverse genetics system will be highly valuable in the HNV toolbox to study putative differences in pathogenesis and vaccine design between HNVs of bat and rodent origin, and significant resource to the ReVAMPP network's objectives. **Third**, generation of BSL-2 surrogate platforms that are based on rCedV and VSV will allow for a wide-variety of *in vitro* studies to support infection and host response studies and to screen in a high throughput manner the neutralization activity of immune sera and monoclonal antibodies (mAbs) and nanobodies (nabs) without the requirement for high containment. **Fourth**, considering that the LayV and AngV F and G proteins are functionally fusion active, the generation of viable rCedV chimeras bearing either LayV or AngV F and G glycoproteins is feasible.

Approach

Scientific Premise

To date, the most extensively studied NiV/HeV vaccine is a soluble, recombinant, oligomeric form (dimers and/or tetramers) of the G glycoprotein of HeV (HeV-sG)[11,27,33], and has been demonstrated to confer complete protection against lethal challenge with NiV-M (Malaysia), NiV-B (Bangladesh) or HeV in multiple animal models[34-40]. It has most recently been shown to provide complete protection of AGMs against NiV-B challenge performed one week post-immunization (single dose)[41]. The HeV-sG vaccine is marketed by Zoetis, Inc., for equine use in Australia since 2012 and is the first commercialized vaccine against any BSL-4 pathogen. HeV-sG /Alum intramuscular formulation is currently in clinical trial as an anti-NiV vaccine. **RP2** proposes to enhance the formulation, immunogenicity, and delivery of recombinant subunit protein based HNV vaccines.

We have developed rCedVs as new tools to study HNV host cell interactions, including ephrin receptor use and infection tropism, and in the development of HNV countermeasures. Experiments with rCedV can be conducted in a BSL-2 containment setting. The rCedV reverse genetics system[42,43] allowed us to develop a rCedV chimeric platform, where the CedV F and G glycoproteins are replaced with that of either NiV-B or with HeV, generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV). We have successfully rescued and characterized the chimeric viruses with and without reporter genes[44]. The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G specific mAbs against these rCedV chimeras highly correlate with the potencies obtained for the same antibodies to neutralize authentic NiV-B and HeV by plaque reduction neutralization tests (PRNTs). The green fluorescent protein (GFP) reporter chimeras were employed to develop a rapid, high-throughput, and quantitative fluorescence reduction neutralization test (FRNT). The antibody potencies to neutralize the reporter gene containing chimeras in the FRNT highly corelated with the respective potencies obtained by PRNT[44]. The rCedV chimera assay has been employed in several studies and its high-throughput ability has also allowed for the conduct of NiV/HeV mAb synergy neutralization tests[45-47]. The rCedV chimeras have their most important application in an authentic HNV-based surrogate neutralization assay that is rapid, cost-effective, and can be utilized in BSL-2 containment. We believe that the rCedV reverse genetics system may be able to serve as a plug-and-play platform for other emerging or pathogenic HNVs. To accomplish this, **RP2** will develop a variety of rCedV variants with reporter genes that will serve as important tools for the PABVAX Center RPs and Cores, and also for the NIAID, ReVAMPP network for pandemic preparedness.

Scientific Rigor

The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed. rCedV laboratory manipulation guidelines and standard operating procedures under BSL-2 conditions were developed for the laboratory and staff, and all procedures were reviewed and approved by the Uniformed Services University Institutional Biosafety Committee in accordance with NIH guidelines. The rCedV tools described here have been transferred to multiple institutions and research organizations which have also reviewed and approved their use at BSL-2 containment.

Aim 1: Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV subunit vaccines.

Rationale and Preliminary Data. It is well documented that the HNV G and F glycoproteins are the primary targets for virus neutralizing antibodies[27,45,48-53]. As such, HeV-sG subunit has clinically advanced in the development of HeV and NiV vaccines. Building on this work, Aim 1 will compare the immunogenicity and efficacy of alternate NiV vaccine immunogens (NiV-sG_{tet}, NiV-sF_{stb}), HeV vaccine immunogen (HeV-sF_{stb}), and a bivalent HNV vaccine candidate (NiV-sF_{stb}+HeV-sG) along with the clinical subunit HeV-sG. All immunogens will be delivered as polyphasphazene (PPZ) adjuvanted MNP vaccines in three animal species (mice, ferrets, and non-human primates-NHPs (AGMs)). Immunogenicity will initially be performed in mice by **Core C**. The neutralizing potencies of the immunized mouse sera will then be evaluated by *in vitro* neutralization assays using rCedV-NiV-B-GFP and rCedV-HeV-GFP[44]. The superior neutralizing antibody inducing MNP vaccine type(s) will be evaluated by animal challenge studies ferrets (first-tier) challenged with NiV-B or HeV and the lead vaccine candidate(s) from those studies will then be evaluated in AGM (second-tier) challenge studies.

Adjuvants are included in vaccine designs to generate a robust and durable vaccine induced immune response. We will utilize a synthetic PPZ macromolecule as the adjuvant to complement the proposed NiV and HeV vaccine immunogens. The PPZ macromolecule has many distinctive properties that make it an attractive choice as an adjuvant; 1) water-soluble and biodegradable, 2) spontaneously self-assembles with antigens in aqueous solutions, 3) forms nanoassemblies that mimick the dimensions of a virus (60-100nm)[54,55] and 4) is completely compatible with MNPs[56-58], the delivery mechanism that will be employed for the HNV immunogens. The extensive clinical use and experimental evidence supports the safety and potency of PPZ in enhancing vaccine-induced protective immune responses[54-60]. In particular, the most commonly used PPZ adjuvant is the clinical stage *poly[di(carboxylato-phenoxy)phosphazene] (PCPP)* and its structural homologue *poly[di(carboxylato-ethylphenoxy) phosphazene] (PCEP)*, which, significantly boosts vaccine immunogenicity without the requirement for large amounts of antigen while providing long-lasting protection in live viral challenge studies[60-62]. MNPs are promising resources to achieve systemic effects by transdermal delivery. They are a minimally invasive, painless system, which can bypass the stratum corneum and overcome the potential drawbacks of subcutaneous injections[63]. (See **Core C** Research Plan).

Manufacturing and characterization of HeV-sG±PCEP MNPs. The technology for fabricating MNPs pioneered by Bediz et al.,[64] was developed and is described in detail in the **Core C** Research Plan). We have conducted preliminary studies with the dissolvable MNPs incorporating HeV-sG antigen with or without PPZ adjuvants including PCEP (HeV-sG±PCEP MNPs) in collaboration with the investigators of **Core C**. Spin-casting of carboxymethyl cellulose and trehalose was used to create MNPs incorporating HeV-sG AF555±PCEP into obelisk-shaped microneedles having the vaccine components at the tip region to maximize the vaccine delivery efficiency by locating the vaccine components at the skin-penetrating regions of the microneedles (**Figure 1a**). The HeV-sG±PCEP MNPs can have a pre-determined array size of 10x10mm (for mice studies) and 20x20mm or greater (for larger animal studies). Images of the HeV-sG AF555±PCEP MNPs before (**Figure 1a**) and after (**Figure 1b**) application (15-20 mins), confirm a nearly complete dissolution of the microneedles into the skin demonstrating that the vaccine was effectively deposited into the skin. To facilitate the characterization and to visualize skin penetration of the developed HeV-sG±PCEP MNPs, HeV-sG was fluorescently labeled with Alexa Fluor 555 dye, HeV-sG AF555. To test the intracutaneous delivery capacity, HeV-sG AF555±PCEP MNPs were applied to the abdomen skin of C57BL/6 mice for 15 min and then removed. The HeV-sG AF555±PCEP MNP-treated mice were imaged before and after *in vivo* skin applications by using an *in vivo* live animal fluorescent imaging system (IVIS Lumina XR) and the acquired images were post-processed using Living Image software (PerkinElmer). **Figures 1c** and **1d** further corroborate the microscopy analysis of these MNPs and show effective deposition of vaccine components to murine skin (**Figure 1c**: HeV-sG AF555 and **Figure 1d**: HeV-sG AF555+PCEP). Together, these data support the feasibility of our PPZ MNP vaccine platform for the translational development of skin-targeted HNV vaccines that could also be employed using recombinant protein immunogens (or even virus-like particles) from other viral agents from a wide-variety of virus families.

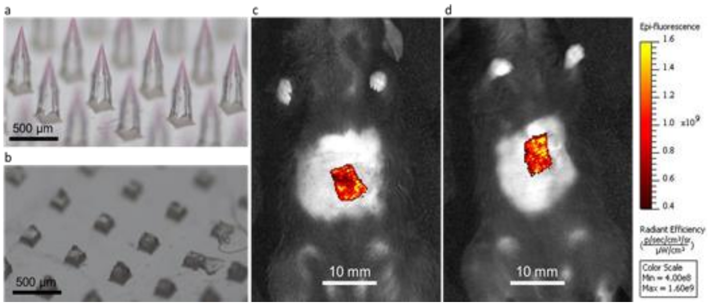


Figure 1: *In vivo* intracutaneous delivery efficacy of HeV-sG±PCEP MNPs in mice. An optical stereomicroscopy image of HeV-sG AF555+PCEP CMC/Trehalose microneedles before (**a**) and after (**b**) *in vivo* skin application. Administration of HeV-sG AF555 **(c)** and HeV-sG AF555+PCEP **(d)** to mouse skin *in vivo* with MNPs, captured by using a fluorescence *in vivo* imaging system (IVIS).

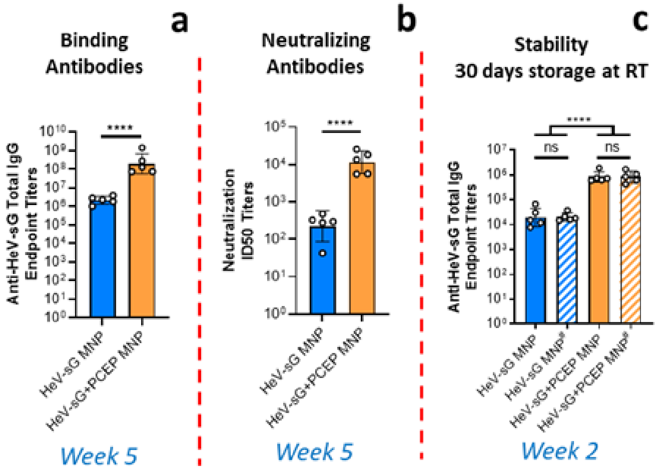


Figure 2: MNPs induced humoral responses and initial MNP stability data. Mice immunized prime/boost. MNPs applied to the abdominal skin of C57BL/6 mice 15 min, D0 (prime) and D21 (boost). Endpoint binding titers **(a)** ELISA. Neutralizing ID_{50} titers **(b)** by FRNT. Stability of MNPs **(c)** measured at D14 endpoint binding titers using a 30-day-old MNP compared to fresh MNP. Neutralizing ID_{50} titers with HeV-sG MNP (1:272) and HeV-sG+PCEP MNP (1:11,445) against rCedV-HeV-GFP.

HeV-sG±PCEP MNPs with unlabeled HeV-sG were fabricated and the immunogenicity tested in C57BL/6 mice. Groups of 5 mice were immunized on Day 0 (D0) (prime) and D21 (boost) with HeV-sG±PCEP MNPs. Sera were collected on D35 and analyzed by ELISA for total binding antibodies (endpoint titers) (**Figure 2a**) and HNV neutralizing capacity of serum in the rCedV chimeras FRNT[44] (**Figure 2b**). These results show that MNPs are highly effective in inducing antigen-specific binding and neutralization antibodies, and improved with PCEP adjuvant. Additional data also demonstrate that the PPZ MNP platforms are superior to intramuscular injection of the current clinical Alum-formulated HeV-sG vaccine (**Core C; Figure 4a**). In addition, temperature stability was tested by storing the HeV-sG±PCEP MNPs at room temperature without refrigeration. Mice were immunized with fresh (group 1) and 30-day old MNP (group 2) by single dose immunization. Mice were bled on D14 post immunization and the endpoint titers of total IgG levels were determined by ELISA. **Figure 2c** suggests that MNP-embedded vaccine components retain their immunogenicity for at least a month without refrigeration with no statistically significant differences in the antibody binding titers between fresh and the 30-day-old MNPs.

Experimental Design.

Aim 1.1: Manufacturing and characterization of PPZ MNPs with HNV glycoprotein antigens. We have tested GLP-like HeV-sG antigen (above), manufactured by Catalent Pharma Solutions, and it is currently available. The remaining 3 immunogens (NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb}$) will be prepared by Genovac, Inc., and **Core C** will prepare MNPs with each of NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb}$, HeV-sG+NiV-sF$_{stb}$ and HeV-sG, all adjuvanted with PCEP. Each fabricated batch of PPZ MNPs will be tested *in vitro* for geometric integrity, mechanical integrity, vaccine loading accuracy and reproducibility, biological integrity, endotoxin content, vaccine stability, and *in vivo* skin delivery performance before the animal immunization studies. The specific quantitative release criteria are as follows: 1) geometric integrity and accuracy – >95% high-quality microneedles across a PPZ MNP vaccine and <10% deviation from the target geometric values; 2) structural integrity – factor of safety (FOS) > 2 (FOS is equal to the failure forces/penetration forces); 3) vaccine loading accuracy and reproducibility – 10% deviation from the mean of theoretical amounts and <10% standard deviation of the mean value; 4) biological integrity – no significant aggregation of adjuvant-antigen complexes and no significant loss of antigenicity ($p>0.05$); 5) endotoxin content – < 1 endotoxin unit per PPZ MNP vaccine; 6) thermostability – biological integrity at elevated temperatures; 7) skin delivery performance – <10% of remaining materials in terms volume and <15% of remaining materials in terms of vaccine after 20 min murine skin application *in vivo* and 20 min the RP-specific animal-skin application *ex vivo* (See **Core C** application for more details). Based on the preliminary data and experience with fabricating HeV-sG MNP, we expect the technology to be plug and play applicable with the new HNV immunogens and with the combination of two different HNV immunogens.

Aim 1.2: Immunogenicity assessment HeV/NiV antigen PPZ MNPs in mice. To evaluate the immunogenicity of PCEP MNP platform with NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb,}$ HeV-sG, and HeV-sG+NiV-sF$_{stb}$, murine immunization studies will be conducted (**Core C)**. The immunization strategy will be performed as single-dose (D0) and prime(D0)-boost(D21) with female C57BL/6 mice (n = 5 mice per group). A schematic of the immunization and bleeding schedule is shown in **Figure 3**. Naïve or unimmunized mice will serve as negative controls and mice immunized with the intramuscular injection of Alum-complexed antigens will serve as positive controls. Serum levels of antigen-specific antibodies (IgM, total IgG, IgG1, IgG2c, and IgA) will be assayed by ELISA. The sera will be tested for neutralization activity using the respective reporter rCedV chimeras – rCedV-HeV-GFP and rCedV-NiV-B-GFP in the FRNT assay[44].

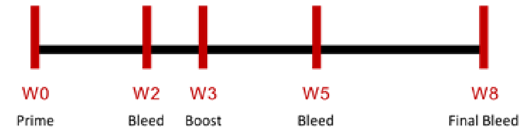


Figure 3. Immunization - bleeding schedule for mouse immunogenicity study.

Cellular immune responses will also be evaluated as described in the **Core C** Research Strategy. Briefly, mice will be sacrificed five days after the booster dose. Mouse blood PBMCs and cells from lymph nodes and spleen will be stimulated with a pool of antigenic peptides derived from the respective immunogen sequence(s). Antigen-specific polyfunctional cellular responses (IFN-γ, TNF-α, IL-2, IL-4, IL-17: CD4+ and CD8+ T-cell) will be determined by using multi-color flow cytometry and intracellular cytokine staining[65,66]. Studying the cellular responses for HNV immunogens has recently gained more interest and preliminary mouse studies have just begun using the HeV-sG vaccine subunit – antigenic peptides have been identified.

Aim 1.3: Evaluation of the immunogenicity and protection efficacy of PPZ MNP HNV vaccines in ferrets. The immunogenicity and efficacy of the lead PPZ MNP HNV vaccine candidates defined in Aim 1.2 will be tested in ferret challenge studies in Year 2 of the project (**Core E)**. Ferrets (n = 6 ferrets per group) will be immunized with the lead PPZ MNP HNV vaccine candidates via either single-dose (D0) or prime(D0)-boost(D21) immunization regimen. Unimmunized ferrets will serve as negative controls. (*Group 1*: Lead PPZ MNP HNV vaccine candidate 1 with the prime-boost regimen**.** *Group 2*: Lead PPZ MNP HNV vaccine candidate 2 with the prime-boost regimen. *Group 3*: Lead PPZ MNP HNV vaccine candidate 1 with the single-dose regimen. *Group 4*: Lead PPZ MNP HNV vaccine candidate 2 with the single-dose regimen. *Group 5*: Naïve/Unimmunized controls). The five groups will be challenged with a lethal dose (5000 TCID$_{50}$) of either NiV-B or HeV. Immunization, challenge and blood and swab sampling schedules are diagrammed in **Figure 4**. Vaccine-induced humoral responses in ferrets prior to virus challenge will be evaluated by **Core E** and **RP2**. All protected animals and survived controls will be euthanized on day 21 post-infection challenge (end of study) and lung and brain tissue along with swabs (oral and rectal) and blood samples will be collected. These will be tested for the presence of viral RNA by TaqMan PCR performed by using the primer sets and probes[35]. Virus isolation will only be attempted from specimens that were PCR positive for NiV-B or HeV genomes. Virus titration will be performed by plaque assay on Vero cells. The limit for detection will be 25 pfu/ml. The experimental details of the challenge studies in ferrets are described in **Core E**. End of study sera neutralization titers will also be measured by FRNT.

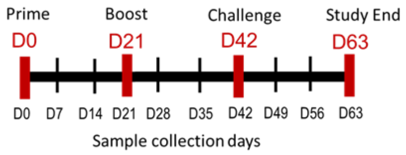


Figure 4. Expected schedule to immunize ferrets and AGMs in the planned efficacy studies. Blood and swabs, oral and rectal, samples will be taken weekly, on days 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63. Single dose groups do not receive boost immunization on D21.

Antigen-specific stimulation of cytokine production by ferret PBMCs will be assayed. The custom-synthesized antigen-specific Peptivators obtained from **Core C** will be compatible with these studies, but we acknowledge that there is a lack of ferret-specific reagents for comprehensive evaluation of cytokine production of peptivator-stimulated PBMCs. Unlike comprehensive studies in mice and AGMs, these studies will quantify the production of cytokines, for which ELISA kits are commercially available, such as ferret IL-17A, IFN-γ, TNF-α, and IL-2, 48 hr after stimulation (See, e.g.,[67]).

Aim 1.4: Immunogenicity and protection efficacy of the lead PPZ MNP HNV vaccine in AGMs. This challenge study will be in year 3 (**Core E**). The immunogen(s) and MNP vaccine type that provides the best cross-protection in the ferret studies will be tested in AGM NiV-B or HeV challenge studies. Fresh MNPs will be fabricated for this study. The study will be conducted by **Core E** and will entail four groups of AGMs (n = 3 AGMs per group). Immunizations with the lead PPZ MNP HNV vaccine candidate will be either as a single-dose (D0) or prime(D0)-boost(D21) regimen. Groups 1 and 2 will be immunized as a prime-boost strategy while groups 3 and 4 will be immunized with a single dose. Naïve unimmunized AGMs will serve as negative controls. The immunization, challenge and blood and swab sampling schedules will follow that as outlined in **Figure 4**. Groups 1 and 3 will be challenged with a lethal dose of NiV-B, while groups 2 and 4 will be challenged with a lethal dose of HeV (lethal dose = $1x10^5$ TCID$_{50}$). Sera from blood samples taken before the challenge will be tested by **RP2** to measure the serum neutralization titers using the same procedure as for mice and ferret sera. Antigen-specific total IgG levels will be determined by commercially available ELISA. Virus-specific neutralization activity against each of HeV and NiV-B will be tested by FRNT assay in the same way as proposed for mice and ferret sera samples. The AGM sera samples will also be tested for neutralization activity against authentic viruses (**Core E**) by using the BSL-4 PRNT assay. Antigen-specific stimulation of key cytokine production by AGM PBMCs, which will be obtained prior to live virus challenge studies, and will also be assessed as described in Aim 1.2.

Aim 1.5: Identifying the correlates of immunization with the lead PPZ MNP HNV vaccine across multiple species. Here we propose to determine the correlates of immunization with the lead PPZ MNP HNV vaccine candidate across multiple species – mice, ferrets, and AGMs. This aim will be inherently integrated with the Aims 1.2-1.4. During these experiments, 3hr post immunization, skin biopsies and serum samples will be obtained from mice, ferrets and AGMs. Prior to challenge studies in AGMs, serum and skin samples from the three different species (mice, ferrets, and AGMs) immunized with the lead PPZ MNP vaccine candidate will be assayed to quantify their cytokine levels (IL-6, TNF-α, IL-17A, IL-10, IL-1β, IL-6, IL-4, IL-5, TNF-α, IFN-α, IFN-β, IFN-γ, and IL-12p70). We note a limitation that while the multiplex assays are commercially available for mice and AGMs, we will have to use the cytokine-specific ELISAs for ferrets to determine only those cytokine levels for which ELISA kits are available, such as ferret IL-17A, IFN-γ, and TNF-α. We expect to identify within the obtained results correlates of immunization across the three species for the lead PPZ MNP vaccine candidate. We also expect that these correlates of immunization might be predictive for the vaccine protective efficacy.

Aim 1.6: Evaluate stability of the lead PPZ MNP HNV vaccine candidate. Here, we will test the thermostability of the lead PPZ MNP HNV vaccine candidates based on retention of their immunogenicity upon storage at room temperature and at 40°C for an extended period of time (1-, 3-, 6-, and 12-month storage). Mice (n = 5 mice per group) will be immunized with fresh and stored PPZ MNP HNV vaccines in a single-dose regimen and blood samples will be collected 2 weeks after immunization. To test the stability of PPZ MNP HNV vaccine candidates, serum levels of total IgG antibodies will be determined by **Core C** and the virus-specific neutralization capacity of these antibodies will be evaluated by the FRNT assay (**RP2**). Stability will be indicated by no statistically significant differences between binding antibody levels and neutralization titers induced by fresh PPZ MNP HNV vaccines compared to those elicited by stored PPZ MNP HNV vaccines. Additional stability testing will be initiated with the freshly manufactured MNP for the AGM challenge study.

Expected results, potential pitfalls and alternatives. Prior data on HNV vaccines support our expectations that we will be able to assess the potency and longevity of vaccine induced responses of lead PPZ MNP HNV vaccine candidates from the proposed studies. The HeV-sG immunogen has been tested as a HNV vaccine in multiple (2-dose prime-boost) prior studies and that protective pre-challenge ID$_{50}$ neutralizing titers against NiV-

M can be as low as of 1:32 in the feline model[35,38]; that more than 1 year post-vaccination and pre-challenge neutralizing titers as low as 1:16 to 1:128 in the ferret model are protective against NiV-M[40]; as an equine vaccine, horses with pre-challenge HeV neutralizing titers ranging from 1:16 to 1:32 at 6 months post-vaccination are completely protected[36]; and in AGMs pre-challenge ID_{50} neutralizing titers ranging from 1:28 to 1:379 against NiV-M are completely protective[34]; and most recently, a HeV-sG/Alum formulation protects against NiV-B challenge in AGMs with pre-challenge ID_{50} titers ranging from only 1:10 to 1:160[41]. Almost without exception, complete protection from challenge in these studies also shows no gross or histologic evidence of infection in any of the vaccinated subjects at study completions, with all tissues examined negative for viral antigen, with no viral genome detected. We expect to obtain protection in AGMs with the lead PPZ MNP HNV vaccine candidate. From the stability study, we will determine the shelf-life of the lead PPZ MNP HNV vaccine candidate. We are confident that if the preliminary mouse immunogenicity studies reveal neutralizing antibody responses are robust, this will translate to protection in the planned challenge studies in ferrets. Indeed, preliminary data has shown robust neutralizing ID_{50} titers from the mouse immunizations. The non-adjuvanted HeV-sG MNP induced an ID_{50} titer of 1:272 and the adjuvanted form HeV-sG+PCEP MNP induced an ID_{50} titer of 1:11,445 against the HeV chimera; and an ID_{50} titer of 1:331 against the NiV chimera. There is a minimal risk of not finding any protecting immunogen (MNP vaccine type or immunogen combination). The PABVAX Center team is sufficiently experienced to handle and resolve any possible unforeseen issues and pitfalls.

Aim 2: Rescue Langya virus and construct LayV and AngV glycoprotein bearing reporter virus tools.

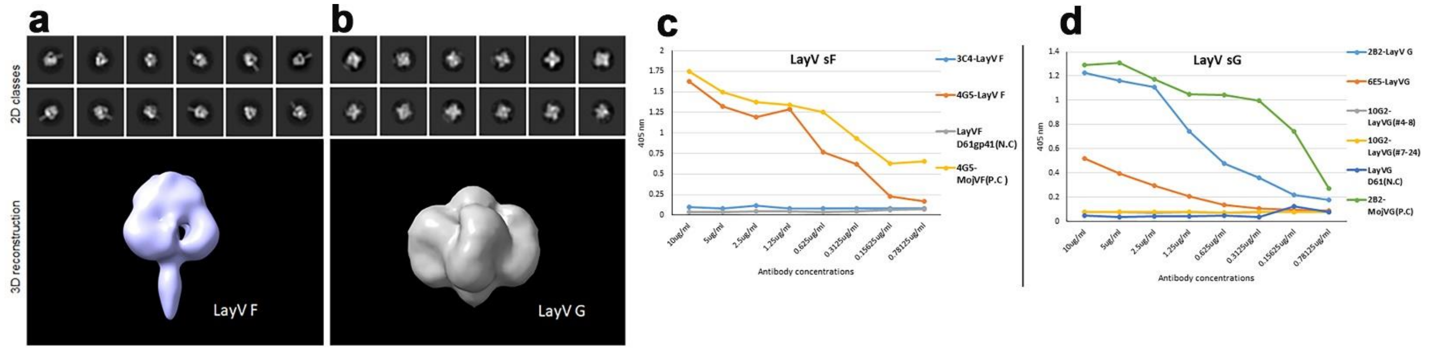


Figure 5. Negative stain EM analysis of LayV sF_{stb} and sG_{tet}. 2D and 3D classifications of LayV F trimers (**a**) and LayV G tetramers (**b**). ELISA cross-reactivity MojV F mAb (**c**, yellow) with LayV-sF and MojV G mAb (**d**, green) with LayV-sG.

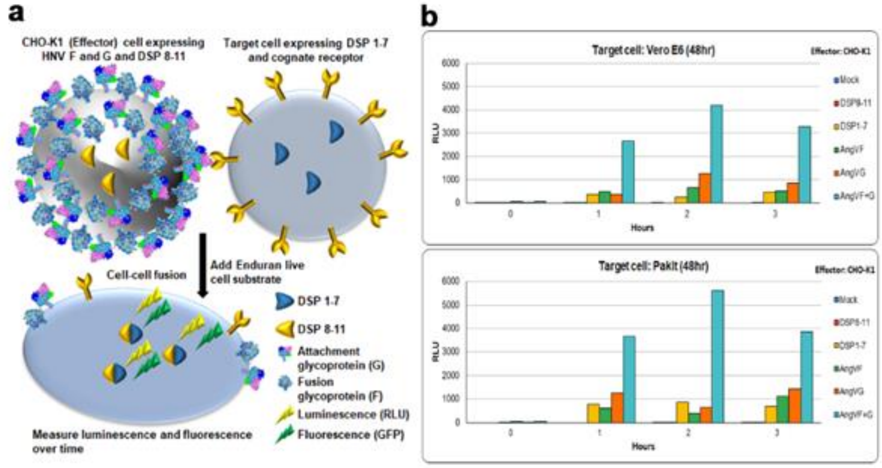


Figure 6. AngV F and G cell-cell fusion using a dual split luciferase. A schematic representation of the dual split-luc cell-cell fusion assay (**a**). Split-luc cell-cell fusion assay with AngV F and G plasmid constructs (**b**).

Rationale and Preliminary Data: Negative stain electron microscopy (EM) was performed on LayV F and G glycoproteins. The 2D and 3D classifications of LayV F (**Figure 5a**) and G (**Figure 5b**) revealed distinct trimer and tetramer assembly, respectively. In addition, using LayV-sF and LayV-sG, our previously generated mAbs to MojV F and G tested by ELISAs demonstrated that there was a dose dependent increase in LayV-sF (**Figure 5c**) and LayV-sG (**Figure 5d**) binding. This data forms the premise for rescuing a rCedV bearing LayV F and G glycoproteins (the sequences from LayV are likely correct). We will construct a full-length recombinant LayV sequence that encodes GFP. The LayV F and G coding sequences are the LayV isolate SDQD_H1801 (GenBank: OM101125.1); LayV F and LayV G; UUV47205.1 and UUV47206.1, respectively. The replication competent rCedV will be used. We will design, construct and rescue a new rCedV chimera that encodes the envelope glycoproteins (F and G glycoproteins) of LayV, i.e., a rCedV-LayV reporter virus encoding GFP. We will characterize it using cell-based infection tropism and growth kinetic assays. We have also utilized an established quantitative fusion assay based on a dual-split-luciferase reporter assay[42,43,68,69] to determine functionality of AngV envelope glycoproteins (F and G glycoproteins). Content mixing between F/G bearing effector cells and a permissive target cell restores luciferase and a signal can be measured[43] (**Figure 6a**). In addition, we have already constructed AngV sG$_{tet}$ and sF proteins and their structural solutions (both pre-fusion and post-fusion sF and the tetrameric structure of sG$_{tet}$) have been made by the PL of **RP5**, and cell-fusion is observed in Vero E6 cells and the bat kidney cell line, PaKiT, indicating that full-length AngV F/G are functional (**Figure 6b**). Taken together, these data suggest that the rescue of reporter viruses with LayV and AngV F/G is feasible. We will characterize viruses in cell-based infection tropism and growth kinetics assays. Alternatively, we will also construct a set of reporter gene-encoding VSV-based (LayV and AngV F/G bearing) reporter viruses as previously done with Australian bat lyssavirus (ABLV) G glycoproteins[70,71] and authentic ABLV[72,73].

Experimental Design.

Aim 2.1: Design and construct a recombinant Langya virus reporter virus. Figure 7 diagrams each of the proposed virus constructs that will be generated. The genome design, length and position of the green fluorescent protein gene are shown, and the experimental plans are described below. LayV helper plasmids for the N, P, and L genes will be synthesized. To construct the rLayV-GFP antigenome plasmid, large gene fragments of LayV will be synthesized and sequentially cloned into pOLTV5$_{opt}$ (**Figure 7a**). Unique restriction sites will be chosen to facilitate ligation of the LayV DNA fragments and to ensure adherence to the genomic "rule of six". All cloning will be performed with *E.coli* Stbl2 cells. The fully sequenced plasmids will be transferred to **Core E**. Virus rescue and subsequent virus characterization by cell-based assays will be conducted in collaboration with **Core E**. In addition, rLayV will be examined by **Core E** in animal model development studies such including the hamster and/or ferret, both successful for the prototype HNVs.

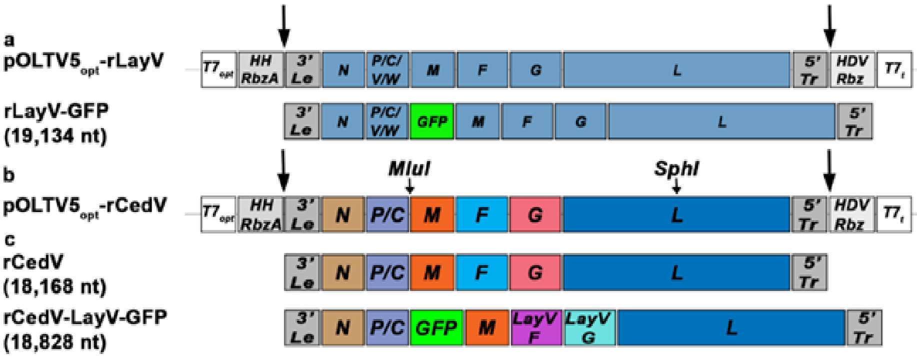


Figure 7. Schematic representation of recombinant reporter viruses. The pOLTV5$_{opt}$-rLayV plasmid (**a** top) and rLayV-GFP genome (**a** bottom). The arrows indicate regions of self-cleavage. The pOLTV5$_{opt}$-rCedV plasmid (**b**). The rLayV-GFP genome (**c** bottom). The long arrows indicate regions of self-cleavage. Shown are the unique restriction sites MluI and SphI that will be used to construct rCedV-LayV-GFP.

Aim 2.2: Construct, rescue and characterize a recombinant Cedar reporter virus encoding Langya virus envelope glycoproteins.

Generation of rCedV encoding LayV envelope glycoproteins. rCedV-LayV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the LayV F and G coding sequences. The CedV non-coding intergenic regions are retained to adhere to the genomic "rule of six". Unique restriction sites, will facilitate the insertion of the LayV F and G gene cassette into the genome clone (pOLTV5$_{opt}$-rCedV) (**Figure 7b**) generating rCedV-LayV-GFP (**Figure 7c**). All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-LayV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (HNV-CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. When maximal syncytia and GFP is observed (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[42-44,74].

***In vitro* characterization of rCedV-LayV-GFP**. The growth kinetics of rCedV-LayV-GFP compared to rCedV or rCedV-NiV/HeV chimeras will be assessed by infecting Vero cells with MOIs of 0.01, 0.1 and 1 and progeny virus recovered in 24hr intervals over a 72hr period and titered. In parallel experiments, infected cells will also be imaged for fluorescence and syncytia (HNV-CPE). **Figure 8** illustrates the syncytia (indicated by the yellow arrows) formed in Vero E6 cells infected with either rCedV-NiV-B-GFP or rCedV-HeV-GFP chimeras. The rCedV-LayV-GFP neutralization assays will be conducted by FRNT[44]. We have also already generated several murine mAbs to LayV F and G (3C4-LY and 4G5-LY to LayV F; and 2B2-LY and 6E5-LY to LayV F) and these will be used in neutralization assays. In addition, we will develop rabbit sera to LayV N, P and L proteins.

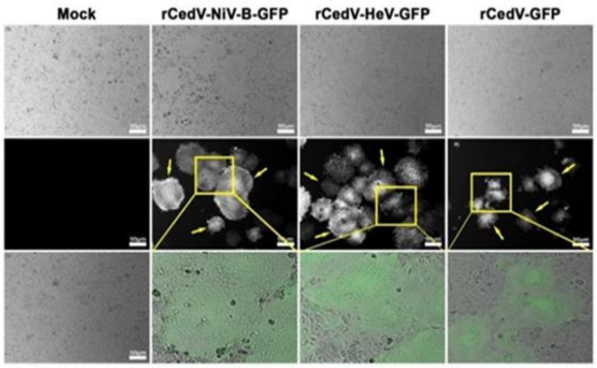


Figure 8. Syncytia induced by rCedV expressing NiV-B or HeV envelope glycoproteins. Vero E6 cells were uninfected (Mock) or infected with either rCedV-NiV-B-GFP, rCedV-HeV-GFP, or rCedV-GFP. All images are 24hrs post-infection. Zoomed-in fluorescence images (3rd row) are the yellow boxes. Arrows indicate giant multinucleated cells (syncytia). Representative images from three independent experiments are shown. Scale bar, 50 µm.

Aim 2.3: Construct, rescue and characterize recombinant reporter viruses encoding Angavokely virus envelope glycoproteins.

Generation of rCedV encoding AngV envelope glycoproteins. Figure 9 diagrams each of the proposed virus constructs that will be prepared. The genome designs, lengths and position of the *GFP* and *Luc* reporter genes are also shown. rCedV-AngV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the AngV F and G coding sequences. The AngV F and G coding sequences will be based on the GenBank: ON613535.1 sequence. The GenBank accession numbers for AngV F and AngV G are UVG43988.1 and UVG43989.1, respectively.

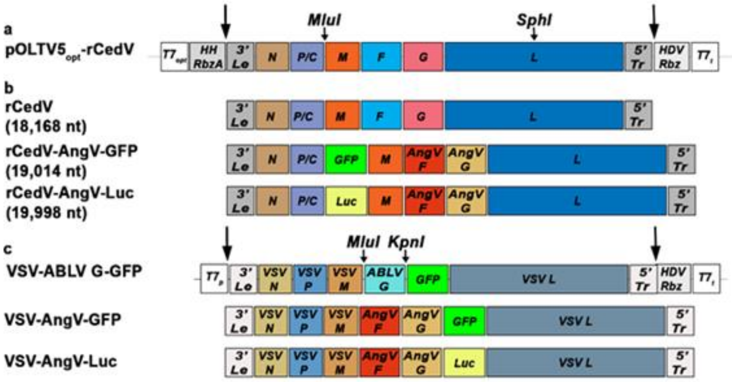


Figure 9. Schematic representation of the AngV chimeric reporter viruses. The pOLTV5$_{opt}$-rCedV plasmid (**a**). Shown are the unique restriction sites MluI and SphI that will be used to construct rCedV-AngV-GFP and rCedV-AngV-Luc. The rCedV-AngV-GFP and rCedV-AngV-Luc genome (**b**). Shown are the unique restriction sites MluI and KpnI that will be used to construct VSV-AngV-GFP and VSV-AngV-Luc (**c**). The long arrows indicate regions of self-cleavage.

CedV non-coding intergenic regions are retained to adhere to the genomic "rule of six". Unique restriction sites, will facilitate the insertion of the AngV F and G gene cassette into the rCedV antigenome plasmid (**Figure 9a**) generating rCedV-AngV-GFP (**Figure 9b**). The rCedV-AngV-Luc clone will be produced by swapping the reporter gene coding sequences. All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-AngV-GFP or pOLTV5$_{opt}$-rCedV-AngV-Luc antigenome plasmid. After 4 days cultures are examined for GFP expression and syncytia (HNV-CPE). BSR-T7/5 cells and supernatants from successful rescue are collected and passaged in Vero 76 cells (T-75 flasks) to prepare a virus stock. When maximal syncytia and GFP is observed (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[42-44,74].

Generation of a VSV encoding AngV envelope glycoproteins. We will also construct reporter gene encoding versions of a replication competent VSV encoding the AngV F and G glycoproteins (GFP and Luc) (**Figure 9c)**. A large gene cassette comprising VSV untranslated intergenic regions flanking the AngV F and G coding sequences will be synthesized. Unique restriction enzyme sites, will facilitate the insertion of the AngV F and G gene cassette into the VSV backbone plasmid to generate VSV-AngV-GFP (**Figure 9c**). The VSV-AngV-Luc clone will be produced by swapping the reporter gene coding sequences. Cloning procedures are performed in *E. coli* Stbl2 cells and all plasmid clones verified by sequencing. Rescue of VSV-AngV-GFP and VSV-AngV-Luc, will be performed in BSR-T7/5 cells, in 12-well plates, transfected with helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-AngV-GFP or VSV-AngV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus uses HEK293T cells (6-well plates) by inoculation with the supernatant from step 1. Typically, when GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above and the virus stocks stored at -80°C. Larger viral stocks are prepared in T-150 flasks in HEK293T cells, and harvested virus supernatants are purified by buffered sucrose cushion centrifugation and virus pellets resuspended in 10% sucrose/TNE buffer, and small volume single use aliquots stored at -80°C. All virus stocks are deep sequenced. Virus stocks (GFP) are titered (infectious units/ml (IU/ml)) by serial dilution on HEK293T cells in 96-well black walled plates. At 24hrs post-infection plates are fixed with 4% formaldehyde, washed 3x with diH_20 and imaged (fluorescence) with a CTL S6 analyzer. Luciferase encoding VSV stocks are titered by plaque assay[75].

Characterization of the recombinant reporter viruses in cell-based infection assays. Replication kinetics of rCedV-based and VSV-based AngV F/G viruses are assayed by Vero cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in 24hr intervals and titered. rCedV-AngV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Similarly, VSV-AngV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G[71]. The rCedV-AngV-GFP and VSV-AngV-GFP assays will be conducted by FRNTs[44] and rCedV-AngV-Luc and VSV-AngV-Luc in luciferase assays[42].

Expected results, potential pitfalls and alternatives. We expect that rCedV-LayV-GFP and rCedV-AngV reporter viruses can be rescued. In addition, we expect that these chimeric viruses will induce syncytia and replicate to high titers. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. In the event that the rescue of rCedV chimeric viruses are not viable, multiple strategies may be examined. 1) test different ratios of helper plasmids and antigenome plasmid; 2) perform rescue in different cell lines; 3) construct alternate versions of the LayV and/or AngV F and G genes within the antigenome plasmids, such as, fuse the LayV and/or AngV F and G ectodomains with the transmembrane and/or cytoplasmic tail domains of the corresponding CedV F and G elements which may facilitate virus particle formation and membrane fusion activity.

<u>Aim 3:</u> Evaluate HNV-specific antibodies and nanobodies developed by the Center's RPs and Core D.

Rationale and Preliminary Data. The rCedV chimeric system we established replaces the need for authentic NiV-B and HeV in neutralization assays. Neutralization values of well-characterized NiV/HeV cross-reactive neutralizing mAbs highly correlated between rCedV-NiV-B-GFP and rCedV-HeV-GFP and authentic NiV-B and HeV at BSL-4 when tested by conventional PRNT[44]. The Pearson correlation analysis between the rCedV chimeras BSL-2 PRNT and the NiV-B or HeV BSL-4 PRNT is shown in **Figure 10a**. We further developed the rCedV-NiV-B-GFP and rCedV-HeV-GFP neutralization assay into a high-throughput and quantitative assay that is based on the reduction of GFP foci, a FRNT[44]. The Pearson correlation analysis between the chimeras PRNT and FRNT is shown in **Figure 10b**. Taken together, these data demonstrate that rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeric viruses are an ideal set of suitable surrogate viruses for conducting a rapid FRNT-based assay for assessing NiV and HeV antibody neutralization.

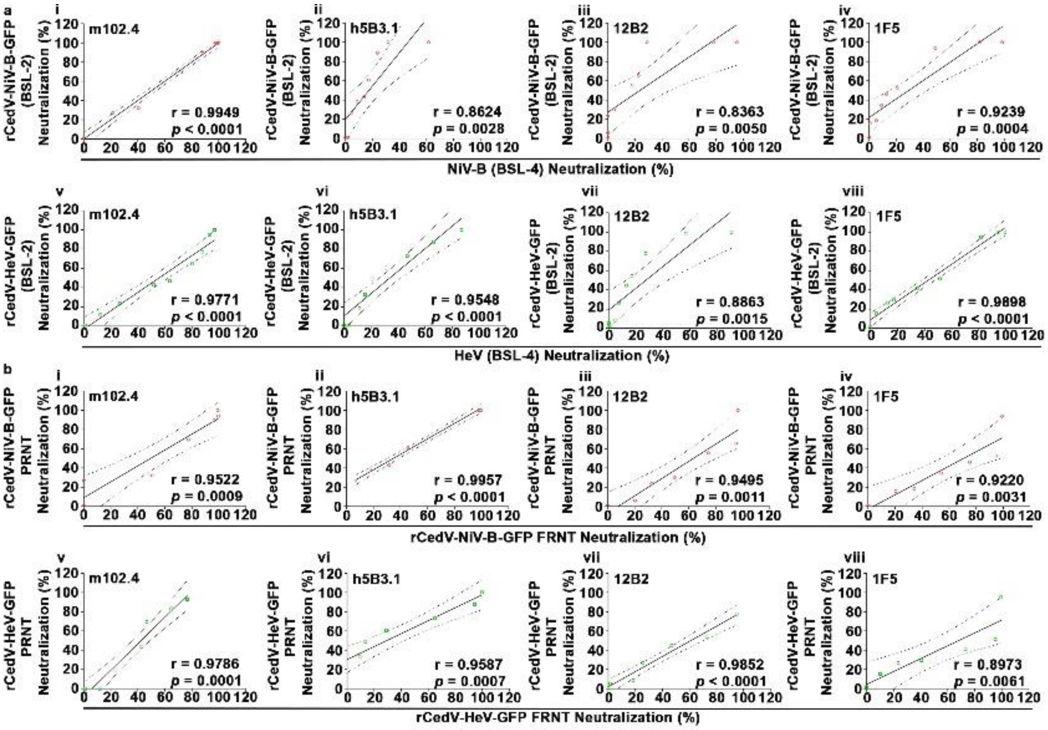


Figure 10. Pearson correlation analysis of rCedV chimeras. Pearson correlation analysis of PRNT neutralization (%) values of rCedV-NiV-B-GFP versus NiV-B (**a**, **i-iv**) and rCedV-HeV-GFP versus HeV (**a**, **v-viii**). Pearson correlation analysis from PRNTs (y-axes) and FRNTs (x-axes) of rCedV-NiV-B-GFP (**b**, **i-iv**) and rCedV-HeV-GFP (**b**, **v-viii**). Neutralization with mAbs m102.4, h5B3.1, 12B2 or 1F5 are shown. The Pearson correlation coefficient 'r,' p-value (two-tailed), linear regression line (solid lines), and 95% confidence intervals (dashed lines).

Experimental Design.

Here, using the new tools (rCedV-LayV-GFP, rCedV-AngV-GFP, rCedV-AngV-Luc, VSV-AngV-GFP and VSV-AngV-Luc) we will assess neutralizing activity, of HNV specific mAbs and/or sera from HNV immunized mice that will be generated by **Core D**. The FRNT is high throughput and performed in 96-well plates to establish a 7-point dose response. Serially diluted immune sera or mAbs are mixed with either rCedV-NiV-B-GFP or rCedV-HeV-GFP for 2hrs. The virus-mAb or virus-sera mixtures are then added in triplicate to confluent pre-seeded Vero 76 cells in black-walled clear bottom 96-well plates and incubated for an additional 24hrs. Plates are then fixed and imaged and fluorescent foci counted using the CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) will be determined as the mAb concentration or serum dilution at which there was a 50% reduction in fluorescent foci versus untreated control wells. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9.

Expected results, potential pitfalls and alternatives. We do not anticipate any unexpected limitations in assessing the neutralization potential of antibodies and sera generated from other research projects and/or cores as we have successfully demonstrated that the rCedV-GFP chimeric FRNT platform can serve as a surrogate BSL-2 neutralization assay that is high throughput with a fast turnaround time.

Aim 4: Validate PPZ-MNP platform with another paramyxovirus-specific antigen in Phase 2.

At completion of Phase I we will have i) a procedure to fabricate PPZ adjuvanted MNP using sG, sF or a combination of both viral glycoproteins; ii) generated rLayV with an associated animal model in collaboration with **Core E** with reporter virus construct (rCedV-LayV-GFP); and iii) generated reporter viruses AngV (rCedV-AngV-GFP, rCedV-AngV-Luc, VSV-AngV-GFP and VSV-AngV-Luc). Here, in Phase II we propose to test the hypothesis that the PPZ-MNP vaccine platform technology can be adapted and transferred as a 'plug and play' strategy for other paramyxoviruses, namely the divergent HNVs LayV and AngV. The major goal rests on the rescue of LayV and establishing an infection/disease model of this most recently discovered pathogenic, zoonotic, HNV. Nevertheless, if rescue of rLayV with an associated animal model is not achieved, we will still be able to test the MNP approach as proof-of-concept to the extent of generating robust immune responses in animals and testing neutralizing antibody responses using both LayV and AngV reporter virus systems.

PPZ-MNP manufacture. The fabrication of the MNPs by **Core C** will be prepared for both the F and G viral glycoproteins: LayV-sG, LayV-sF, AngV-sG and AngV-sF depending on the data and outcomes, and vaccine down-selection in Phase I we may also include a combination of sG and sF. Vaccine antigens will have been prepared by **Core D**. It may be important to evaluate both the attachment and fusion glycoprotein immunogens for LayV and AngV in Phase II. Although the preponderance of data to date for the pathogenic prototype HNVs (NiV/HeV) indicates that the attachment glycoprotein (G) alone can serve as an effective vaccine immunogen, this is presently unknown for these two divergent HNVs, so test both sF and sG for these HNVs is needed.

Immunogenicity. As in Aim 1, groups of 5 mice will be used for each immunogen(s) and a control of nonimmunized subjects. Sera will be drawn as scheduled in **Figure 3**. ELISA titers of sera to vaccine immunogens will be evaluated by **Core C** and sera will be evaluated for virus neutralization with newly developed reporter viruses. Cellular immune responses will be assessed as described in Aim 1.

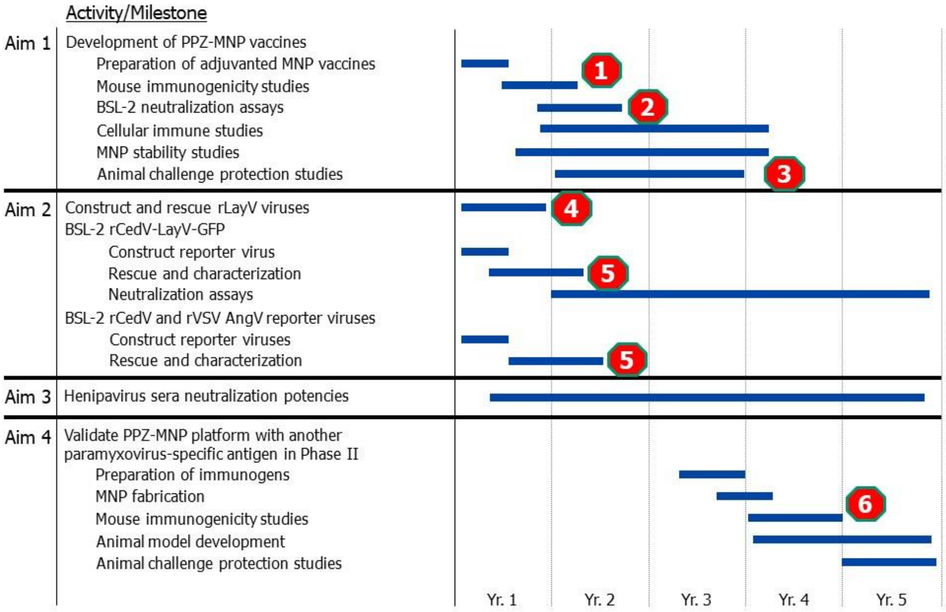
Animal challenge studies. The experimental design for these studies will be adjusted once the animal model has been developed. The basic design will follow those for ferrets and AGMs described in Aim 1. The immunogen will be chosen based on the results from the mouse sera neutralization tests.

Expected results, potential pitfalls and alternatives. We expect that LayV / AngV glycoproteins MNPs fabrication will be possible. Mouse immunization studies with MNPs are likely to be uncomplicated and we will be able to assess immune responses. These data will determine whether the sG or sF is the immunogen of choice for best virus neutralization antibody induction. If rescue of rLayV and establishing an animal model are not met, the ability to measure inhibition of virus in cell-based assays, using either the new reporter viruses or a cell-cell fusion surrogate assays will be done. The mouse immunogenicity studies, together with the virus neutralization assays will provide a foundation for potential anti-LayV and anti-AngV MNP vaccine approaches.

Project Milestones, Timelines, and Go/No-go decisions. The **RP2** Aims, milestones, and timeline with activities having periodic assessments by Center leaders, SAB, and NIAID staff as shown in the Gannt Chart.

Aim 1: Develop PPZ-MNP-based sG$_{tet}$ and sF$_{stb}$ prototype HNV subunit vaccines

Milestone 1: Adjuvanted MNP vaccines are successfully prepared: In year 1, the MNP vaccine fabrication by **Core C** will commence and formulation of recombinant antigens with PCEP adjuvant in the context of MNPs will be assessed. We are confident that this stage of the research plan will be achievable because we have already had success in MNP fabrication with the HeV-sG formulated with two different PPZ adjuvants (PCEP and PCPP) and the preliminary mouse immunization and cellular immune responses and induction of NiV/HeV cross-neutralizing antibody titers was excellent, with ID$_{50}$ titers significantly greater than previously measured pre-challenge ID$_{50}$ titers that provided complete protection across several prior studies in cats, ferrets, horses, and AGMs (preliminary data). We expect that the formulation of the remaining 3 prototype HNV immunogens (NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb}$ (and the combination test: HeV-sG+NiV-sFstb)) adjuvanted with PCEP and applied to the MNPs will be fully achievable. Analysis will also include antigen stability studies by re-release of sG$_{tet}$ and sF$_{stb}$ immunogens and recognition by select conformation dependent mAbs. A go/no-go decision may be made pending the results of MNP fabrication and antigen analysis of each MNP vaccine type, poor fabrication or loss of antigen integrity will trigger termination of those individual vaccines prior to immunogenicity studies.



Gannt chart outlining project milestones (left). Solid lines represent periods of activity to achieve milestones. ***Go/No-go*** decision points are indicated by red octagons as follows:

(1) Successful fabrication of vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity is a ***No-go*** for the respective individual vaccine MNP.

(2) An SNT <10 is a ***No-go*** for the tested immunogen. The highest SNT >10 is a ***Go*** for the respective immunogen.

(3) Successful protection is a ***Go*** to the project Phase II. No protection is a ***No-go*** for the MNP vaccine platform.

(4) Successful rescue of rLayV is a ***Go*** to the development animal models in Aim 4. Otherwise is a ***No-go*** for the animal models development and the challenge protection studies in Aim 4.

(5) Successful rescue of a reporter virus is a ***Go*** for neutralization assays development. Inability to rescue a reporter virus is a ***No-go*** to that reporter virus neutralization assay development.

(6) Successful fabrication of vaccine MNPs is a ***Go***. Poor fabrication, loss of antigen integrity is a ***No-go*** for the respective individual vaccine MNP.

Milestone 2: High immunogenicity and best in class immunogen(s) identified: **Core C** will conduct immunogenicity studies in mice as detailed in the preliminary data and experimental plan and the Core research plan; multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction, virus-neutralization, and polyfunctional T-cell responses by in vitro stimulation). As sera are made available from the mice MNP vaccine cohorts, we will assess the NiV/HeV-neutralizing antibody activities of each of the MNP vaccine formulations of the prototype HNV immunogens (NiV-sG$_{tet}$, NiV-sF$_{stb}$, HeV-sF$_{stb}$, HeV-sG, and HeV-sG+NiV-sF$_{stb}$). The criteria to judge success and go/no-go decisions will be levels of cross-neutralizing antibody responses (neutralization of both HeV and NiV-B) induced by each vaccine type. We expect to be able to down-select to 'best-in-class' vaccine as a single antigen or perhaps the combination antigen MNP vaccine formulation at this stage prior to progressing to the first-round ferret challenge models.

Milestone 3: MNP vaccination protection in animal challenge studies: Here, the determination of whether MNP vaccination affords protection in animal model challenge experiments will be made. For the NiV and HeV prototypes the animal models (ferret and AGM) are extremely well-developed and the PABVAX Center leadership and staff have extensive experience in this area, and outcomes at this stage should be readily assessable. If we are successful in developing and validating the PPZ adjuvanted MNP recombinant protein vaccine approach for the HNV prototypes (NiV/HeV) and demonstrate protection in the planned homologous and heterologous HNV challenge studies, this will be expected to be a 'go' decision point in year 3, and the proposed Specific Aim 4 tasks (below) will continue in preparation for Phase II of the PABVAX Center. If in the unlikely event that the MNP vaccine approach fails (no-go) then further consultation with industry and regulatory expertise within **Core D** and partners and the **SAB** will be geared towards using the previously identified 'best-in-class' immunogens with alternative vaccine platforms (IM formulations; virus vectored such as a VSV technology held by the PABVAX Center PI and RP1 PI at UTMB; or mRNA, Moderna Inc., has provided the Center a letter of support).

Aim 2: Rescue Langya virus and construct LayV and AngV glycoprotein bearing reporter virus tools.

Milestone 1: Successful rescue of recombinant Langya virus. Since the genetic data on LayV is derived from an actual human virus isolate, it suggests that rescue of rLayV will be possible. **RP2** staff have extensive experience in HNV reverse genetics with rCedV (and VSV) with unique newly developed reagents. Virus rescue is in collaboration with **Core E**, followed by virus characterization in cell-based assays. **Core E** will then initiate small animal model development studies such as the hamster and/or ferret, both successful for the prototype HNVs. If rescue and animal modeling is successful, it will be very significant advancement for this pandemic preparedness initiative and objectives, and the virus, reagents, tools and models supplied to the ReVAMPP network. If rescue is not possible a no-go decision is obvious for animal model development. However, even if a LayV isolate remains unavailable by other means, we can still test the MNP approach with LayV and also our alternative divergent HNV prototype (AngV) by characterizing G and/or F vaccine-induced neutralizing antibodies using alternative reporter viruses (Milestone 2 below) or cell-based (cell-cell fusion assays; **Figure 6B**).

<u>*Milestone 2: Construction of rCedV and rVSV reporter viruses encoding LayV and AngV F/G glycoproteins:*</u> Here, we have reasonable confidence that one or both alternate reporter virus platforms will be possible. Successful rescue of these reporter viruses will enable the determination of immune sera neutralization titers and is a 'go' for the development of the neutralization assays. An unsuccessful rescue of either of the LayV or AngV reporters will be a no-go for those assay developments. If neither reporter virus is rescued we will proceed to cell-cell fusion assays development to study the immune sera neutralization titers. We have already successfully generated recombinant sF_{stb} and sG_{tet} glycoproteins from AngV and LayV based on available sequence data (AngV, **RP2** in collaboration with **Dr. Cara Brook,** The University of Chicago, and letter of support included in the PABVAX Overall component; and LayV, in collaboration with **RP5** leaders and **Genovac, Inc.**). In our prior experience, success in making soluble and secreted oligomeric forms of virus glycoproteins strongly indicates that genetic sequences are correct which should also translate into functional proteins. Rescued viruses will be characterized in various cell-based assays. These new virus tools will be used in collaboration with **RP4**, **RP5** and **Core D**, in assessing newly developed mAbs and nabs against LayV and AngV, and also vaccine-induced neutralizing antibody responses in PABVAX Center activities in Phase II.

Aim 3: Is also shown which is the collaborative support provided by **RP2** in assessing specific antibody development activities to HNVs by **RP4** and **RP5** is also included.

<u>***Aim 4: Validate PPZ-MNP platform with another paramyxovirus-specific antigen in Phase 2***</u>. At this stage one or both of the divergent HNV prototypes (LayV and/or AngV) would be selected and the milestones for progress would include MNP vaccine manufacture; mouse immunogenicity studies; and virus challenge protection. These milestones will be similar as described for Specific Aim 1. We have obtained preliminary structural characterizations of sF_{stb} and sG_{tet} glycoproteins from AngV and LayV (native-like oligomeric and stabilized glycoproteins) in collaboration with **RP5** leaders, and have also determined (preliminary data) that the LayV sF_{stb} and sG_{tet} glycoproteins are recognized by 3 previously generated mAbs to MojV F/G from our lab (**RP2**). As discussed above, the primary HNV target of **RP2** is LayV, and the alternate is AngV, but the go/no-go decisions on the virus target will depend on the progress and outcomes of the earlier studies. Also, as discussed above, even in the absence of rLayV rescue or its availability from another source and its characterization in animal models by **Core E**; or the possible isolation of AngV and its work-up *in vivo* and pathogenesis studies by Core E; we expect to still be able to assess and validate the recombinant subunit protein MNP vaccine approach with our already prepared immunogens and the other virus tools we plan on developing.

Industry Expertise and Regulatory Considerations

Consultation with industry and regulatory expertise within **Cores A and D** and the **SAB** will be critical and occur with regularity as the project progresses by quarterly virtual meetings, written quarterly reports and data analysis, and annual meetings with Center participants. Relative to the major goal of **RP2** (MNP vaccines): Stability, manufacturability testing, and early process development of the MNP-based vaccines will be researched in collaboration with **Core C** and **Core D** industry experts, and interface with the **SAB**. As vaccines are regulated by The Center for Drug Evaluation and Research (CDER) of the U.S. Food and Drug Administration (FDA), performance of all activities will be as per regulatory expectations. Consultation and input from **Core D** vaccine industrial partners (Emergent Biosolutions, Inc., and Moderna, Inc.; letters of support provided) will be integrated into the translational development efforts of the MNP-based HNV vaccine platform. The expectation in the out years of Phase I of the PABVAX period, leaders of Center will hold a pre-IND (type B) meeting with the FDA to discuss the Chemistry Manufacturing and Controls (CMC) section (topics such as cleavage or tag removal strategies if an issue for any recombinant protein vaccine candidate(s) for Drug Substance and strategy for manufacturing and releasing Drug Product), along with the all efficacy data from **RP1/RP2/Core E** and future IND-enabling toxicology studies to be supported by other NIAID mechanisms. Feedback from such meetings will be valuable for the translational development of any PPZ-MNP vaccines toward future "plug and play" pandemic readiness efforts, including the PABVAX Phase II plans, and the overall goal of the NIAID, ReVAMPP Centers.

If new neutralizing mAbs to the F glycoprotein of NiV/HeV (cross-reactive) or to LayV or AngV or nabs or bi-specific nabs that show antiviral activities to NiV/HeV, from the collaborations of **RP2, RP3, RP4** and **RP5** are found, we will seek industry and regulatory expertise in **Core D** and the **SAB** in areas such as; identification of correlates of protection, lead optimization, stability and manufacturability testing. Antibodies may be engineered (such as altered N-glycans, Fc mutations for effector functions and extended half-life, multimeric formats) by **Core D** and further evaluated to correlate mechanisms of action with maximal efficacy by **Core E** (authentic HNVs) and **RP3** (rCedV chimera model). This work will identify lead prototypes to transition to early process development. Further details on antibody development, milestones, and go/no-go decisions are discussed in the relevant RPs and **Cores D** and **E**.

References cited

1 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33 (1995).

2 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

3 Chua, K. B. Nipah virus outbreak in Malaysia. *J Clin Virol* **26**, 265-275 (2003).

4 Chua, K. B. *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**, 1257-1259 (1999). https://doi.org:10.1016/S0140-6736(99)04299-3 [doi]

5 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res* **100**, 8-13 (2013). https://doi.org:10.1016/j.antiviral.2013.06.012

6 Geisbert, T. W., Feldmann, H. & Broder, C. C. in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 153-177 (Springer, Berlin, Heidelberg, 2012).

7 Mehand, M. S., Al-Shorbaji, F., Millett, P. & Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral Res* **159**, 63-67 (2018).

8 *Priority Diseases 2021*, <https://cepi.net/research_dev/priority-diseases/> (2021).

9 Brende, B. *et al.* CEPI-a new global R&D organisation for epidemic preparedness and response. *Lancet* **389**, 233-235 (2017). https://doi.org:10.1016/s0140-6736(17)30131-9

10 Anonymous. Hendra virus - Australia (02): (QL) horse. Report No. 20220710.8704331, (International Society for Infectious Diseases, 2022).

11 Broder, C. C., Weir, D. L. & Reid, P. A. Hendra virus and Nipah virus animal vaccines. *Vaccine* **34**, 3525-3534 (2016). https://doi.org:10.1016/j.vaccine.2016.03.075

12 Homaira, N. *et al.* Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect* **138**, 1630-1636 (2010). https://doi.org:10.1017/S0950268810000695 [doi]

13 Homaira, N. *et al.* Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One* **5**, e13570 (2010). https://doi.org:10.1371/journal.pone.0013570 [doi]

14 Luby, S. P. *et al.* Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis* **15**, 1229-1235 (2009).

15 Rahman, M. A. *et al.* Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector borne and zoonotic diseases (Larchmont, N.Y.)* **12**, 65-72 (2012). https://doi.org:10.1089/vbz.2011.0656

16 Anonymous. Nipah virus - Bangladesh (05). Report No. 20230208.8708217, (International Society for Infectious Diseases, 2023).

17 Lee, S. H. *et al.* Discovery and Genetic Characterization of Novel Paramyxoviruses Related to the Genus Henipavirus in Crocidura Species in the Republic of Korea. *Viruses* **13** (2021). https://doi.org:10.3390/v13102020

18 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836 (2012). https://doi.org:10.1371/journal.ppat.1002836

19 Zhang, X. A. *et al.* A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med* **387**, 470-472 (2022). https://doi.org:10.1056/NEJMc2202705

20 Drexler, J. F. *et al.* Henipavirus RNA in African bats. *PLoS One* **4**, e6367 (2009). https://doi.org:10.1371/journal.pone.0006367 [doi]

21 Madera, S. *et al.* Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol* **96**, e0092122 (2022). https://doi.org:10.1128/jvi.00921-22

22 Vanmechelen, B. *et al.* The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol* **8**, veac061 (2022).

23 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066 (2014). https://doi.org:10.3201/eid2006.131022

24 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses and public health* **60**, 69-83 (2013). https://doi.org:10.1111/j.1863-2378.2012.01501.x

25 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951 (2011). https://doi.org:10.4269/ajtmh.2011.10-0567 [doi]

26 Schountz, T. *et al.* Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **11** (2019). https://doi.org:10.3390/v11030291

27 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473 (2020). https://doi.org:10.1146/annurev-virology-021920-113833

28 Broder, C. C. *et al.* in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 197-223 (Springer, Berlin, Heidelberg, 2012).

29 Basler, C. F. in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 123-152 (Springer, Berlin, Heidelberg, 2012).

30 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral Res* **124**, 69-76 (2015). https://doi.org:10.1016/j.antiviral.2015.09.017

31 Marsh, G. A. & Wang, L. F. Hendra and Nipah viruses: why are they so deadly? *Curr Opin Virol* **2**, 242-247 (2012). https://doi.org:10.1016/j.coviro.2012.03.006

32 NIH, C. a. (ed U.S. Department of Health and Human Services) 248-250 (US Government Printing Office, Washington, 2020).

33 Bossart, K. N. *et al.* Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol* **79**, 6690-6702 (2005).

34 Bossart, K. N. *et al.* A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Science translational medicine* **4**, 146ra107 (2012). https://doi.org:10.1126/scitranslmed.3004241

35 McEachern, J. A. *et al.* A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* **26**, 3842-3852 (2008). https://doi.org:10.1016/j.vaccine.2008.05.016

36 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379 (2014). https://doi.org:10.3201/eid2003.131159

37 Mire, C. E. *et al.* A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol* **88**, 4624-4631 (2014). https://doi.org:10.1128/jvi.00005-14

38 Mungall, B. A. *et al.* Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol* **80**, 12293-12302 (2006).

39 Pallister, J. *et al.* A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine* **29**, 5623-5630 (2011). https://doi.org:10.1016/j.vaccine.2011.06.015

40 Pallister, J. A. *et al.* Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J* **10**, 237 (2013). https://doi.org:10.1186/1743-422x-10-237

41 Geisbert, T. W. *et al.* A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. *NPJ Vaccines* **6**, 23 (2021).

42 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084 (2021). https://doi.org:10.1016/j.antiviral.2021.105084

43 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018). https://doi.org:10.1186/s12985-018-0964-0

44 Amaya, M. *et al.* A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses* **15** (2023). https://doi.org:10.3390/v15051077

45 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36**, 109628 (2021). https://doi.org:10.1016/j.celrep.2021.109628

46 Wang, Z. *et al.* Architecture and antigenicity of the Nipah virus attachment glycoprotein. *Science* **375**, 1373-1378 (2022). https://doi.org:10.1126/science.abm5561

47 Wang, Z. *et al.* Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. *Proc Natl Acad Sci U S A* **119**, e2122769119 (2022). https://doi.org:10.1073/pnas.2122769119

48 Avanzato, V. A. *et al.* A structural basis for antibody-mediated neutralization of Nipah virus reveals a site of vulnerability at the fusion glycoprotein apex. *Proc Natl Acad Sci U S A* **116**, 25057-25067 (2019). https://doi.org:10.1073/pnas.1912503116

49 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987 (2019). https://doi.org:10.1038/s41594-019-0308-9

50 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434 (2021). https://doi.org:10.1038/s41594-021-00584-8

51 Dong, J. *et al.* Potent Henipavirus Neutralization by Antibodies Recognizing Diverse Sites on Hendra and Nipah Virus Receptor Binding Protein. *Cell* **183**, 1536-1550 e1517 (2020). https://doi.org:10.1016/j.cell.2020.11.023

52 Mire, C. E. *et al.* A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis* **221**, S471-S479 (2020). https://doi.org:10.1093/infdis/jiz515

53 Playford, E. G. *et al.* Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis* (2020). https://doi.org:10.1016/s1473-3099(19)30634-6

54 Andrianov, A. K., Marin, A. & Fuerst, T. R. Molecular-Level Interactions of Polyphosphazene Immunoadjuvants and Their Potential Role in Antigen Presentation and Cell Stimulation. *Biomacromolecules* **17**, 3732-3742 (2016). https://doi.org:10.1021/acs.biomac.6b01251

55 Andrianov, A. K. *et al.* In Vivo and In Vitro Potency of Polyphosphazene Immunoadjuvants with Hepatitis C Virus Antigen and the Role of Their Supramolecular Assembly. *Mol Pharm* **18**, 726-734 (2021). https://doi.org:10.1021/acs.molpharmaceut.0c00487

56 Andrianov, A. K. & Langer, R. Polyphosphazene immunoadjuvants: Historical perspective and recent advances. *J Control Release* **329**, 299-315 (2021). https://doi.org:10.1016/j.jconrel.2020.12.001

57 Chand, D. J., Magiri, R. B., Wilson, H. L. & Mutwiri, G. K. Polyphosphazenes as Adjuvants for Animal Vaccines and Other Medical Applications. *Front Bioeng Biotechnol* **9**, 625482 (2021). https://doi.org:10.3389/fbioe.2021.625482

58 Eng, N. F. *et al.* The potential of polyphosphazenes for delivery of vaccine antigens and immunotherapeutic agents. *Curr Drug Deliv* **7**, 13-20 (2010). https://doi.org:10.2174/156720110790396481

59 Andrianov, A. K. *et al.* Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A* **106**, 18936-18941 (2009). https://doi.org:10.1073/pnas.0908842106

60 Valencia, S. M. *et al.* Improvement of RG1-VLP vaccine performance in BALB/c mice by substitution of alhydrogel with the next generation polyphosphazene adjuvant PCEP. *Hum Vaccin Immunother* **17**, 2748-2761 (2021). https://doi.org:10.1080/21645515.2021.1875763

61 Magiri, R. *et al.* Intradermal immunization with inactivated swine influenza virus and adjuvant polydi(sodium carboxylatoethylphenoxy)phosphazene (PCEP) induced humoral and cell-mediated immunity and reduced lung viral titres in pigs. *Vaccine* **36**, 1606-1613 (2018). https://doi.org:10.1016/j.vaccine.2018.02.026

62 Magiri, R. B., Lai, K. J., Mutwiri, G. K. & Wilson, H. L. Experimental PCEP-Adjuvanted Swine Influenza H1N1 Vaccine Induced Strong Immune Responses but Did Not Protect Piglets against Heterologous H3N2 Virus Challenge. *Vaccines (Basel)* **8** (2020). https://doi.org:10.3390/vaccines8020235

63 Guillot, A. J. *et al.* Microneedle-Based Delivery: An Overview of Current Applications and Trends. *Pharmaceutics* **12** (2020). https://doi.org:10.3390/pharmaceutics12060569

64 Bediz, B. *et al.* Dissolvable microneedle arrays for intradermal delivery of biologics: fabrication and application. *Pharm Res* **31**, 117-135 (2014). https://doi.org:10.1007/s11095-013-1137-x

65 Al-Attiyah, R., El-Shazly, A. & Mustafa, A. S. Comparative analysis of spontaneous and mycobacterial antigen-induced secretion of Th1, Th2 and pro-inflammatory cytokines by peripheral blood mononuclear cells of tuberculosis patients. *Scand J Immunol* **75**, 623-632 (2012). https://doi.org:10.1111/j.1365-3083.2012.02692.x

66 Balmert, S. C. *et al.* A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. *iScience* **25**, 105045 (2022). https://doi.org:10.1016/j.isci.2022.105045

67 van de Ven, K., de Heij, F., van Dijken, H., Ferreira, J. A. & de Jonge, J. Systemic and respiratory T-cells induced by seasonal H1N1 influenza protect against pandemic H2N2 in ferrets. *Commun Biol* **3**, 564 (2020). https://doi.org:10.1038/s42003-020-01278-5

68 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715 (2019). https://doi.org:10.1073/pnas.1911773116

69 Navaratnarajah, C. K., Rosemarie, Q. & Cattaneo, R. A Structurally Unresolved Head Segment of Defined Length Favors Proper Measles Virus Hemagglutinin Tetramerization and Efficient Membrane Fusion Triggering. *J Virol* **90**, 68-75 (2016). https://doi.org:10.1128/JVI.02253-15

70 Weir, D. L., Laing, E. D., Smith, I. L., Wang, L. F. & Broder, C. C. Host cell virus entry mediated by Australian bat lyssavirus G envelope glycoprotein occurs through a clathrin-mediated endocytic pathway that requires actin and Rab5. *Virol J* **11**, 40 (2014). https://doi.org:10.1186/1743-422X-11-40

71 Weir, D. L., Smith, I. L., Bossart, K. N., Wang, L. F. & Broder, C. C. Host cell tropism mediated by Australian bat lyssavirus envelope glycoproteins. *Virology* **444**, 21-30 (2013). https://doi.org:10.1016/j.virol.2013.06.016

72 Laing, E. D. *et al.* Enhanced Autophagy Contributes to Reduced Viral Infection in Black Flying Fox Cells. *Viruses* **11** (2019). https://doi.org:10.3390/v11030260

73 Mastraccio, K. E. *et al.* Establishment of a longitudinal pre-clinical model of lyssavirus infection. *J Virol Methods* **281**, 113882 (2020). https://doi.org:10.1016/j.jviromet.2020.113882

74 Weingartl, H. M. *et al.* Recombinant nipah virus vaccines protect pigs against challenge. *J Virol* **80**, 7929-7938 (2006). https://doi.org:10.1128/JVI.00263-06

75 Abdelmageed, A. A. & Ferran, M. C. The Propagation, Quantification, and Storage of Vesicular Stomatitis Virus. *Curr Protoc Microbiol* **58**, e110 (2020). https://doi.org:10.1002/cpmc.110

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Attachments: RP2-Research strategy_formatted-Final-060523.docx
RP2-Research strategy_formatted-Final-060523.pdf

Sherry / all

here is RP2 Res strategy, in case it gets late
Moushimi and Tony are looking at this final version from me

Brian and I have qual exams this PM

Are we supposed to remove the hyperlinks in the References also?

CENTER OVERVIEW: *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center (PABVAX)

RESEARCH STRATEGY

Introduction. Emerging and periodically reemerging pathogenic enveloped RNA viruses are continuous pandemic threats. Among viral taxon associated with human zoonoses, members of the family ***Paramyxoviridae*** and order ***Bunyavirales*** stand out for their capacity to cause debilitating and life-threatening disease**.** Humans can become infected via excreta from animal reservoir hosts such as rodents or bats, through intermediate amplifying hosts such as small or domestic animals, or by arthropod vectors such as mosquitos and ticks[1-4]. Humans often represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur. During this process, RNA viral pathogens may mutate and adapt to more efficiently replicate and shed in the host, posing an epidemic threat[5, 6]. The viral order ***Bunyavirales*** is composed of several viral families containing high priority human pathogens[7]. Notably, the ***Arenaviridae*** and ***Nairoviridae*** families contain viruses that cause severe hemorrhagic fever diseases in humans globally with mortality rates up to 60%[8] and significant, long-term sequelae in survivors[9]. Many bunyaviruses are included on the World Health Organization's (WHO) List of Priority Pathogens[10, 11]. Six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHAPV, GTOV, JUNV, MACV) and one nairovirus, Crimean-Congo hemorrhagic fever virus (CCHFV), are also identified as NIAID Category A pathogens[12] due to their 1) high associated morbidity and mortality rates, 2) ease of dissemination and/or ability to transmit from person-to-person, 3) potential to cause public panic and social disruption, and 4) requirement for special actions in the context of public health preparedness. The ***Paramyxoviridae*** also includes members in the WHO Priority Pathogens, namely Nipah virus (NiV) and Hendra virus (HeV). These henipaviruses (HNVs) are the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease[13]. The genus now includes nine additional reported HNVs, but only HeV and NiV are associated with severe and often fatal henipaviral disease in humans and a number of animal species[14]. Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illness in humans[15].

These collective factors plus the absence of approved vaccines or therapeutics make bunyaviruses and paramyxoviruses a significant threat to global public health. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for developing vaccine and monoclonal antibody (mAb) countermeasures to prototype bunyavirus and paramyxovirus pathogens (**Table 1**). In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old World) Machupo (New World)	Lujo (Old World) Chapare (New World)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Table 1. PABVAX selected prototype and test case viral agents

Arenaviridae: The *Arenaviridae* is composed of five genera: *Mammarenavirus*, *Reptarenavirus*, *Innmovirus*, *Antennavirus*, and *Hartmanivirus*[16]. The genus *Mammarenavirus* contains a large number of rodent-borne viruses, some of which cause severe hemorrhagic fever, neurological damage, death, and permanent sequelae; hearing loss, vision impairment, and balance issues in survivors[17]. While these genera exhibit a global distribution, two geographically distinct serogroups exist that diverged[18]. Old World complex mammarenaviruses (OWAV) in the Eastern Hemisphere include LASV, lymphocytic choriomeningitis virus (LCMV), and the newly discovered LUJV; New World complex mammarenaviruses (NWAV) in the Western Hemisphere include JUNV, MACV, Sabia Virus (SABV), and CHAPV. There are no FDA approved vaccines or therapeutics for mammarenaviruses, though the attenuated JUNV "Candid-1" vaccine is approved for use in Argentina.

Disease severity and case fatality rates (CFR) vary across mammarenaviruses even within the same clades and species. Up to 500,000 cases of LASV infection occur per year with CFRs sometimes approaching 70% in hospitalized cases[19]. Before the introduction of the Candid-1 vaccine, it is believed that an upwards of 60,000 cases of Argentine Hemorrhagic Fever (AHF) from infection with JUNV occurred per year with CFRs ranging from 15-30%. Convalescent plasma from JUNV survivors is still the primary means to treat acute AHF in Argentina[20]. Over 1000 cases were reported for the initial outbreak of MACV with CFRs ranging from 5-30%; enhanced rodent abatement practices and potentially cross-protective immunity in populations that have taken the JUNV Candid-1 vaccine may have reduced recent outbreak frequency[21]. All mammarenaviruses share a common genomic organization consisting of two ambisense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix) and L protein (polymerase), and the glycoprotein precursor (GPC) and nucleoprotein (NP)[17]. Despite general

similarity of their genomic architecture, mammarenaviruses exhibit marked differences in their replication life cycles including attachment factors, entry mechanisms, and immune evasion; nonetheless, the dominant antigens driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Despite the abundant genetic diversity of LASV, high levels of cross reactivity have been seen with antigen-based diagnostics across seven known lineages, suggesting the generation of cross-protective immunogens and antibodies against mammarenaviruses is achievable[22]. Monoclonal antibodies derived from human LASV survivors were shown to protect nonhuman primates (NHP) against lethal challenge[23]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[24]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, indicating this domain could serve as an antigenic target. Investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccines to test this approach and have demonstrated protection against lethal LASV lineage IV Josiah challenge. Specifically, a stabilized prefusion trimeric GPe, an immunogen containing a highly conserved NP NTD epitope, or a combination of these two approaches conferred protection in a lethal outbred guinea pig model. Nevertheless, even with the considerable molecular diversity in LASV strains, conservation at key antigenic sites within the GP and NP suggest a pan-LASV vaccine may be possible[22, 25, 26]. **RP1** and **Core E** investigators have demonstrated that a recombinant vesicular stomatitis virus (rVSV)-based vaccine expressing the LASV-Josiah GPC is protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[27], and against heterologous challenge with Lineage II[28], III, V[29], and VI viruses (Cross and Geisbert, unpublished data).

In **Phase I of RP1**, the ability of an optimized LASV GPe/NP subunit vaccine delivered intramuscularly (IM) will be assessed in guinea pig and NHP models across multiple viral lineages. We will also assess a microneedle skin patch (MNP) formulation optimized in **Core C** to determine if a needle-free vaccination route affords more potent vaccine-mediated responses and protection. Similarly, we have generated MACV GPe immunogens and will design a MACV NP-NTD subunit antigen to test the same approach across multiple MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing both OWAV (LUJV) and NWAV (CHAPV) complexes, for which there are no vaccines available. A unique advantage of the PABVAX Center is its access to Biosafety Level (BSL)-4 containment, allowing us to work with authentic versus surrogate arenaviruses. The BSL-4 facilities described in **Core E** are capable of evaluating countermeasures in small animal models and NHPs. The cynomolgus macaque is the current “gold standard” animal model for LASV, JUNV, and MACV research as disease recapitulates human infection and there are a multitude of immunological reagents available[30-32]. The evaluation of prototype OWAV and NWAV vaccines and therapies in the NHP model is likely pivotal for IND applications and for future licensure of medical countermeasures for human use.

<u>Nairoviridae:</u> Unique to most bunyaviruses, orthonairoviruses are transmitted primarily by ticks[2]. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[33], Issyk-Kul virus[34], Songling virus[35]), CCHFV is the most recognized public health threat. Outbreaks of CCHFV have occurred across Europe, Africa, the Middle East, and Asia. There is growing concern for expansion of the CCHFV endemic region due to increased range of its tick host (*Hyalomma* spp.) tied to bird migration changes, livestock trade, and global warming. For example, CCHFV cases have been reported in Spain[36], a previously non-endemic region, and *Hyalomma* ticks have been found in Sweden[37]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to a severe hemorrhagic fever and death in 30% of cases[38]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[39]. Ribavirin has been used to treat infections with limited success[40].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and GPC [38]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[38]. Conversely, the CCHFV GPC is considerably less conserved (< 75% amino acid identity)[38]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location. Thus, creating a pan-CCHFV vaccine or therapeutic based solely on one clade poses a challenge. Nevertheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a rVSV vectored vaccine expressing the full GPC of CCHFV[41].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP immunogens with the highest level of homology across clades. We will assess their capacity to protect against lethal challenge with isolates from multiple CCHFV clades in established mouse[41, 42] and NHP[43] models with **Core E**. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best formulation of CCHFV GP/NP MNP vaccine against the emerging test case nairovirus, Kasokero virus, for which there are no vaccines available.

Paramyxoviridae: The HNVs NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality[4, 44]. HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality[45]. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs, which served as amplifying hosts[46]. NiV and HeV can infect at least 18 animal species across 6 orders of mammals, and among these they cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans[14,47]. The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission[5]. Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and Southeast Asia and Australia. Their broad host tropism and ability to infect livestock also causes increased risk of human infections. HNVs are single-stranded, negative sense, enveloped RNA viruses and their biology lends themselves to be synthetically produced. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS). The Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents[48] because the virus could be engineered for mass dissemination and it possesses the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the R&D Blueprint list of priority pathogens with epidemic potential[49].

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb exist. These postexposure treatments conferred protecion against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP)[50-53]. The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia[54]. In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs, and several vaccine platforms have clear potential for use in people[14]. The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies[14]. Such preventive vaccines would have utility for lab workers, first responders or individuals at high-risk exposure, and in mitigating the spread of an outbreak. In the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent[55]. It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection[47, 56]. The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished data)[57]. The divergent LayV will be the focus of the test case studies. The choice is justifiable for several important reasons: 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; and 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including rescuing recombinant LayV (rLayV), exploring animal model development, constructing additional LayV and AngV reporter virus tools, and then translating and testing the MNP vaccine “plug-and-play” approach. Finally, we will evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME. A strength of the PABVAX is that a majority of the Center Research Project (RP) and Scientific Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center’s RPs and Core leaders currently have active and productive collaborative research programs in related areas, most spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

The pandemic preparedness research gaps addressed by PABVAX are diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **arenaviruses** and **nairoviruses**, and **RP2** will target the prototype **HNVs**. Both RP1 and RP2 will develop new prototype virus tools (recombinant prototype viruses) along with existing tools, to analyze vaccine responses and evaluate new mAbs and nabs from the other RPs. A third RP will develop a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant *Cedar henipavirus* (rCedV) *in vivo* and a bioluminescence detection method that can longitudinally trace the dynamics and anatomical progression of infection with a luciferase-encoding virus in individual animals (**RP3**). There will also be two RPs that focus on the development of broadly reactive human mAbs and nanobodies to the PABVAX selected viral prototypes. **RP4** will isolate human mAbs derived from NiV-infected survivors with a specific focus on F glycoprotein-targeting mAbs. Additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will engage in the manufacture, quality control, and testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**). Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core (**Core A**) will provide organizational and programmatic structure to promote research productivity and scientific interactions along with compliance with regulations concerning select agent, vertebrate animal, and any human subject use. **Core A** will coordinate all activities with the external Scientific Advisory Board (**SAB**) and make data available through the Data Management Core (**Core B**).

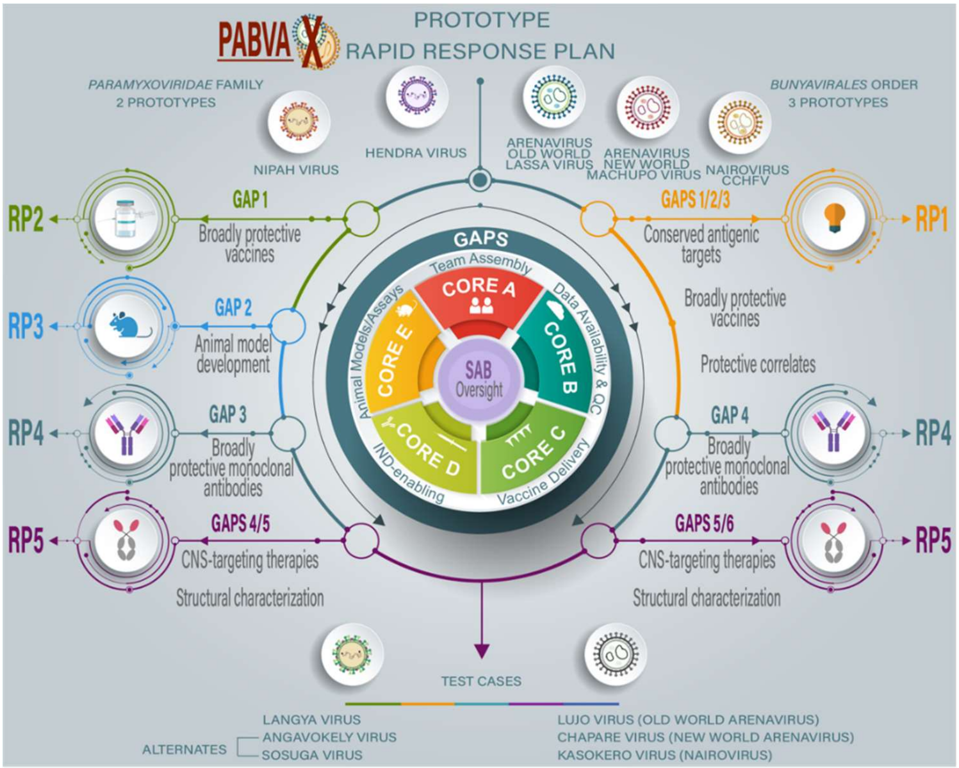


Figure 1: Research gaps addressed by the *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center- (PABVAX).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform. The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**). This project tested a dissolvable MNP-based vaccine technology ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine[58]. The MNP vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable microneedles that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants[59] to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as to facilitate improved vaccine storage and distribution due to their improved thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, Scientific **Core C** (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ)-adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (*Paramyxoviridae*). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro*, along with analysis of polyfunctional T-cell responses by antigenic peptide stimulation, followed by intracellular cytokine staining (ICS) and flow cytometry.

Discover effective antibody-based prophylactics and therapies. Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah[60], Hendra[61], Ebola[62], Marburg[63] and Lassa[64]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, etc.), a feature to be exploited in **Core D** by Dr. Gunn. In addition to therapeutic applications, mAbs with Fc mutations dramatically extend serum half-life[65-67] and offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g*. rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization, and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures. Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal Model Development and Preclinical Evaluation Scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective efficacy of antivirals and vaccines, as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation, including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within

RP1. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they are generally less laborious and can be adapted to higher throughput platforms and/or integrated with other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput fluorescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic NiV and HeV vaccine-induced immune sera, mAbs (**RP4**), nbs (**RP5**), and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either HeV or NiV-B envelope GPs) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals to explore nab based therapeutics to CNS resident HNVs[68].

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the HNVs (*Paramyxoviridae*) and the *Bunyavirales* (*Arenaviridae* and *Nairoviridae*) that will bolster vaccine endurance to storage and transportation; we also anticipate improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

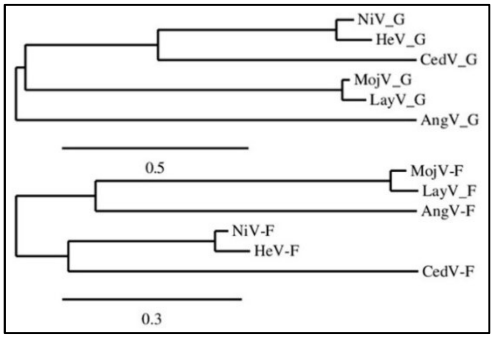
The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology, and mAb and vaccine translational drug development along with Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Paramyxoviruses: NiV and HeV are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis[4, 44]. HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals. These viruses are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of CedV, Gamak virus, Daeryong virus, and (LayV)[69] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[70-73]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and

Figure 2: Phylogenetic analysis of select HNV G and F glycoproteins. Trees were constructed for using the PhyML program's maximum likelihood method and aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.



AngV, are old world pteropid fruit bats[70, 74]. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province)[15]. The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**. Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal HNV disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters[47], and AGMs (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P). The P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists[75-79]. The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections[80, 81]. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature[82]. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2[83] and CedV is now recognized as a BSL-2 restricted agent[84]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore **LayV as part of the Center's Phase II "plug and play"** research plan (**RP2** and **PR5** with **Cores C, D,** and **E**).

Arenaviruses (old world): For **phase 1**, **RP1** will utilize **LASV as the prototype virus** due its significant public health burden throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[85]. LASV is the most imported viral hemorrhagic fever (VHF) next to Dengue virus and has been documented in several countries outside of Africa over 35 times[86]. CFRs approaching 70% in hospitalized cases and marked long term sequelae are the result of the lack of approved vaccines or therapeutics, although several live attenuated virus candidates are currently being evaluated with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI)[87]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize only LASV GP as the target immunogen.

Investigators in **RP1**, **Core D**, and **Core E** have already demonstrated protective efficacy against challenge with LASV in outbred guinea pigs vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These immunogens have been engineered to express homologous antigenic regions to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach suggests a similar strategy could be implemented against other arenaviruses. The **OWAV test pathogen, LUJV,** was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia, which quickly spread nosocomially[88]. The CFR for this infectious virus was 80% in this small outbreak of 5 people. There are currently no approved vaccines or therapeutics available for this virus and its genetic divergence from suggest current LASV countermeasures may be of limited use. LUJV will serve as an ideal "test case" to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Arenaviruses (new world): The live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[89], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[90, 91]. Furthermore, there is the potential for pathogenic reversion. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest exploring other JUNV countermeasures are warranted. Building on the success of the LASV GPe/NP-NTD, investigators in **RP1 and Cores D and E** will engineer broadly protective MACV GPe and NP-NTD antigens and perform efficacy testing in established guinea pig and NHP models. We will extend this plug-and-play approach to the **NWAV test case, CHAPV**. First reported in 2003 in Bolivia, CHAPV is a rodent-borne mammarenavirus associated with the development of severe hemorrhagic fever with case fatality rates up to 60%[92, 93]. Recent studies have demonstrated clear human-to-human transmission during the acute phase evidenced by several nosocomial infections; isolation of viable virus from semen at 86 days after symptom onset demonstrates a clear need to implement specialized management

and treatment [93]. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Nairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as it represents the largest global public health burden within the orthonairovirus family with outbreaks annually across Europe, Africa, and Asia. There are no FDA-approved vaccines for CCHFV; however, a suckling mouse brain-derived, adjuvanted whole virus vaccine for CCHFV has been approved for use in Bulgaria. This vaccine has numerous safety concerns and has limited demonstrated efficacy. Several experimental vaccine approaches have been evaluated ranging from viral vectored approaches to DNA-based vaccines with varying efficacy, advantages, and disadvantages[94]; however, only a Modified Vaccinia Ankara virus vaccine (MVA-CCHFV) has begun to advance towards clinical trials[95]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test these for protective efficacy from challenge with 6 different CCHFV lineages using established mouse and NHP models. Next, we will deploy the same approach to the **test case nairovirus, Kasokero virus (KASV),** for which no vaccine or treatment exists.

In 1977, KASV was first isolated from Ugandan bats and caused several cases of acute febrile illness of laboratory workers from the same lab that processed the infected bats[96]. Surveys of 109 laboratory workers from the same facility demonstrated 13% seropositivity and clinical symptoms in individuals 4 to 15 months after the virus was isolated. These findings suggest either continued laboratory-associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the tick reservoir, *Ornithodoros faini,* to participate in enzootic maintenance of KASV[97]. While most chiropteran ticks exhibit host specificity, humans entering caves these bats inhabit have reported bites from *O.faini* ticks. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics suggest investment in countermeasure development against this virus is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Management Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**; arenavirus and nairovirus projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine active and passive immunization approaches to further improve the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

An additional and outstanding advantage of the PABVAX Center is the **Animal Model Development and Preclinical Evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL. Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for the Center RPs will be performed by the same BSL-4 staff under the

same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for members of the *Bunyavirales* and *Paramyxoviridae*.**
2. **Develop new tools and animal models for cell-based and *in vivo* therapeutic evaluation.**
3. **Develop antibodies and nanobodies to the selected prototype and test viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

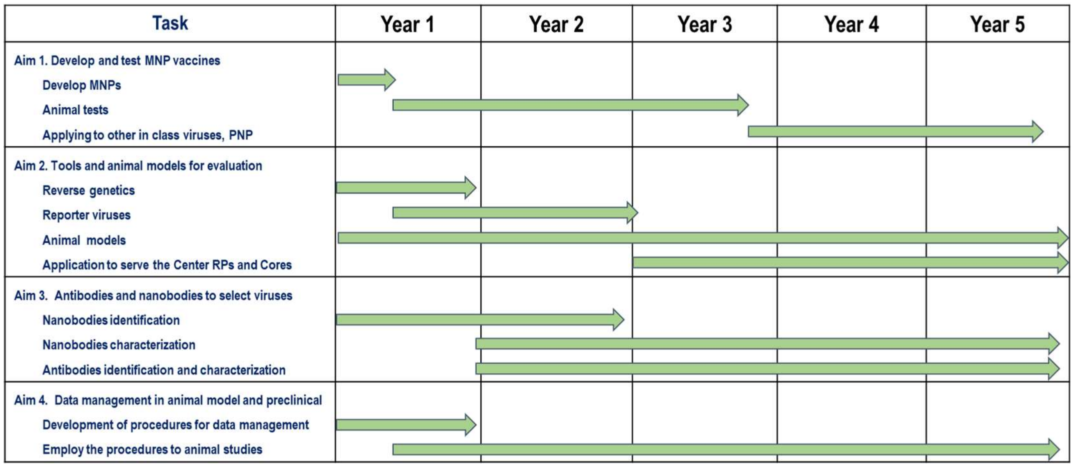


Figure 3: Projected PABVAX timeline to achieve the 4 overarching tasks.

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**. Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (details are specified within the Administration **Core A** Research Strategy). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommending new directions as appropriate, and providing Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

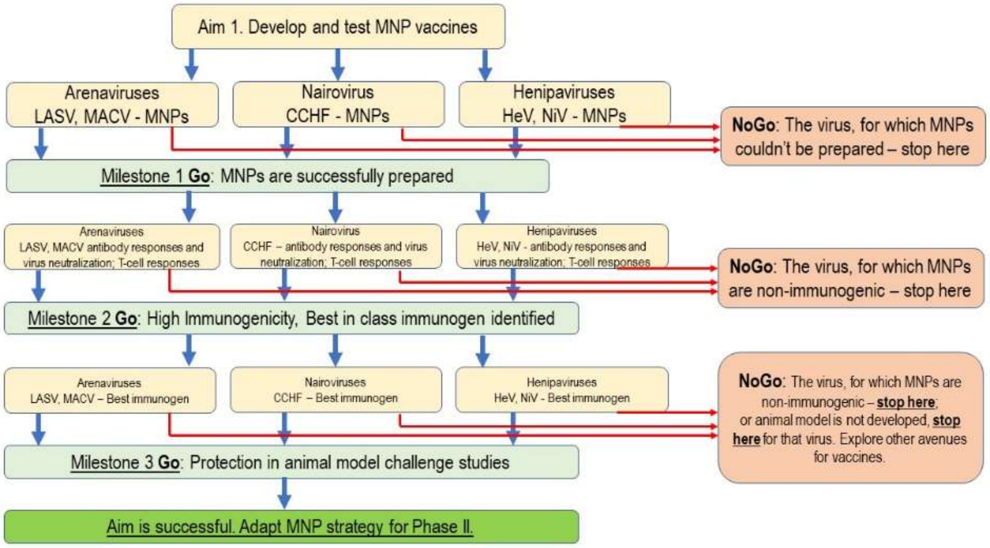


Figure 4. Milestones and criteria for **Aim 1** of the PABVAX Center's overall objectives.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

The first milestone will address whether formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made (**Figure 4**). The determination of MNP vaccine potential success will first be established using immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen-specific antibody induction, virus neutralization, and polyfunctional T-cell responses by *in vitro* stimulation of isolated cells). Go/no go decisions will be made based on outcomes of these first-tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as a success. Comparisons will also be made such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of the best-in-class immunogens, or immunogen combinations, will also be explored to down-select vaccines. The third milestone will determine whether MNP vaccination affords protection in animal model challenge experiments. In the case of NiV and HeV prototypes, the animal models chosen (ferrets and African green monkeys (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales*, there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASV, and guinea pig models exist for some but not all of these lineages. For MACV, there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is the limitations of cost and availability of rhesus and cynomolgus monkeys. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be developing and evaluating animal models within the PABVAX program, including further development of AGM models for arenaviruses and possibly CCHFV as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for cell-based and in vivo therapeutics evaluation.

The second overall objective of PABVAX is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation (**Figure 5**). These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed because genomic data is incomplete. rLayV pathogenesis and animal model development will be conducted by **Core E**. If successful, a large battery of new materials will be available to share with the ReVAMPP network and be key milestones for execution of Phase II of the PABVAX Center program.

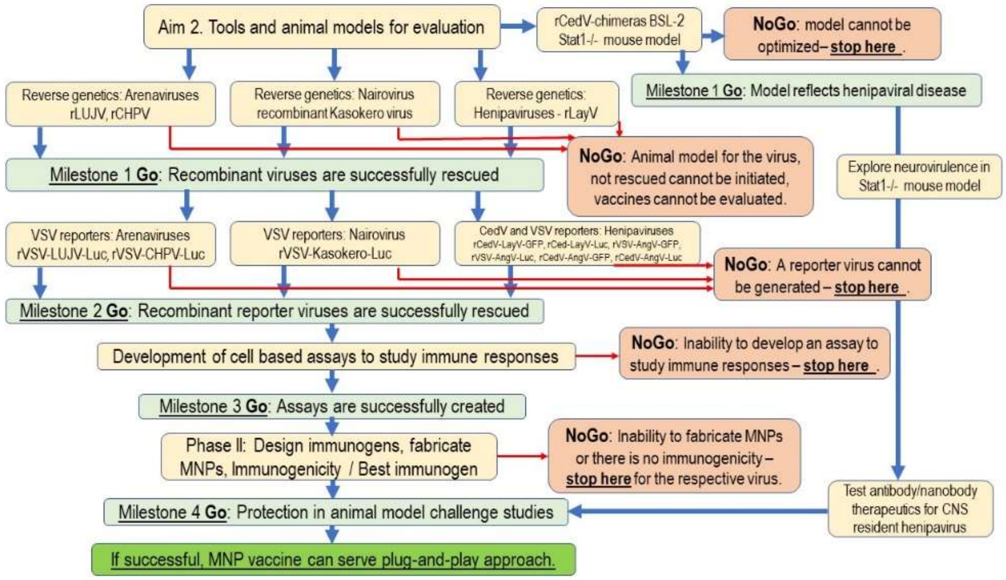


Figure 5. Milestones and criteria for **Aim 2** of the PABVAX Center's overall objectives.

The translation of the MNP vaccine plug-and-play approach will be in Phase II (**Table 1**). Milestones will be similar to those in **Figure 4**. New virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new *in vivo* bioluminescence-based immune deficient mouse model developed by the PIs of **PR2** and **RP3** that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease *in vivo* safely and expediently at BSL-2 containment. The model

will be deployed to test a critically important therapeutic need against CNS resident HNV infection, and **RP3** will collaborate with **RP5** in the testing of engineered nabs.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

New human mAbs and nbs as tools and potential therapeutics will be developed by **RP4** and **RP5** in collaboration with **RP2** and **Core D** (**Figure 6**). NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs, and in collaboration with **RP2**, neutralization potencies will be rapidly assessed (as well as evaluating synergistic cell-based mAb neutralization) that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

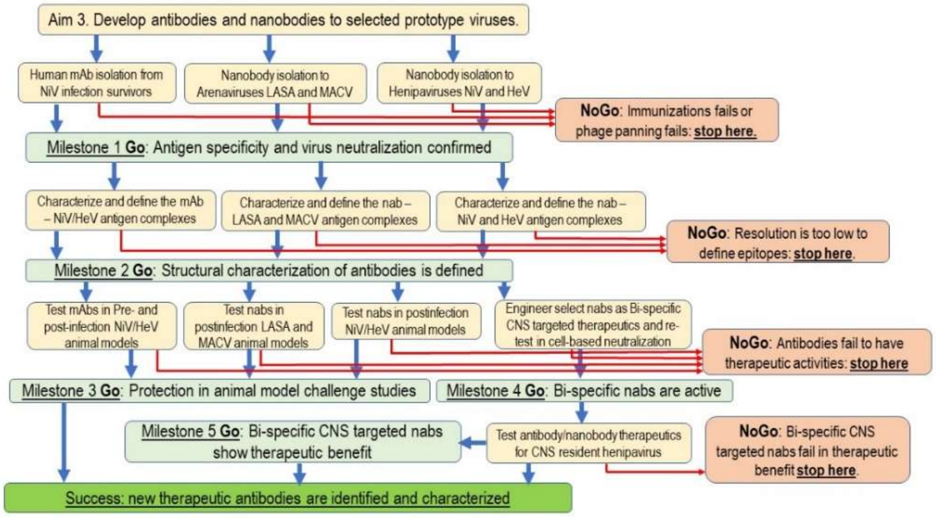


Figure 6. Milestones and criteria for **Aim 3** of the PABVAX Center's overall objectives.

Nbs, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nbs can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nbs targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus), and select animal models of LASV and MACV by **Core E**. Success will be measured by the 1) identification of potent virus-neutralizing nbs, 2) discovery of more broadly reactive nbs particularly to the multiple LASV clades, 3) evidence of therapeutic benefit in animal models, and 4) the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

Industry Partnerships.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience in developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and

make an ideal partner for PABVAX. Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

SPECIAL FEATURES OF THE PABVAX

Galveston National Laboratory (GNL). The GNL is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics, and therapeutics. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370 NHP studies employing some 2,772 animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses have been performed. These studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder, and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP facility and a staff of full-time veterinarians. **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above, another special feature is the location of the WRCEVA virus collection within the GNL.

***Core E (Animal model development and preclinical evaluation)*. Core E** operations will be conducted at the GNL, and in conjunction with **Core A**, will also include a regulatory expert from the UTMB Institutional Office of Regulated Nonclinical Studies (ORNcS). The ORNcS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL, and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses, arenaviruses, and henipaviruses. He has published over 250 peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI), was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs). Mapp Biopharmaceutical has deep experience with antigens and mAbs against these antigens. Their early development and research group has expertise in immunology, infectious diseases, and the production of antigens, antibodies, and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [98] [99]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1[100]. Dr. Gunn and Genovac will join the team to analyze antibody Fc effector functions and to provide antigen/antibody discovery resources, respectively. Dr. Geisbert will contribute his experience with high containment animal models and vaccine development. As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

BIBLIOGRAPHY AND REFERENCES CITED

1. Lamb RA, Parks GD. Paramyxoviridae: the viruses and their replication. In Fields BN, Knipe DM, Howley PM, editors, Fields Virology: Sixth Edition. Vol. 1. Lippincott, Williams, and Wilkins. 2013. p. 957-995.
2. Boshra H. An Overview of the Infectious Cycle of Bunyaviruses. Viruses. 2022;14(10). Epub 20220928. doi: 10.3390/v14102139. PubMed PMID: 36298693; PMCID: PMC9610998.
3. Horne KM, Vanlandingham DL. Bunyavirus-vector interactions. Viruses. 2014;6(11):4373-97. Epub 20141113. doi: 10.3390/v6114373. PubMed PMID: 25402172; PMCID: PMC4246228.
4. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia), p 1070-1085.
5. Luby SP. The pandemic potential of Nipah virus. Antiviral research. 2013;100(1):38-43. Epub 20130730. doi: 10.1016/j.antiviral.2013.07.011. PubMed PMID: 23911335.
6. Kummer S, Kranz DC. Henipaviruses-A constant threat to livestock and humans. PLoS Negl Trop Dis. 2022;16(2):e0010157. Epub 20220218. doi: 10.1371/journal.pntd.0010157. PubMed PMID: 35180217; PMCID: PMC8856525.
7. Maes P, Adkins S, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, DeRisi JL, Digiaro M, Drebot M, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hewson R, Hughes HR, Jain RK, Jonson MG, Junglen S, Klempa B, Klingström J, Kormelink R, Lambert AJ, Langevin SA, Lukashevich IS, Marklewitz M, Martelli GP, Mielke-Ehret N, Mirazimi A, Mühlbach HP, Naidu R, Nunes MRT, Palacios G, Papa A, Pawęska JT, Peters CJ, Plyusnin A, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Schmaljohn C, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Tesh RB, Turina M, Wèi T, Whitfield AE, Yeh SD, Zerbini FM, Zhang YZ, Zhou X, Kuhn JH. Taxonomy of the order Bunyavirales: second update 2018. Arch Virol. 2019;164(3):927-41. doi: 10.1007/s00705-018-04127-3. PubMed PMID: 30663021; PMCID: PMC6581445.
8. Silva-Ramos CR, Montoya-Ruíz C, Faccini-Martínez Á A, Rodas JD. An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever. Arch Virol. 2022;167(9):1727-38. Epub 20220517. doi: 10.1007/s00705-022-05453-3. PubMed PMID: 35579715; PMCID: PMC9110938.
9. Garry RF. Lassa fever - the road ahead. Nat Rev Microbiol. 2023;21(2):87-96. Epub 20220912. doi: 10.1038/s41579-022-00789-8. PubMed PMID: 36097163; PMCID: PMC9466315.
10. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. Antiviral research. 2018;159:63-7. Epub 20180924. doi: 10.1016/j.antiviral.2018.09.009. PubMed PMID: 30261226; PMCID: PMC7113760.
11. WHO. List of Blueprint Priority Diseases. 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
12. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2018 [04 Jun 2023]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
13. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. Nat Rev Microbiol. 2006;4(1):23-35. doi: 10.1038/nrmicro1323. PubMed PMID: 16357858; PMCID: PMC7097447.
14. Amaya M, Broder CC. Vaccines to Emerging Viruses: Nipah and Hendra. Annu Rev Virol. 2020;7(1):447-73. doi: 10.1146/annurev-virology-021920-113833. PubMed PMID: 32991264; PMCID: PMC8782152.
15. Zhang XA, Li H, Jiang FC, Zhu F, Zhang YF, Chen JJ, Tan CW, Anderson DE, Fan H, Dong LY, Li C, Zhang PH, Li Y, Ding H, Fang LQ, Wang LF, Liu W. A Zoonotic Henipavirus in Febrile Patients in China. N Engl J Med. 2022;387(5):470-2. doi: 10.1056/NEJMc2202705. PubMed PMID: 35921459.
16. Radoshitzky SR, Buchmeier MJ, Charrel RN, Clegg JCS, Gonzalez JJ, Günther S, Hepojoki J, Kuhn JH, Lukashevich IS, Romanowski V, Salvato MS, Sironi M, Stenglein MD, de la Torre JC, Ictv Report C. ICTV Virus Taxonomy Profile: Arenaviridae. J Gen Virol. 2019;100(8):1200-1. Epub 20190613. doi: 10.1099/jgv.0.001280. PubMed PMID: 31192784.
17. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
18. Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient Evolution of Mammarenaviruses: Adaptation via Changes in the L Protein and No Evidence for Host-Virus Codivergence. Genome Biol Evol. 2018;10(3):863-74. doi: 10.1093/gbe/evy050. PubMed PMID: 29608723.

19. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF, the Viral Hemorrhagic Fever C. Lassa Fever in Post-Conflict Sierra Leone. PLOS Neglected Tropical Diseases. 2014;8(3):e2748. doi: 10.1371/journal.pntd.0002748.

20. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Antiviral research. 2008;78(1):132-9. Epub 2007/12/07. doi: 10.1016/j.antiviral.2007.10.010. PubMed PMID: 18054395.

21. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

22. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia I, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Scientific Reports. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.

23. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nature Medicine. 2017;23(10):1146-9. doi: 10.1038/nm.4396.

24. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.

25. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.

26. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Scientific Reports. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.

27. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLOS Medicine. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.

28. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

29. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLOS Neglected Tropical Diseases. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

30. Hensley LE, Smith MA, Geisbert JB, Fritz EA, Daddario-DiCaprio KM, Larsen T, Geisbert TW. Pathogenesis of Lassa fever in cynomolgus macaques. Virol J. 2011;8:205. Epub 20110506. doi: 10.1186/1743-422x-8-205. PubMed PMID: 21548931; PMCID: PMC3104370.
31. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proceedings of the National Academy of Sciences. 2021;118(11):e2023332118. doi: doi:10.1073/pnas.2023332118.
32. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure. Veterinary Pathology. 2015;52(1):26-37. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.
33. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.
34. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.
35. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.
36. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.
37. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.
38. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.
39. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral research. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.
40. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infectious Diseases. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.
41. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 20190523. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.
42. Bente DA, Alimonti JB, Shieh WJ, Camus G, Ströher U, Zaki S, Jones SM. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. J Virol. 2010;84(21):11089-100. Epub 20100825. doi: 10.1128/jvi.01383-10. PubMed PMID: 20739514; PMCID: PMC2953203.
43. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 20200813. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.
44. Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York, p 519-536.
45. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B, et al. A morbillivirus that caused fatal disease in horses and humans. Science. 1995;268(5207):94-7. doi: 10.1126/science.7701348. PubMed PMID: 7701348.

46. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BW. Nipah virus: a recently emergent deadly paramyxovirus. Science (New York, NY). 2000;288(5470):1432-5. doi: 10.1126/science.288.5470.1432. PubMed PMID: 10827955.
47. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. Curr Top Microbiol Immunol. 2012;359:153-77. doi: 10.1007/82_2012_208. PubMed PMID: 22476556; PMCID: PMC7120132.
48. Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087.
49. Sweileh WM. Global research trends of World Health Organization's top eight emerging pathogens. Global Health. 2017;13(1):9. Epub 20170208. doi: 10.1186/s12992-017-0233-9. PubMed PMID: 28179007; PMCID: PMC5299748.
50. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng Y-R, Brining D, Scott D, Wang Y, Dimitrov AS, Callison J, Chan Y-P, Hickey AC, Dimitrov DS, Broder CC, Rockx B. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. Science translational medicine. 2011;3(105):105ra3. doi: 10.1126/scitranslmed.3002901. PubMed PMID: 22013123.
51. Geisbert TW, Mire CE, Geisbert JB, Chan Y-P, Agans KN, Feldmann F, Fenton KA, Zhu Z, Dimitrov DS, Scott DP, Bossart KN, Feldmann H, Broder CC. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Science translational medicine. 2014;6(242):242ra82. doi: 10.1126/scitranslmed.3008929. PubMed PMID: 24964990.
52. Mire CE, Satterfield BA, Geisbert JB, Agans KN, Borisevich V, Yan L, Chan YP, Cross RW, Fenton KA, Broder CC, Geisbert TW. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. Sci Rep. 2016;6:30916. Epub 20160803. doi: 10.1038/srep30916. PubMed PMID: 27484128; PMCID: PMC4971471.
53. Mire CE, Chan YP, Borisevich V, Cross RW, Yan L, Agans KN, Dang HV, Veesler D, Fenton KA, Geisbert TW, Broder CC. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. J Infect Dis. 2020;221(Suppl 4):S471-s9. doi: 10.1093/infdis/jiz515. PubMed PMID: 31686101; PMCID: PMC7199785.
54. Playford EG, Munro T, Mahler SM, Elliott S, Gerometta M, Hoger KL, Jones ML, Griffin P, Lynch KD, Carroll H, El Saadi D, Gilmour ME, Hughes B, Hughes K, Huang E, de Bakker C, Klein R, Scher MG, Smith IL, Wang LF, Lambert SB, Dimitrov DS, Gray PP, Broder CC. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. Lancet Infect Dis. 2020;20(4):445-54. Epub 20200203. doi: 10.1016/s1473-3099(19)30634-6. PubMed PMID: 32027842.
55. Middleton D, Pallister J, Klein R, Feng Y-R, Haining J, Arkinstall R, Frazer L, Huang J-A, Edwards N, Wareing M, Elhay M, Hashmi Z, Bingham J, Yamada M, Johnson D, White J, Foord A, Heine HG, Marsh GA, Broder CC, Wang L-F. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerging infectious diseases. 2014;20(3):372-9. doi: 10.3201/eid2003.131159. PubMed PMID: 24572697.
56. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, Smith MA, Chan YP, Wang LF, Mattapallil JJ, Geisbert JB, Bossart KN, Broder CC. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One. 2010;5(5):e10690. Epub 20100518. doi: 10.1371/journal.pone.0010690. PubMed PMID: 20502528; PMCID: PMC2872660.
57. Cheliout Da Silva S, Yan L, Dang HV, Xu K, Epstein JH, Veesler D, Broder CC. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. Viruses. 2021;13(3). Epub 20210322. doi: 10.3390/v13030517. PubMed PMID: 33809833; PMCID: PMC8004131.
58. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo LD, Jr. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. Expert Opin Drug Deliv. 2021;18(2):151-67. Epub 20201006. doi: 10.1080/17425247.2021.1823964. PubMed PMID: 32924651; PMCID: PMC9355143.
59. Andrianov AK, DeCollibus DP, Gillis HA, Kha HH, Marin A, Prausnitz MR, Babiuk LA, Townsend H, Mutwiri G. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. Proc Natl Acad Sci U S A. 2009;106(45):18936-41. Epub 20091028. doi: 10.1073/pnas.0908842106. PubMed PMID: 19864632; PMCID: PMC2770009.
60. Geisbert TW, Mire CE, Geisbert JB, Chan YP, Agans KN, Feldmann F, Fenton KA, Zhu Z, Dimitrov DS, Scott DP, Bossart KN, Feldmann H, Broder CC. Therapeutic treatment of Nipah virus infection in nonhuman

primates with a neutralizing human monoclonal antibody. Sci Transl Med. 2014;6(242):242ra82. doi: 10.1126/scitranslmed.3008929. PubMed PMID: 24964990; PMCID: PMC4467163.

61. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng YR, Brining D, Scott D, Wang Y, Dimitrov AS, Callison J, Chan YP, Hickey AC, Dimitrov DS, Broder CC, Rockx B. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. Sci Transl Med. 2011;3(105):105ra3. doi: 10.1126/scitranslmed.3002901. PubMed PMID: 22013123; PMCID: PMC3313625.

62. Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti JB, Fausther-Bovendo H, Wei H, Aviles J, Hiatt E, Johnson A, Morton J, Swope K, Bohorov O, Bohorova N, Goodman C, Kim D, Pauly MH, Velasco J, Pettitt J, Olinger GG, Whaley K, Xu B, Strong JE, Zeitlin L, Kobinger GP. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature. 2014;514(7520):47-53. doi: 10.1038/nature13777. PubMed PMID: 25171469; PMCID: PMC4214273.

63. Mire CE, Geisbert JB, Borisevich V, Fenton KA, Agans KN, Flyak AI, Deer DJ, Steinkellner H, Bohorov O, Bohorova N, Goodman C, Hiatt A, Kim DH, Pauly MH, Velasco J, Whaley KJ, Crowe JE, Jr., Zeitlin L, Geisbert TW. Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. Sci Transl Med. 2017;9(384). doi: 10.1126/scitranslmed.aai8711. PubMed PMID: 28381540; PMCID: PMC5719873.

64. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. Epub 2017/09/05. doi: 10.1038/nm.4396. PubMed PMID: 28869611; PMCID: PMC5719877.

65. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. mAbs. 2011;3(5):422-30. Epub 2011/11/04. doi: 10.4161/mabs.3.5.16983. PubMed PMID: 22048693; PMCID: PMC3225846.

66. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study. Antimicrobial agents and chemotherapy. 2013. Epub 2013/10/02. doi: 10.1128/aac.01285-13. PubMed PMID: 24080653.

67. Gaudinski MR, Coates EE, Houser KV, Chen GL, Yamshchikov G, Saunders JG, Holman LA, Gordon I, Plummer S, Hendel CS, Conan-Cibotti M, Lorenzo MG, Sitar S, Carlton K, Laurencot C, Bailer RT, Narpala S, McDermott AB, Namboodiri AM, Pandey JP, Schwartz RM, Hu Z, Koup RA, Capparelli E, Graham BS, Mascola JR, Ledgerwood JE. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. PLoS Med. 2018;15(1):e1002493. Epub 2018/01/25. doi: 10.1371/journal.pmed.1002493. PubMed PMID: 29364886; PMCID: PMC5783347.

68. Mastraccio KE, Huaman C, Laing ED, Broder CC, Schaefer BC. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. Methods Mol Biol. 2022;2524:369-94. doi: 10.1007/978-1-0716-2453-1_30. PubMed PMID: 35821488.

69. Rima B, Balkema-Buschmann A, Dundon WG, Duprex P, Easton A, Fouchier R, Kurath G, Lamb R, Lee B, Rota P, Wang L, Ictv Report C. ICTV Virus Taxonomy Profile: Paramyxoviridae. J Gen Virol. 2019;100(12):1593-4. Epub 20191011. doi: 10.1099/jgv.0.001328. PubMed PMID: 31609197; PMCID: PMC7273325.

70. Madera S, Kistler A, Ranaivoson HC, Ahyong V, Andrianiaina A, Andry S, Raharinosy V, Randriambolamanantsoa TH, Ravelomanantsoa NAF, Tato CM, DeRisi JL, Aguilar HC, Lacoste V, Dussart P, Heraud JM, Brook CE. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. J Virol. 2022;96(18):e0092122. Epub 20220830. doi: 10.1128/jvi.00921-22. PubMed PMID: 36040175; PMCID: PMC9517717.

71. Vanmechelen B, Meurs S, Horemans M, Loosen A, Joly Maes T, Laenen L, Vergote V, Koundouno FR, Magassouba N, Konde MK, Condé IS, Carroll MW, Maes P. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. Virus Evol. 2022;8(2):veac061. Epub 20220630. doi: 10.1093/ve/veac061. PubMed PMID: 35854826; PMCID: PMC9290864.

72. Drexler JF, Corman VM, Müller MA, Maganga GD, Vallo P, Binger T, Gloza-Rausch F, Cottontail VM, Rasche A, Yordanov S, Seebens A, Knörnschild M, Oppong S, Adu Sarkodie Y, Pongombo C, Lukashev AN, Schmidt-Chanasit J, Stöcker A, Carneiro AJ, Erbar S, Maisner A, Fronhoffs F, Buettner R, Kalko EK, Kruppa T, Franke CR, Kallies R, Yandoko ER, Herrler G, Reusken C, Hassanin A, Krüger DH, Matthee S, Ulrich RG,

Leroy EM, Drosten C. Bats host major mammalian paramyxoviruses. Nat Commun. 2012;3:796. Epub 20120424. doi: 10.1038/ncomms1796. PubMed PMID: 22531181; PMCID: PMC3343228.
73. Wu Z, Yang L, Yang F, Ren X, Jiang J, Dong J, Sun L, Zhu Y, Zhou H, Jin Q. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. Emerg Infect Dis. 2014;20(6):1064-6. doi: 10.3201/eid2006.131022. PubMed PMID: 24865545; PMCID: PMC4036791.
74. Voigt K, Hoffmann M, Drexler JF, Müller MA, Drosten C, Herrler G, Krüger N. Fusogenicity of the Ghana Virus (Henipavirus: Ghanaian bat henipavirus) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. Viruses. 2019;11(9). Epub 20190829. doi: 10.3390/v11090800. PubMed PMID: 31470664; PMCID: PMC6784138.
75. Childs K, Randall R, Goodbourn S. Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction. J Virol. 2012;86(7):3411-21. Epub 20120201. doi: 10.1128/jvi.06405-11. PubMed PMID: 22301134; PMCID: PMC3302505.
76. Davis ME, Wang MK, Rennick LJ, Full F, Gableske S, Mesman AW, Gringhuis SI, Geijtenbeek TB, Duprex WP, Gack MU. Antagonism of the phosphatase PP1 by the measles virus V protein is required for innate immune escape of MDA5. Cell Host Microbe. 2014;16(1):19-30. doi: 10.1016/j.chom.2014.06.007. PubMed PMID: 25011105; PMCID: PMC4120867.
77. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. J Virol. 2002;76(22):11476-83. doi: 10.1128/jvi.76.22.11476-11483.2002. PubMed PMID: 12388709; PMCID: PMC136769.
78. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. J Virol. 2005;79(10):6078-88. doi: 10.1128/jvi.79.10.6078-6088.2005. PubMed PMID: 15857993; PMCID: PMC1091709.
79. Basler CF. Nipah and hendra virus interactions with the innate immune system. Curr Top Microbiol Immunol. 2012;359:123-52. doi: 10.1007/82_2012_209. PubMed PMID: 22491899.
80. Yoneda M, Guillaume V, Sato H, Fujita K, Georges-Courbot MC, Ikeda F, Omi M, Muto-Terao Y, Wild TF, Kai C. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. PLoS One. 2010;5(9):e12709. Epub 20100915. doi: 10.1371/journal.pone.0012709. PubMed PMID: 20856799; PMCID: PMC2939873.
81. Satterfield BA, Cross RW, Fenton KA, Borisevich V, Agans KN, Deer DJ, Graber J, Basler CF, Geisbert TW, Mire CE. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. J Virol. 2016;90(14):6326-43. Epub 20160624. doi: 10.1128/jvi.00215-16. PubMed PMID: 27147733; PMCID: PMC4936148.
82. Marsh GA, de Jong C, Barr JA, Tachedjian M, Smith C, Middleton D, Yu M, Todd S, Foord AJ, Haring V, Payne J, Robinson R, Broz I, Crameri G, Field HE, Wang LF. Cedar virus: a novel Henipavirus isolated from Australian bats. PLoS Pathog. 2012;8(8):e1002836. Epub 20120802. doi: 10.1371/journal.ppat.1002836. PubMed PMID: 22879820; PMCID: PMC3410871.
83. Amaya M, Cheng H, Borisevich V, Navaratnarajah CK, Cattaneo R, Cooper L, Moore TW, Gaisina IN, Geisbert TW, Rong L, Broder CC. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral research. 2021;193:105084. Epub 20210530. doi: 10.1016/j.antiviral.2021.105084. PubMed PMID: 34077807; PMCID: PMC8631057.
84. CDC. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. [updated June 2020]. Available from: https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.
85. Organization WH. Lassa fever fact sheet. World Health Organization. 2017.
86. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.
87. Purushotham J, Lambe T, Gilbert SC. Vaccine platforms for the prevention of Lassa fever. Immunol Lett. 2019;215:1-11. Epub 20190423. doi: 10.1016/j.imlet.2019.03.008. PubMed PMID: 31026485; PMCID: PMC7132387.
88. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.
89. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

90. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.
91. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.
92. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.
93. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.
94. Tipih T, Burt FJ. Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development. Biores Open Access. 2020;9(1):137-50. Epub 20200512. doi: 10.1089/biores.2019.0057. PubMed PMID: 32461819; PMCID: PMC7247048.
95. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.
96. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.
97. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.
98. Mulangu S, Dodd LE, Davey RT, Jr., Tshiani Mbaya O, Proschan M, Mukadi D, Lusakibanza Manzo M, Nzolo D, Tshomba Oloma A, Ibanda A, Ali R, Coulibaly S, Levine AC, Grais R, Diaz J, Lane HC, Muyembe-Tamfum JJ, Group PW, Sivahera B, Camara M, Kojan R, Walker R, Dighero-Kemp B, Cao H, Mukumbayi P, Mbala-Kingebeni P, Ahuka S, Albert S, Bonnett T, Crozier I, Duvenhage M, Proffitt C, Teitelbaum M, Moench T, Aboulhab J, Barrett K, Cahill K, Cone K, Eckes R, Hensley L, Herpin B, Higgs E, Ledgerwood J, Pierson J, Smolskis M, Sow Y, Tierney J, Sivapalasingam S, Holman W, Gettinger N, Vallee D, Nordwall J, Team PCS. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. N Engl J Med. 2019;381(24):2293-303. Epub 2019/11/28. doi: 10.1056/NEJMoa1910993. PubMed PMID: 31774950.
99. Group PIW, Multi-National PIIST, Davey RT, Jr., Dodd L, Proschan MA, Neaton J, Neuhaus Nordwall J, Koopmeiners JS, Beigel J, Tierney J, Lane HC, Fauci AS, Massaquoi MBF, Sahr F, Malvy D. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. N Engl J Med. 2016;375(15):1448-56. doi: 10.1056/NEJMoa1604330. PubMed PMID: 27732819; PMCID: PMC5086427.
100. Politch JA, Cu-Uvin S, Moench TR, Tashima KT, Marathe JG, Guthrie KM, Cabral H, Nyhuis T, Brennan M, Zeitlin L, Spiegel HML, Mayer KH, Whaley KJ, Anderson DJ. Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial. PLoS Med. 2021;18(2):e1003495. Epub 2021/02/04. doi: 10.1371/journal.pmed.1003495. PubMed PMID: 33534791.

From: Geisbert, Thomas W. on behalf of Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
To: Haller, Sherry; Stutz, Sonja J.; CHRISTOPHER BRODER
Subject: UTMB and USUHS ReVAMPP Overall Research Strategy Final
Date: Monday, June 5, 2023 12:01:01 PM
Attachments: Overall_Research-Strategy_PABVAX-12pages-Final.docx

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Here is the final Research Strategy for the Overall for the UTMB and USUHS ReVAMPP Center. I think there is still one question from Larry that Chris needs to address for the Overall Aims.

CENTER OVERVIEW: *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction. Emerging and periodically reemerging pathogenic enveloped RNA viruses are continuous pandemic threats. Among viral taxon associated with human zoonoses, members of the family ***Paramyxoviridae*** and order ***Bunyavirales*** stand out for their capacity to cause debilitating and life-threatening disease**.** Humans can become infected via excreta from animal reservoir hosts such as rodents or bats, through intermediate amplifying hosts such as small or domestic animals, or by arthropod vectors such as mosquitos and ticks[1-4]. Humans often represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur. During this process, RNA viral pathogens may mutate and adapt to more efficiently replicate and shed in the host, posing an epidemic threat[5, 6]. The viral order ***Bunyavirales*** is composed of several viral families containing high priority human pathogens[7]. Notably, the ***Arenaviridae*** and ***Nairoviridae*** families contain viruses that cause severe hemorrhagic fever diseases in humans globally with mortality rates up to 60%[8] and significant, long-term sequelae in survivors[9]. Many bunyaviruses are included on the World Health Organization's (WHO) List of Priority Pathogens[10, 11]. Six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHAPV, GTOV, JUNV, MACV) and one nairovirus, Crimean-Congo Hemorrhagic Fever Virus (CCHFV), are also identified as NIAID Category A pathogens[12] due to their 1) high associated morbidity and mortality rates, 2) ease of dissemination and/or ability to transmit from person-to-person, 3) potential to cause public panic and social disruption, and 4) requirement for special actions in the context of public health preparedness. The ***Paramyxoviridae*** also includes members in the WHO Priority Pathogens, namely Nipah virus (NiV) and Hendra virus (HeV). These henipaviruses (HNVs) are the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease[13]. The genus now includes nine additional reported HNVs, but only HeV and NiV are associated with severe and often fatal henipaviral disease in humans and a number of animal species[14]. Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illness in humans[15].

These collective factors plus the absence of approved vaccines or therapeutics make bunyaviruses and paramyxoviruses a significant threat to global public health. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for developing vaccine and monoclonal antibody (mAb) countermeasures to prototype bunyavirus and paramyxovirus pathogens (**Table 1**). In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old World) Machupo (New World)	Lujo (Old World) Chapare (New World)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Table 1. PABVAX selected prototype and test case viral agents

Arenaviridae*:* The *Arenaviridae* is composed of five genera: *Mammarenavirus*, *Reptarenavirus*, *Innmovirus*, *Antennavirus*, and *Hartmanivirus*[16]. The genus *Mammarenavirus* contains a large number of rodent-borne viruses, some of which cause severe hemorrhagic fever, neurological damage, death, and permanent sequelae; hearing loss, vision impairment, and balance issues in survivors[17]. While these genera exhibit a global distribution, two geographically distinct serogroups exist that diverged[18]. Old World complex mammarenaviruses (OWAV) in the Eastern Hemisphere include LASV, Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered LUJV; New World complex mammarenaviruses (NWAV) in the Western Hemisphere include JUNV, MACV, Sabia Virus (SABV), and CHAPV. There are no FDA approved vaccines or therapeutics for mammarenaviruses, though the attenuated JUNV "Candid-1" vaccine is approved for use in Argentina.

Disease severity and case fatality rates (CFR) vary across mammarenaviruses even within the same clades and species. Up to 500,000 cases of LASV infection occur per year with CFRs sometimes approaching 70% in hospitalized cases[19]. Before the introduction of the Candid-1 vaccine, it is believed that an upwards of 60,000 cases of Argentine Hemorrhagic Fever (AHF) from infection with JUNV occurred per year with CFRs ranging from 15-30%. Convalescent plasma from JUNV survivors is still the primary means to treat acute AHF in Argentina[20]. Over 1000 cases were reported for the initial outbreak of MACV with CFRs ranging from 5-30%; enhanced rodent abatement practices and potentially cross-protective immunity in populations that have taken the JUNV Candid-1 vaccine may have reduced recent outbreak frequency[21]. All mammarenaviruses share a common genomic organization consisting of two ambisense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix) and L protein (polymerase), and the glycoprotein precursor (GPC) and nucleoprotein (NP)[17]. Despite general

similarity of their genomic architecture, mammarenaviruses exhibit marked differences in their replication life cycles including attachment factors, entry mechanisms, and immune evasion; nonetheless, the dominant antigens driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Despite the abundant genetic diversity of LASV, high levels of cross reactivity have been seen with antigen-based diagnostics across seven known lineages, suggesting the generation of cross-protective immunogens and antibodies against mammarenaviruses is achievable[22]. Monoclonal antibodies derived from human LASV survivors were shown to protect non-human primates (NHP) against lethal challenge[23]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[24]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, indicating this domain could serve as an antigenic target. Investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccines to test this approach and have demonstrated protection against lethal LASV lineage IV Josiah challenge. Specifically, a stabilized prefusion trimeric GPe, an immunogen containing a highly conserved NP NTD epitope, or a combination of these two approaches conferred protection in a lethal outbred guinea pig model. Nevertheless, even with the considerable molecular diversity in LASV strains, conservation at key antigenic sites within the GP and NP suggest a pan-LASV vaccine may be possible[22, 25, 26]. **RP1** and **Core E** investigators have demonstrated that a recombinant vesicular stomatitis virus (rVSV)-based vaccine expressing the LASV-Josiah GPC is protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[27], and against heterologous challenge with Lineage II[28], III, V[29], and VI viruses (Cross and Geisbert, unpublished data).

In **Phase I of RP1**, the ability of an optimized LASV GPe/NP subunit vaccine delivered intramuscularly (IM) will be assessed in guinea pig and NHP models across multiple viral lineages. We will also assess a microneedle skin patch (MNP) formulation optimized in **Core C** to determine if a needle-free vaccination route affords more potent vaccine-mediated responses and protection. Similarly, we have generated MACV GPe immunogens and will design a MACV NP-NTD subunit antigen to test the same approach across multiple MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing both OWAV (LUJV) and NWAV (CHAPV) complexes, for which there are no vaccines available. A unique advantage of the PABVAX Center is its access to biosafety level (BSL)-4 containment, allowing us to work with authentic versus surrogate arenaviruses. The BSL-4 facilities described in **Core E** are capable of evaluating countermeasures in small animal models and NHPs. The cynomolgus macaque is the current "gold standard" animal model for LASV, JUNV, and MACV research as disease recapitulates human infection and there are a multitude of immunological reagents available[30-32]. The evaluation of prototype OWAV and NWAV vaccines and therapies in the NHP model is likely pivotal for IND applications and for future licensure of medical countermeasures for human use.

<u>Nairoviridae:</u> Unique to most bunyaviruses, orthonairoviruses are transmitted primarily by ticks[2]. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[33], Issyk-Kul virus[34], Songling virus[35]), CCHFV is the most recognized public health threat. Outbreaks of CCHFV have occurred across Europe, Africa, the Middle East, and Asia. There is growing concern for expansion of the CCHFV endemic region due to increased range of its tick host (*Hyalomma* spp.) tied to bird migration changes, livestock trade, and global warming. For example, CCHFV cases have been reported in Spain[36], a previously non-endemic region, and *Hyalomma* ticks have been found in Sweden[37]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to a severe hemorrhagic fever and death in 30% of cases[38]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[39]. Ribavirin has been used to treat infections with limited success[40].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and GPC [38]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[38]. Conversely, the CCHFV GPC is considerably less conserved (< 75% amino acid identity)[38]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location. Thus, creating a pan-CCHFV vaccine or therapeutic based solely on one clade poses a challenge. Nevertheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a rVSV vectored vaccine expressing the full GPC of CCHFV[41].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP immunogens with the highest level of homology across clades. We will assess their capacity to protect against lethal challenge with isolates from multiple CCHFV clades in established mouse[41, 42] and NHP[43] models with **Core E**. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best formulation of CCHFV GP/NP MNP vaccine against the emerging test case nairovirus, Kasokero virus, for which there are no vaccines available.

Paramyxoviridae: The HNVs NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality[4, 44]. HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality[45]. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs, which served as amplifying hosts[46]. NiV and HeV can infect at least 18 animal species across 6 orders of mammals, and among these they cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans[14,47]. The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission[5]. Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and Southeast Asia and Australia. Their broad host tropism and ability to infect livestock also causes increased risk of human infections. HNVs are single-stranded, negative sense, enveloped RNA viruses and their biology lends themselves to be synthetically produced. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS). The Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents[48] because the virus could be engineered for mass dissemination and it possesses the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the R&D Blueprint list of priority pathogens with epidemic potential[49].

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb exist. These postexposure treatments conferred protecion against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP)[50-53]. The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia[54]. In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs, and several vaccine platforms have clear potential for use in people[14]. The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies[14]. Such preventive vaccines would have utility for lab workers, first responders or individuals at high-risk exposure, and in mitigating the spread of an outbreak. In the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent[55]. It is widely considered that the African green monkey (AGM) is the current "gold standard" animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection[47, 56]. The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished)[57]. The divergent LayV will be the focus of the test case studies. The choice is justifiable for several important reasons: 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; and 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including rescuing recombinant LayV (rLayV), exploring animal model development, constructing additional LayV and AngV reporter virus tools, and then translating and testing the MNP vaccine "plug-and-play" approach. Finally, we will evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME. A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center's RPs and Core leaders currently have active and productive collaborative research programs in related areas, most spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

The pandemic preparedness research gaps addressed by PABVAX are diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **arenaviruses** and **nairoviruses**, and **RP2** will target the prototype **HNVs**. Both RP1 and RP2 will develop new prototype virus tools (recombinant prototype viruses) along with existing tools, to analyze vaccine responses and evaluate new mAbs and nabs from the other RPs. A third RP will develop a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant *Cedar henipavirus* (rCedV) *in vivo* and a bioluminescence detection method that can longitudinally trace the dynamics and anatomical progression of infection with a luciferase-encoding virus in individual animals (**RP3**). There will also be two RPs that focus on the development of broadly reactive human mAbs and nanobodies to the PABVAX selected viral prototypes. **RP4** will isolate human mAbs derived from NiV-infected survivors with a specific focus on F glycoprotein-targeting mAbs. Additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will engage in the manufacture, quality control, and testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**). Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core (**Core A**) will provide organizational and programmatic structure to promote research productivity and scientific interactions along with compliance with regulations concerning select agent, vertebrate animal, and human subject use. **Core A** will coordinate all activities with the Scientific Advisory Board (**SAB**) and make data available through the Data Management Core (**Core B**).

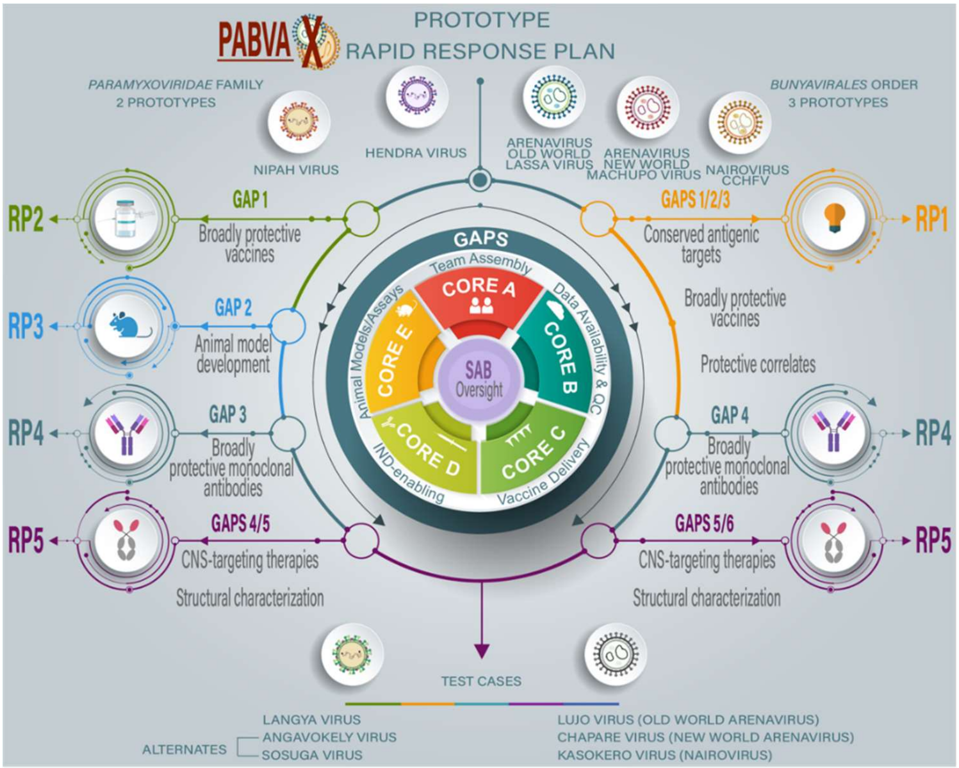


Figure 1: Research gaps addressed by the *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center- (PABVAX).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform. The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**). This project tested a dissolvable MNP-based vaccine technology ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine[58]. The MNP vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable microneedles that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants[59] to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as to facilitate improved vaccine storage and distribution due to their improved thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, Scientific **Core C** (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ)-adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (*Paramyxoviridae*). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro*, along with analysis of polyfunctional T-cell responses by antigenic peptide stimulation, followed by intracellular cytokine staining (ICS) and flow cytometry.

Discover effective antibody-based prophylactics and therapies. Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah[60], Hendra[61], Ebola[62], Marburg[63] and Lassa[64]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, etc.), a feature to be exploited in **Core D** by Dr. Gunn. In addition to therapeutic applications, mAbs with Fc mutations dramatically extend serum half-life[65-67] and offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g*. rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization, and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures. Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal Model Development and Preclinical Evaluation Scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective efficacy of antivirals and vaccines, as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation, including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within

RP1. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they are generally less laborious and can be adapted to higher throughput platforms and/or integrated with other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput fluorescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic NiV and HeV vaccine-induced immune sera, mAbs (**RP4**), nbs (**RP5**), and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either HeV or NiV-B envelope GPs) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals to explore nab based therapeutics to CNS resident HNVs[68].

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the HNVs (*Paramyxoviridae*) and the *Bunyavirales* (*Arenaviridae* and *Nairoviridae*) that will bolster vaccine endurance to storage and transportation; we also anticipate improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

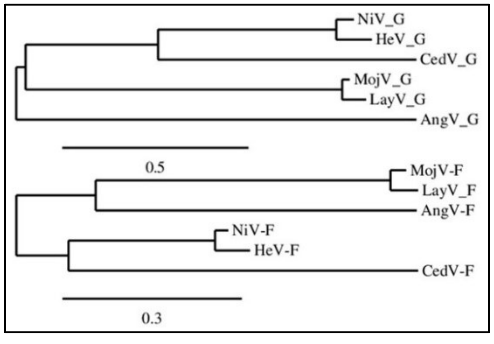
The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology, and mAb and vaccine translational drug development along with Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Paramyxoviruses: NiV and HeV are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis[4, 44]. HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals. These viruses are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of CedV, Gamak virus, Daeryong virus, and (LayV)[69] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[70-73]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and

Figure 2: Phylogenetic analysis of select HNV G and F glycoproteins. Trees were constructed for using the PhyML program's maximum likelihood method and aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.



AngV, are old world pteropid fruit bats[70, 74]. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province)[15]. The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**. Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal HNV disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters[47], and AGMs (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P). The P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists[75-79]. The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections[80, 81]. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature[82]. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2[83] and CedV is now recognized as a BSL-2 restricted agent[84]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II "plug and play" research plan (**RP2** and **PR5** with **Cores C, D,** and **E**).

Arenaviruses (old world): For **phase 1**, **RP1** will utilize **LASV as the prototype virus** due its significant public health burden throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[85]. LASV is the most imported viral hemorrhagic fever (VHF) next to Dengue virus and has been documented in several countries outside of Africa over 35 times[86]. CFRs approaching 70% in hospitalized cases and marked long term sequelae are the result of the lack of approved vaccines or therapeutics, although several live attenuated virus candidates are currently being evaluated with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI)[87]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize only LASV GP as the target immunogen.

Investigators in **RP1**, **Core D**, and **Core E** have already demonstrated protective efficacy against challenge with LASV in outbred guinea pigs vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These immunogens have been engineered to express homologous antigenic regions to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach suggests a similar strategy could be implemented against other arenaviruses. The **OWAV test pathogen, LUJV,** was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia, which quickly spread nosocomially[88]. The CFR for this infectious virus was 80% in this small outbreak of 5 people. There are currently no approved vaccines or therapeutics available for this virus and its genetic divergence from suggest current LASV countermeasures may be of limited use. LUJV will serve as an ideal "test case" to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Arenaviruses (new world): The live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[89], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[90, 91]. Furthermore, there is the potential for pathogenic reversion. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest exploring other JUNV countermeasures are warranted. Building on the success of the LASV GPe/NP-NTD, investigators in **RP1 and Cores D and E** will engineer broadly protective MACV GPe and NP-NTD antigens and perform efficacy testing in established guinea pig and NHP models. We will extend this plug-and-play approach to the **NWAV test case, CHAPV**. First reported in 2003 in Bolivia, CHAPV is a rodent-borne mammarenavirus associated with the development of severe hemorrhagic fever with case fatality rates up to 60%[92, 93]. Recent studies have demonstrated clear human-to-human transmission during the acute phase evidenced by several nosocomial infections; isolation of viable virus from semen at 86 days after symptom onset demonstrates a clear need to implement specialized management

and treatment [93]. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Nairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as it represents the largest global public health burden within the orthonairovirus family with outbreaks annually across Europe, Africa, and Asia. There are no FDA-approved vaccines for CCHFV; however, a suckling mouse brain-derived, adjuvanted whole virus vaccine for CCHFV has been approved for use in Bulgaria. This vaccine has numerous safety concerns and has limited demonstrated efficacy. Several experimental vaccine approaches have been evaluated ranging from viral vectored approaches to DNA-based vaccines with varying efficacy, advantages, and disadvantages[94]; however, only a Modified Vaccinia Ankara virus vaccine (MVA-CCHFV) has begun to advance towards clinical trials[95]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test these for protective efficacy from challenge with 6 different CCHFV lineages using established mouse and NHP models. Next, we will deploy the same approach to the **test case nairovirus, Kasokero virus (KASV),** for which no vaccine or treatment exists.

In 1977, KASV was first isolated from Ugandan bats and caused several cases of acute febrile illness of laboratory workers from the same lab that processed the infected bats[96]. Surveys of 109 laboratory workers from the same facility demonstrated 13% seropositivity and clinical symptoms in individuals 4 to 15 months after the virus was isolated. These findings suggest either continued laboratory-associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the tick reservoir, *Ornithodoros faini,* to participate in enzootic maintenance of KASV[97]. While most chiropteran ticks exhibit host specificity, humans entering caves these bats inhabit have reported bites from *O.faini* ticks. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics suggest investment in countermeasure development against this virus is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Management Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**; arenavirus and nairovirus projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine active and passive immunization approaches to further improve the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

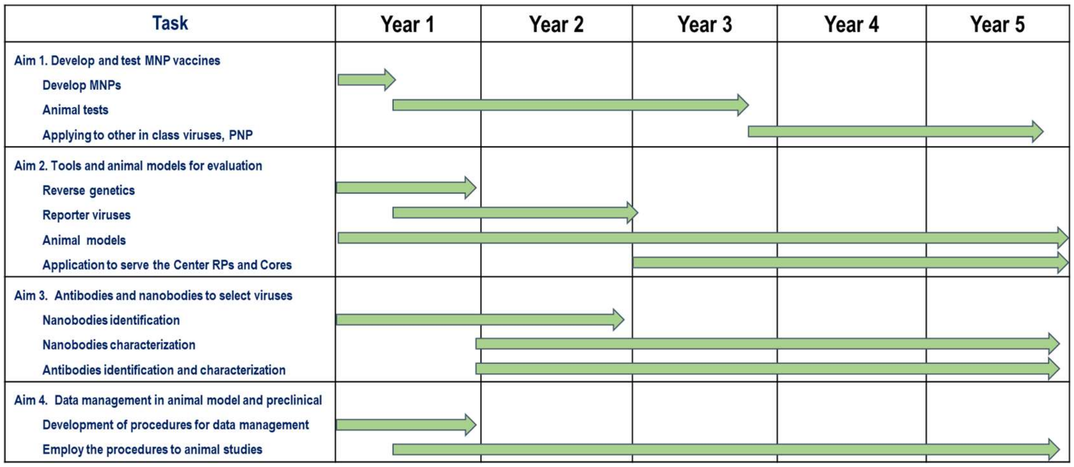
An additional and outstanding advantage of the PABVAX Center is the **Animal Model Development and Preclinical Evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL . Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for the Center RPs will be performed by the same BSL-4 staff under the

same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for members of the *Bunyavirales* and *Paramyxoviridae*.**
2. **Develop new tools and animal models for cell-based and *in vivo* therapeutic evaluation.**
3. **Develop antibodies and nanobodies to the selected prototype and test viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**



Task	Year 1	Year 2	Year 3	Year 4	Year 5
Aim 1. Develop and test MNP vaccines					
Develop MNPs	→				
Animal tests	→	→	→		
Applying to other in class viruses, PNP				→	→
Aim 2. Tools and animal models for evaluation					
Reverse genetics	→				
Reporter viruses	→	→			
Animal models	→	→	→	→	→
Application to serve the Center RPs and Cores			→	→	→
Aim 3. Antibodies and nanobodies to select viruses					
Nanobodies identification	→	→			
Nanobodies characterization		→	→	→	→
Antibodies identification and characterization		→	→	→	→
Aim 4. Data management in animal model and preclinical					
Development of procedures for data management	→				
Employ the procedures to animal studies	→	→	→	→	→

Figure 3: Projected PABVAX timeline to achieve the 4 overarching tasks.

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**. Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (details are specified within the Administration **Core A** Research Strategy). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommending new directions as appropriate, and providing Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

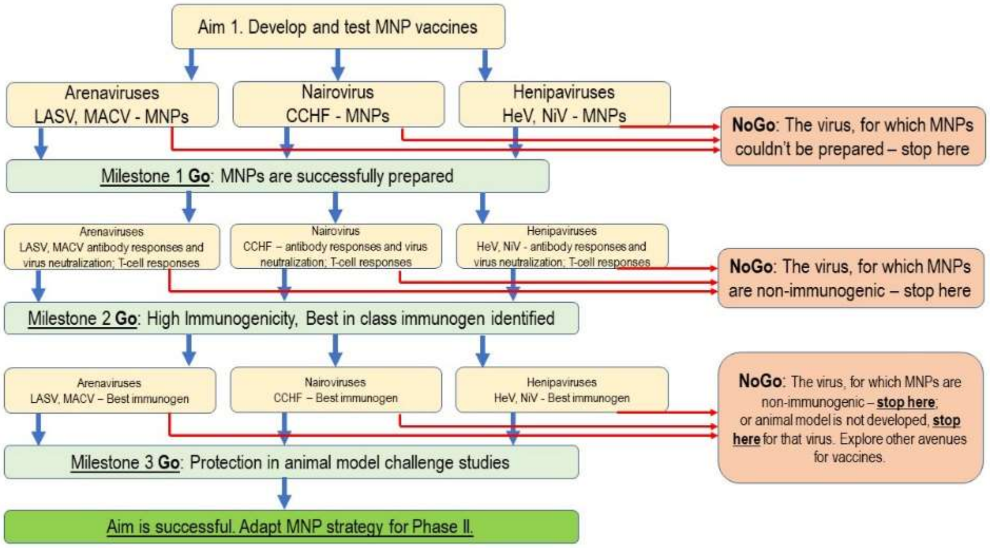


Figure 4. Milestones and criteria for **Aim 1** of the PABVAX Center's overall objectives.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

The first milestone will address whether formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made (**Figure 4**). The determination of MNP vaccine potential success will first be established using immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen-specific antibody induction, virus neutralization, and polyfunctional T-cell responses by *in vitro* stimulation of isolated cells). Go/no go decisions will be made based on outcomes of these first-tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as a success. Comparisons will also be made such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of the best-in-class immunogens, or immunogen combinations, will also be explored to down-select vaccines. The third milestone will determine whether MNP vaccination affords protection in animal model challenge experiments. In the case of NiV and HeV prototypes, the animal models chosen (ferrets and African green monkeys (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales*, there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASV, and guinea pig models exist for some but not all of these lineages. For MACV, there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is the limitations of cost and availability of rhesus and cynomolgus monkeys. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be developing and evaluating animal models within the PABVAX program, including further development of AGM models for arenaviruses and possibly CCHFV as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for cell-based and in vivo therapeutics evaluation.

The second overall objective of PABVAX is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation (**Figure 5**). These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed because genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If successful, a large battery of new materials will be available to share with the ReVAMPP network and be key milestones for execution of Phase II of the PABVAX Center program.

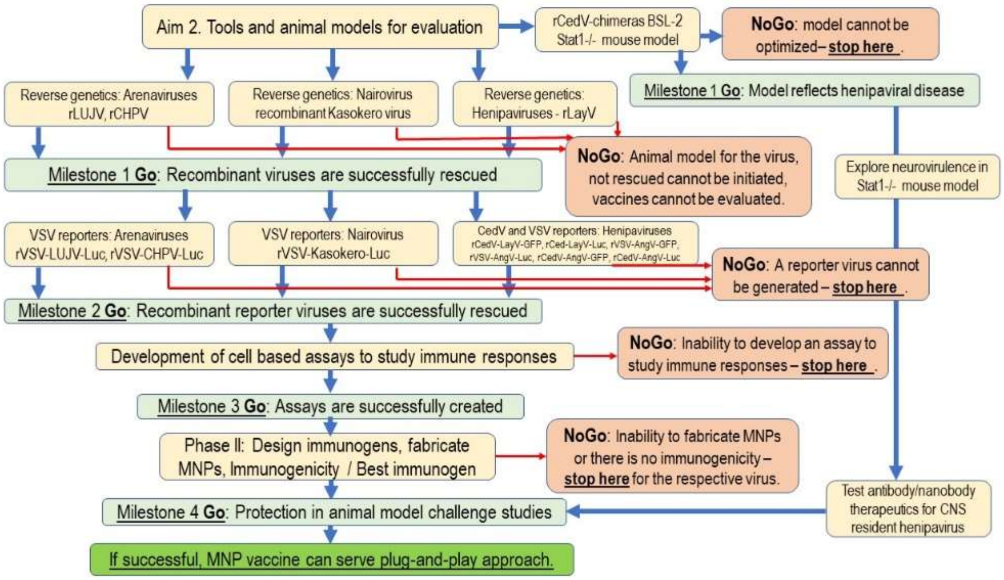


Figure 5. Milestones and criteria for **Aim 2** of the PABVAX Center's overall objectives.

The translation of the MNP vaccine plug-and-play approach will be in Phase II (**Table 1**). Milestones will be similar to those in **Figure 4**. New virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new *in vivo* bioluminescence-based immune deficient mouse model developed by the PIs of **PR2** and **RP3** that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease *in vivo* safely and expediently at BSL-2 containment. The model

will be deployed to test a critically important therapeutic need against CNS resident HNV infection, and **RP3** will collaborate with **RP5** in the testing of engineered nabs.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

New human mAbs and nbs as tools and potential therapeutics will be developed by **RP4** and **RP5** in collaboration with **RP2** and **Core D** (**Figure 6**). NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs, and in collaboration with **RP2**, neutralization potencies will be rapidly assessed (as well as evaluating synergistic cell-based mAb neutralization) that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

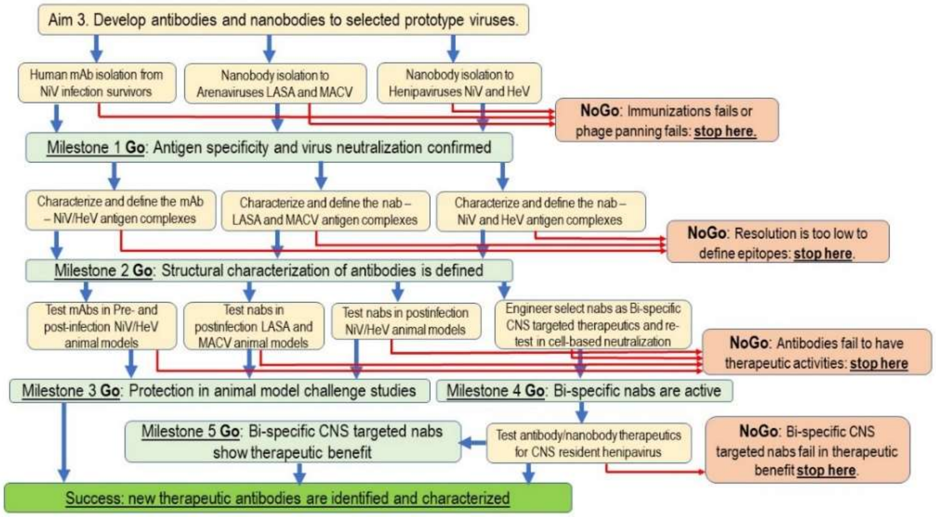


Figure 6. Milestones and criteria for **Aim 3** of the PABVAX Center's overall objectives.

Nbs, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nbs can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nbs targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus), and select animal models of LASV and MACV by **Core E**. Success will be measured by the 1) identification of potent virus-neutralizing nbs, 2) discovery of more broadly reactive nbs particularly to the multiple LASV clades, 3) evidence of therapeutic benefit in animal models, and 4) the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

Industry Partnerships.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience in developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and

make an ideal partner for PABVAX. Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

SPECIAL FEATURES OF THE PABVAX

Galveston National Laboratory (GNL). The GNL is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics, and therapeutics. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370 NHP studies employing some 2,772 animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses have been performed. These studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder, and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP facility and a staff of full-time veterinarians. **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above, another special feature is the location of the WRCEVA virus collection within the GNL.

***Core E (Animal model development and preclinical evaluation)*. Core E** operations will be conducted at the GNL, and in conjunction with **Core A**, will also include a regulatory expert from the UTMB Institutional Office of Regulated Nonclinical Studies (ORNcS). The ORNcS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL, and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses, arenaviruses, and henipaviruses. He has published over 250 peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI), was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs). Mapp Biopharmaceutical has deep experience with antigens and mAbs against these antigens. Their early development and research group has expertise in immunology, infectious diseases, and the production of antigens, antibodies, and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [98] [99]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1[100]. Dr. Gunn and Genovac will join the team to analyze antibody Fc effector functions and to provide antigen/antibody discovery resources, respectively. Dr. Geisbert will contribute his experience with high containment animal models and vaccine development. As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

BIBLIOGRAPHY AND REFERENCES CITED

1. Lamb RA, Parks GD. Paramyxoviridae: the viruses and their replication. In Fields BN, Knipe DM, Howley PM, editors, Fields Virology: Sixth Edition. Vol. 1. Lippincott, Williams, and Wilkins. 2013. p. 957-995.
2. Boshra H. An Overview of the Infectious Cycle of Bunyaviruses. Viruses. 2022;14(10). Epub 20220928. doi: 10.3390/v14102139. PubMed PMID: 36298693; PMCID: PMC9610998.
3. Horne KM, Vanlandingham DL. Bunyavirus-vector interactions. Viruses. 2014;6(11):4373-97. Epub 20141113. doi: 10.3390/v6114373. PubMed PMID: 25402172; PMCID: PMC4246228.
4. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia), p 1070-1085.
5. Luby SP. The pandemic potential of Nipah virus. Antiviral research. 2013;100(1):38-43. Epub 20130730. doi: 10.1016/j.antiviral.2013.07.011. PubMed PMID: 23911335.
6. Kummer S, Kranz DC. Henipaviruses-A constant threat to livestock and humans. PLoS Negl Trop Dis. 2022;16(2):e0010157. Epub 20220218. doi: 10.1371/journal.pntd.0010157. PubMed PMID: 35180217; PMCID: PMC8856525.
7. Maes P, Adkins S, Alkhovsky SV, Avšič-Županc T, Ballinger MJ, Bente DA, Beer M, Bergeron É, Blair CD, Briese T, Buchmeier MJ, Burt FJ, Calisher CH, Charrel RN, Choi IR, Clegg JCS, de la Torre JC, de Lamballerie X, DeRisi JL, Digiaro M, Drebot M, Ebihara H, Elbeaino T, Ergünay K, Fulhorst CF, Garrison AR, Gāo GF, Gonzalez JJ, Groschup MH, Günther S, Haenni AL, Hall RA, Hewson R, Hughes HR, Jain RK, Jonson MG, Junglen S, Klempa B, Klingström J, Kormelink R, Lambert AJ, Langevin SA, Lukashevich IS, Marklewitz M, Martelli GP, Mielke-Ehret N, Mirazimi A, Mühlbach HP, Naidu R, Nunes MRT, Palacios G, Papa A, Pawęska JT, Peters CJ, Plyusnin A, Radoshitzky SR, Resende RO, Romanowski V, Sall AA, Salvato MS, Sasaya T, Schmaljohn C, Shí X, Shirako Y, Simmonds P, Sironi M, Song JW, Spengler JR, Stenglein MD, Tesh RB, Turina M, Wèi T, Whitfield AE, Yeh SD, Zerbini FM, Zhang YZ, Zhou X, Kuhn JH. Taxonomy of the order Bunyavirales: second update 2018. Arch Virol. 2019;164(3):927-41. doi: 10.1007/s00705-018-04127-3. PubMed PMID: 30663021; PMCID: PMC6581445.
8. Silva-Ramos CR, Montoya-Ruíz C, Faccini-Martínez Á A, Rodas JD. An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever. Arch Virol. 2022;167(9):1727-38. Epub 20220517. doi: 10.1007/s00705-022-05453-3. PubMed PMID: 35579715; PMCID: PMC9110938.
9. Garry RF. Lassa fever - the road ahead. Nat Rev Microbiol. 2023;21(2):87-96. Epub 20220912. doi: 10.1038/s41579-022-00789-8. PubMed PMID: 36097163; PMCID: PMC9466315.
10. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. Antiviral research. 2018;159:63-7. Epub 20180924. doi: 10.1016/j.antiviral.2018.09.009. PubMed PMID: 30261226; PMCID: PMC7113760.
11. WHO. List of Blueprint Priority Diseases. 2018 [cited 2018 November 14]. Available from: http://www.who.int/blueprint/priority-diseases/en/.
12. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens 2018 [04 Jun 2023]. Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
13. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. Nat Rev Microbiol. 2006;4(1):23-35. doi: 10.1038/nrmicro1323. PubMed PMID: 16357858; PMCID: PMC7097447.
14. Amaya M, Broder CC. Vaccines to Emerging Viruses: Nipah and Hendra. Annu Rev Virol. 2020;7(1):447-73. doi: 10.1146/annurev-virology-021920-113833. PubMed PMID: 32991264; PMCID: PMC8782152.
15. Zhang XA, Li H, Jiang FC, Zhu F, Zhang YF, Chen JJ, Tan CW, Anderson DE, Fan H, Dong LY, Li C, Zhang PH, Li Y, Ding H, Fang LQ, Wang LF, Liu W. A Zoonotic Henipavirus in Febrile Patients in China. N Engl J Med. 2022;387(5):470-2. doi: 10.1056/NEJMc2202705. PubMed PMID: 35921459.
16. Radoshitzky SR, Buchmeier MJ, Charrel RN, Clegg JCS, Gonzalez JJ, Günther S, Hepojoki J, Kuhn JH, Lukashevich IS, Romanowski V, Salvato MS, Sironi M, Stenglein MD, de la Torre JC, Ictv Report C. ICTV Virus Taxonomy Profile: Arenaviridae. J Gen Virol. 2019;100(8):1200-1. Epub 20190613. doi: 10.1099/jgv.0.001280. PubMed PMID: 31192784.
17. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. Front Microbiol. 2018;9:1751. Epub 20180803. doi: 10.3389/fmicb.2018.01751. PubMed PMID: 30123198; PMCID: PMC6085440.
18. Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient Evolution of Mammarenaviruses: Adaptation via Changes in the L Protein and No Evidence for Host-Virus Codivergence. Genome Biol Evol. 2018;10(3):863-74. doi: 10.1093/gbe/evy050. PubMed PMID: 29608723.

19. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M, Momoh M, Fonnie M, Fonnie R, Kanneh L, Koroma VJ, Kargbo K, Ottomassathien D, Muncy IJ, Jones AB, Illick MM, Kulakosky PC, Haislip AM, Bishop CM, Elliot DH, Brown BL, Zhu H, Hastie KM, Andersen KG, Gire SK, Tabrizi S, Tariyal R, Stremlau M, Matschiner A, Sampey DB, Spence JS, Cross RW, Geisbert JB, Folarin OA, Happi CT, Pitts KR, Geske FJ, Geisbert TW, Saphire EO, Robinson JE, Wilson RB, Sabeti PC, Henderson LA, Khan SH, Bausch DG, Branco LM, Garry RF, the Viral Hemorrhagic Fever C. Lassa Fever in Post-Conflict Sierra Leone. PLOS Neglected Tropical Diseases. 2014;8(3):e2748. doi: 10.1371/journal.pntd.0002748.

20. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Antiviral research. 2008;78(1):132-9. Epub 2007/12/07. doi: 10.1016/j.antiviral.2007.10.010. PubMed PMID: 18054395.

21. Kilgore PE, Peters CJ, Mills JN, Rollin PE, Armstrong L, Khan AS, Ksiazek TG. Prospects for the control of Bolivian hemorrhagic fever. Emerg Infect Dis. 1995;1(3):97-100. doi: 10.3201/eid0103.950308. PubMed PMID: 8903174; PMCID: PMC2626873.

22. Heinrich ML, Boisen ML, Nelson DKS, Bush DJ, Cross RW, Koval AP, Hoffmann AR, Beddingfield BJ, Hastie KM, Rowland MM, Aimukanova I, Koval S, Lathigra R, Borisevich V, Momoh M, Sandi JD, Goba A, Odia I, Baimba F, Aiyepada JO, Ebo B, Eromon P, Ugwu C, Folarin O, Olumade T, Onyechi MN, Etafo J, Adeyemi R, Ella EE, Aminu M, Gomerep SS, Eke MA, Ogunsanya O, Akpede GO, Asogun DO, Okogbenin SA, Okokhere PO, Holst J, Shaffer JG, Schieffelin JS, Geisbert TW, Saphire EO, Happi CT, Grant DS, Garry RF, Branco LM. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. Scientific Reports. 2020;10(1):16030. doi: 10.1038/s41598-020-72539-w.

23. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nature Medicine. 2017;23(10):1146-9. doi: 10.1038/nm.4396.

24. Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO. Structural basis for antibody-mediated neutralization of Lassa virus. Science. 2017;356(6341):923-8. doi: 10.1126/science.aam7260. PubMed PMID: 28572385; PMCID: PMC6007842.

25. Sullivan BM, Sakabe S, Hartnett JN, Ngo N, Goba A, Momoh M, Demby Sandi J, Kanneh L, Cubitt B, Garcia SD, Ware BC, Kotliar D, Robles-Sikisaka R, Gangavarapu K, Branco L, Eromon P, Odia I, Ogbaini-Emovon E, Folarin O, Okogbenin S, Okokhere PO, Happi C, de la Torre JC, Sabeti PC, Andersen KG, Garry RF, Grant DS, Schieffelin JS, Oldstone MBA. High crossreactivity of human T cell responses between Lassa virus lineages. PLoS Pathog. 2020;16(3):e1008352. Epub 20200306. doi: 10.1371/journal.ppat.1008352. PubMed PMID: 32142546; PMCID: PMC7080273.

26. Ugwu C, Olumade T, Nwakpakpa E, Onyia V, Odeh E, Duruiheoma RO, Ojide CK, Eke MA, Nwafor IE, Chika-Igwenyi N, Abu AM, Azuogu B, Ajayi N, Ogah E, Ayodeji O, Abejegah C, Adedosu N, Oyejide N, Abah S, Omidele A, Ingbian W, Osoba E, Eromon P, Oluniyi P, Ogunsanya O, Happi A, Otuh P, Nadesalingam A, Carnell G, Krause N, Aguinam E, Kinsley R, Storisteanu DML, Tonks P, Nelson D, McAlister C, Boisen M, Garry R, Wright E, Temperton N, Frost S, Heeney JL, Happi C. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. Scientific Reports. 2022;12(1):22330. doi: 10.1038/s41598-022-26045-w.

27. Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, Liebscher R, Grolla A, Ströher U, Fernando L, Daddario KM, Guttieri MC, Mothé BR, Larsen T, Hensley LE, Jahrling PB, Feldmann H. Development of a New Vaccine for the Prevention of Lassa Fever. PLOS Medicine. 2005;2(6):e183. doi: 10.1371/journal.pmed.0020183.

28. Cross RW, Woolsey C, Prasad AN, Borisevich V, Agans KN, Deer DJ, Geisbert JB, Dobias NS, Fenton KA, Geisbert TW. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. Cell Rep. 2022;40(3):111094. doi: 10.1016/j.celrep.2022.111094. PubMed PMID: 35858566.

29. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. PLOS Neglected Tropical Diseases. 2015;9(4):e0003736. doi: 10.1371/journal.pntd.0003736.

30. Hensley LE, Smith MA, Geisbert JB, Fritz EA, Daddario-DiCaprio KM, Larsen T, Geisbert TW. Pathogenesis of Lassa fever in cynomolgus macaques. Virol J. 2011;8:205. Epub 20110506. doi: 10.1186/1743-422x-8-205. PubMed PMID: 21548931; PMCID: PMC3104370.
31. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proceedings of the National Academy of Sciences. 2021;118(11):e2023332118. doi: doi:10.1073/pnas.2023332118.
32. Bell TM, Shaia CI, Bunton TE, Robinson CG, Wilkinson ER, Hensley LE, Cashman KA. Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure. Veterinary Pathology. 2015;52(1):26-37. doi: 10.1177/0300985814540544. PubMed PMID: 24990481.
33. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. Epidemiol Infect. 1996;116(3):353-61. doi: 10.1017/s0950268800052687. PubMed PMID: 8666081; PMCID: PMC2271429.
34. Brinkmann A, Kohl C, Radonić A, Dabrowski PW, Mühldorfer K, Nitsche A, Wibbelt G, Kurth A. First detection of bat-borne Issyk-Kul virus in Europe. Sci Rep. 2020;10(1):22384. Epub 20201224. doi: 10.1038/s41598-020-79468-8. PubMed PMID: 33361773; PMCID: PMC7759570.
35. Ma J, Lv XL, Zhang X, Han SZ, Wang ZD, Li L, Sun HT, Ma LX, Cheng ZL, Shao JW, Chen C, Zhao YH, Sui L, Liu LN, Qian J, Wang W, Liu Q. Identification of a new orthonairovirus associated with human febrile illness in China. Nat Med. 2021;27(3):434-9. Epub 20210218. doi: 10.1038/s41591-020-01228-y. PubMed PMID: 33603240.
36. Lorenzo Juanes HM, Carbonell C, Sendra BF, López-Bernus A, Bahamonde A, Orfao A, Lista CV, Ledesma MS, Negredo AI, Rodríguez-Alonso B, Bua BR, Sánchez-Seco MP, Muñoz Bellido JL, Muro A, Belhassen-García M. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. Emerg Infect Dis. 2023;29(2):252-9. doi: 10.3201/eid2902.220677. PubMed PMID: 36692301; PMCID: PMC9881766.
37. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, Strube C, Springer A, Albihn A, Jaenson TGT, Omazic A. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. Ticks Tick Borne Dis. 2020;11(3):101403. Epub 20200130. doi: 10.1016/j.ttbdis.2020.101403. PubMed PMID: 32037097.
38. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. Nature Reviews Microbiology. 2023. doi: 10.1038/s41579-023-00871-9.
39. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. Antiviral research. 2011;90(2):85-92. Epub 20110306. doi: 10.1016/j.antiviral.2011.02.010. PubMed PMID: 21362441.
40. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. BMC Infectious Diseases. 2010;10(1):207. doi: 10.1186/1471-2334-10-207.
41. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. Sci Rep. 2019;9(1):7755. Epub 20190523. doi: 10.1038/s41598-019-44210-6. PubMed PMID: 31123310; PMCID: PMC6533279.
42. Bente DA, Alimonti JB, Shieh WJ, Camus G, Ströher U, Zaki S, Jones SM. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. J Virol. 2010;84(21):11089-100. Epub 20100825. doi: 10.1128/jvi.01383-10. PubMed PMID: 20739514; PMCID: PMC2953203.
43. Cross RW, Prasad AN, Borisevich V, Geisbert JB, Agans KN, Deer DJ, Fenton KA, Geisbert TW. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. PLoS Negl Trop Dis. 2020;14(8):e0008637. Epub 20200813. doi: 10.1371/journal.pntd.0008637. PubMed PMID: 32790668; PMCID: PMC7447009.
44. Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York, p 519-536.
45. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B, et al. A morbillivirus that caused fatal disease in horses and humans. Science. 1995;268(5207):94-7. doi: 10.1126/science.7701348. PubMed PMID: 7701348.

46. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS, Gubler DJ, Roehrig JT, Eaton B, Gould AR, Olson J, Field H, Daniels P, Ling AE, Peters CJ, Anderson LJ, Mahy BW. Nipah virus: a recently emergent deadly paramyxovirus. Science (New York, NY). 2000;288(5470):1432-5. doi: 10.1126/science.288.5470.1432. PubMed PMID: 10827955.
47. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. Curr Top Microbiol Immunol. 2012;359:153-77. doi: 10.1007/82_2012_208. PubMed PMID: 22476556; PMCID: PMC7120132.
48. Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087.
49. Sweileh WM. Global research trends of World Health Organization's top eight emerging pathogens. Global Health. 2017;13(1):9. Epub 20170208. doi: 10.1186/s12992-017-0233-9. PubMed PMID: 28179007; PMCID: PMC5299748.
50. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng Y-R, Brining D, Scott D, Wang Y, Dimitrov AS, Callison J, Chan Y-P, Hickey AC, Dimitrov DS, Broder CC, Rockx B. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. Science translational medicine. 2011;3(105):105ra3. doi: 10.1126/scitranslmed.3002901. PubMed PMID: 22013123.
51. Geisbert TW, Mire CE, Geisbert JB, Chan Y-P, Agans KN, Feldmann F, Fenton KA, Zhu Z, Dimitrov DS, Scott DP, Bossart KN, Feldmann H, Broder CC. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Science translational medicine. 2014;6(242):242ra82. doi: 10.1126/scitranslmed.3008929. PubMed PMID: 24964990.
52. Mire CE, Satterfield BA, Geisbert JB, Agans KN, Borisevich V, Yan L, Chan YP, Cross RW, Fenton KA, Broder CC, Geisbert TW. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. Sci Rep. 2016;6:30916. Epub 20160803. doi: 10.1038/srep30916. PubMed PMID: 27484128; PMCID: PMC4971471.
53. Mire CE, Chan YP, Borisevich V, Cross RW, Yan L, Agans KN, Dang HV, Veesler D, Fenton KA, Geisbert TW, Broder CC. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. J Infect Dis. 2020;221(Suppl 4):S471-s9. doi: 10.1093/infdis/jiz515. PubMed PMID: 31686101; PMCID: PMC7199785.
54. Playford EG, Munro T, Mahler SM, Elliott S, Gerometta M, Hoger KL, Jones ML, Griffin P, Lynch KD, Carroll H, El Saadi D, Gilmour ME, Hughes B, Hughes K, Huang E, de Bakker C, Klein R, Scher MG, Smith IL, Wang LF, Lambert SB, Dimitrov DS, Gray PP, Broder CC. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. Lancet Infect Dis. 2020;20(4):445-54. Epub 20200203. doi: 10.1016/s1473-3099(19)30634-6. PubMed PMID: 32027842.
55. Middleton D, Pallister J, Klein R, Feng Y-R, Haining J, Arkinstall R, Frazer L, Huang J-A, Edwards N, Wareing M, Elhay M, Hashmi Z, Bingham J, Yamada M, Johnson D, White J, Foord A, Heine HG, Marsh GA, Broder CC, Wang L-F. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerging infectious diseases. 2014;20(3):372-9. doi: 10.3201/eid2003.131159. PubMed PMID: 24572697.
56. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, Smith MA, Chan YP, Wang LF, Mattapallil JJ, Geisbert JB, Bossart KN, Broder CC. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One. 2010;5(5):e10690. Epub 20100518. doi: 10.1371/journal.pone.0010690. PubMed PMID: 20502528; PMCID: PMC2872660.
57. Cheliout Da Silva S, Yan L, Dang HV, Xu K, Epstein JH, Veesler D, Broder CC. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. Viruses. 2021;13(3). Epub 20210322. doi: 10.3390/v13030517. PubMed PMID: 33809833; PMCID: PMC8004131.
58. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo LD, Jr. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. Expert Opin Drug Deliv. 2021;18(2):151-67. Epub 20201006. doi: 10.1080/17425247.2021.1823964. PubMed PMID: 32924651; PMCID: PMC9355143.
59. Andrianov AK, DeCollibus DP, Gillis HA, Kha HH, Marin A, Prausnitz MR, Babiuk LA, Townsend H, Mutwiri G. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. Proc Natl Acad Sci U S A. 2009;106(45):18936-41. Epub 20091028. doi: 10.1073/pnas.0908842106. PubMed PMID: 19864632; PMCID: PMC2770009.
60. Geisbert TW, Mire CE, Geisbert JB, Chan YP, Agans KN, Feldmann F, Fenton KA, Zhu Z, Dimitrov DS, Scott DP, Bossart KN, Feldmann H, Broder CC. Therapeutic treatment of Nipah virus infection in nonhuman

primates with a neutralizing human monoclonal antibody. Sci Transl Med. 2014;6(242):242ra82. doi: 10.1126/scitranslmed.3008929. PubMed PMID: 24964990; PMCID: PMC4467163.

61. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng YR, Brining D, Scott D, Wang Y, Dimitrov AS, Callison J, Chan YP, Hickey AC, Dimitrov DS, Broder CC, Rockx B. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. Sci Transl Med. 2011;3(105):105ra3. doi: 10.1126/scitranslmed.3002901. PubMed PMID: 22013123; PMCID: PMC3313625.

62. Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti JB, Fausther-Bovendo H, Wei H, Aviles J, Hiatt E, Johnson A, Morton J, Swope K, Bohorov O, Bohorova N, Goodman C, Kim D, Pauly MH, Velasco J, Pettitt J, Olinger GG, Whaley K, Xu B, Strong JE, Zeitlin L, Kobinger GP. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature. 2014;514(7520):47-53. doi: 10.1038/nature13777. PubMed PMID: 25171469; PMCID: PMC4214273.

63. Mire CE, Geisbert JB, Borisevich V, Fenton KA, Agans KN, Flyak AI, Deer DJ, Steinkellner H, Bohorov O, Bohorova N, Goodman C, Hiatt A, Kim DH, Pauly MH, Velasco J, Whaley KJ, Crowe JE, Jr., Zeitlin L, Geisbert TW. Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. Sci Transl Med. 2017;9(384). doi: 10.1126/scitranslmed.aai8711. PubMed PMID: 28381540; PMCID: PMC5719873.

64. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med. 2017;23(10):1146-9. Epub 2017/09/05. doi: 10.1038/nm.4396. PubMed PMID: 28869611; PMCID: PMC5719877.

65. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. mAbs. 2011;3(5):422-30. Epub 2011/11/04. doi: 10.4161/mabs.3.5.16983. PubMed PMID: 22048693; PMCID: PMC3225846.

66. Robbie GJ, Criste R, Dall'acqua WF, Jensen K, Patel NK, Losonsky GA, Griffin MP. A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study. Antimicrobial agents and chemotherapy. 2013. Epub 2013/10/02. doi: 10.1128/aac.01285-13. PubMed PMID: 24080653.

67. Gaudinski MR, Coates EE, Houser KV, Chen GL, Yamshchikov G, Saunders JG, Holman LA, Gordon I, Plummer S, Hendel CS, Conan-Cibotti M, Lorenzo MG, Sitar S, Carlton K, Laurencot C, Bailer RT, Narpala S, McDermott AB, Namboodiri AM, Pandey JP, Schwartz RM, Hu Z, Koup RA, Capparelli E, Graham BS, Mascola JR, Ledgerwood JE. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. PLoS Med. 2018;15(1):e1002493. Epub 2018/01/25. doi: 10.1371/journal.pmed.1002493. PubMed PMID: 29364886; PMCID: PMC5783347.

68. Mastraccio KE, Huaman C, Laing ED, Broder CC, Schaefer BC. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. Methods Mol Biol. 2022;2524:369-94. doi: 10.1007/978-1-0716-2453-1_30. PubMed PMID: 35821488.

69. Rima B, Balkema-Buschmann A, Dundon WG, Duprex P, Easton A, Fouchier R, Kurath G, Lamb R, Lee B, Rota P, Wang L, Ictv Report C. ICTV Virus Taxonomy Profile: Paramyxoviridae. J Gen Virol. 2019;100(12):1593-4. Epub 20191011. doi: 10.1099/jgv.0.001328. PubMed PMID: 31609197; PMCID: PMC7273325.

70. Madera S, Kistler A, Ranaivoson HC, Ahyong V, Andrianiaina A, Andry S, Raharinosy V, Randriambolamanantsoa TH, Ravelomanantsoa NAF, Tato CM, DeRisi JL, Aguilar HC, Lacoste V, Dussart P, Heraud JM, Brook CE. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. J Virol. 2022;96(18):e0092122. Epub 20220830. doi: 10.1128/jvi.00921-22. PubMed PMID: 36040175; PMCID: PMC9517717.

71. Vanmechelen B, Meurs S, Horemans M, Loosen A, Joly Maes T, Laenen L, Vergote V, Koundouno FR, Magassouba N, Konde MK, Condé IS, Carroll MW, Maes P. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. Virus Evol. 2022;8(2):veac061. Epub 20220630. doi: 10.1093/ve/veac061. PubMed PMID: 35854826; PMCID: PMC9290864.

72. Drexler JF, Corman VM, Müller MA, Maganga GD, Vallo P, Binger T, Gloza-Rausch F, Cottontail VM, Rasche A, Yordanov S, Seebens A, Knörnschild M, Oppong S, Adu Sarkodie Y, Pongombo C, Lukashev AN, Schmidt-Chanasit J, Stöcker A, Carneiro AJ, Erbar S, Maisner A, Fronhoffs F, Buettner R, Kalko EK, Kruppa T, Franke CR, Kallies R, Yandoko ER, Herrler G, Reusken C, Hassanin A, Krüger DH, Matthee S, Ulrich RG,

Leroy EM, Drosten C. Bats host major mammalian paramyxoviruses. Nat Commun. 2012;3:796. Epub 20120424. doi: 10.1038/ncomms1796. PubMed PMID: 22531181; PMCID: PMC3343228.
73. Wu Z, Yang L, Yang F, Ren X, Jiang J, Dong J, Sun L, Zhu Y, Zhou H, Jin Q. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. Emerg Infect Dis. 2014;20(6):1064-6. doi: 10.3201/eid2006.131022. PubMed PMID: 24865545; PMCID: PMC4036791.
74. Voigt K, Hoffmann M, Drexler JF, Müller MA, Drosten C, Herrler G, Krüger N. Fusogenicity of the Ghana Virus (Henipavirus: Ghanaian bat henipavirus) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. Viruses. 2019;11(9). Epub 20190829. doi: 10.3390/v11090800. PubMed PMID: 31470664; PMCID: PMC6784138.
75. Childs K, Randall R, Goodbourn S. Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction. J Virol. 2012;86(7):3411-21. Epub 20120201. doi: 10.1128/jvi.06405-11. PubMed PMID: 22301134; PMCID: PMC3302505.
76. Davis ME, Wang MK, Rennick LJ, Full F, Gableske S, Mesman AW, Gringhuis SI, Geijtenbeek TB, Duprex WP, Gack MU. Antagonism of the phosphatase PP1 by the measles virus V protein is required for innate immune escape of MDA5. Cell Host Microbe. 2014;16(1):19-30. doi: 10.1016/j.chom.2014.06.007. PubMed PMID: 25011105; PMCID: PMC4120867.
77. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. J Virol. 2002;76(22):11476-83. doi: 10.1128/jvi.76.22.11476-11483.2002. PubMed PMID: 12388709; PMCID: PMC136769.
78. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. J Virol. 2005;79(10):6078-88. doi: 10.1128/jvi.79.10.6078-6088.2005. PubMed PMID: 15857993; PMCID: PMC1091709.
79. Basler CF. Nipah and hendra virus interactions with the innate immune system. Curr Top Microbiol Immunol. 2012;359:123-52. doi: 10.1007/82_2012_209. PubMed PMID: 22491899.
80. Yoneda M, Guillaume V, Sato H, Fujita K, Georges-Courbot MC, Ikeda F, Omi M, Muto-Terao Y, Wild TF, Kai C. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. PLoS One. 2010;5(9):e12709. Epub 20100915. doi: 10.1371/journal.pone.0012709. PubMed PMID: 20856799; PMCID: PMC2939873.
81. Satterfield BA, Cross RW, Fenton KA, Borisevich V, Agans KN, Deer DJ, Graber J, Basler CF, Geisbert TW, Mire CE. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. J Virol. 2016;90(14):6326-43. Epub 20160624. doi: 10.1128/jvi.00215-16. PubMed PMID: 27147733; PMCID: PMC4936148.
82. Marsh GA, de Jong C, Barr JA, Tachedjian M, Smith C, Middleton D, Yu M, Todd S, Foord AJ, Haring V, Payne J, Robinson R, Broz I, Crameri G, Field HE, Wang LF. Cedar virus: a novel Henipavirus isolated from Australian bats. PLoS Pathog. 2012;8(8):e1002836. Epub 20120802. doi: 10.1371/journal.ppat.1002836. PubMed PMID: 22879820; PMCID: PMC3410871.
83. Amaya M, Cheng H, Borisevich V, Navaratnarajah CK, Cattaneo R, Cooper L, Moore TW, Gaisina IN, Geisbert TW, Rong L, Broder CC. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral research. 2021;193:105084. Epub 20210530. doi: 10.1016/j.antiviral.2021.105084. PubMed PMID: 34077807; PMCID: PMC8631057.
84. CDC. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. [updated June 2020]. Available from: https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.
85. Organization WH. Lassa fever fact sheet. World Health Organization. 2017.
86. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. J Travel Med. 2020;27(4). doi: 10.1093/jtm/taaa035. PubMed PMID: 32219400.
87. Purushotham J, Lambe T, Gilbert SC. Vaccine platforms for the prevention of Lassa fever. Immunol Lett. 2019;215:1-11. Epub 20190423. doi: 10.1016/j.imlet.2019.03.008. PubMed PMID: 31026485; PMCID: PMC7132387.
88. Sewlall NH, Richards G, Duse A, Swanepoel R, Paweska J, Blumberg L, Dinh TH, Bausch D. Clinical features and patient management of Lujo hemorrhagic fever. PLoS Negl Trop Dis. 2014;8(11):e3233. Epub 20141113. doi: 10.1371/journal.pntd.0003233. PubMed PMID: 25393244; PMCID: PMC4230886.
89. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. Vaccines. 2023;11(3):635. PubMed PMID: doi:10.3390/vaccines11030635.

90. Koma T, Patterson M, Huang C, Seregin AV, Maharaj PD, Miller M, Smith JN, Walker AG, Hallam S, Paessler S. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. J Virol. 2016;90(3):1290-7. Epub 20151118. doi: 10.1128/jvi.02615-15. PubMed PMID: 26581982; PMCID: PMC4719595.
91. Jahrling P TR, Barrero O. , editor. Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region; 1988; Mar del Plata, Argentina.
92. Delgado S, Erickson BR, Agudo R, Blair PJ, Vallejo E, Albariño CG, Vargas J, Comer JA, Rollin PE, Ksiazek TG, Olson JG, Nichol ST. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. PLOS Pathogens. 2008;4(4):e1000047. doi: 10.1371/journal.ppat.1000047.
93. Loayza Mafayle R, Morales-Betoulle ME, Romero C, Cossaboom CM, Whitmer S, Alvarez Aguilera CE, Avila Ardaya C, Cruz Zambrana M, Dávalos Anajia A, Mendoza Loayza N, Montaño A-M, Morales Alvis FL, Revollo Guzmán J, Sasías Martínez S, Alarcón De La Vega G, Medina Ramírez A, Molina Gutiérrez JT, Cornejo Pinto AJ, Salas Bacci R, Brignone J, Garcia J, Añez A, Mendez-Rico J, Luz K, Segales A, Torrez Cruz KM, Valdivia-Cayoja A, Amman BR, Choi MJ, Erickson B-R, Goldsmith C, Graziano JC, Joyce A, Klena JD, Leach A, Malenfant JH, Nichol ST, Patel K, Sealy T, Shoemaker T, Spiropoulou CF, Todres A, Towner JS, Montgomery JM. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. New England Journal of Medicine. 2022;386(24):2283-94. doi: 10.1056/NEJMoa2110339. PubMed PMID: 35704480.
94. Tipih T, Burt FJ. Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development. Biores Open Access. 2020;9(1):137-50. Epub 20200512. doi: 10.1089/biores.2019.0057. PubMed PMID: 32461819; PMCID: PMC7247048.
95. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease 2023 [cited 2023 03/06]. Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.
96. Kalunda M, Mukwaya LG, Mukuye A, Lule M, Sekyalo E, Wright J, Casals J. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. The American Journal of Tropical Medicine and Hygiene. 1986;35(2):387-92. doi: 10.4269/ajtmh.1986.35.387.
97. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. Emerg Infect Dis. 2020;26(12):2944-50. doi: 10.3201/eid2612.202411. PubMed PMID: 33219649; PMCID: PMC7706932.
98. Mulangu S, Dodd LE, Davey RT, Jr., Tshiani Mbaya O, Proschan M, Mukadi D, Lusakibanza Manzo M, Nzolo D, Tshomba Oloma A, Ibanda A, Ali R, Coulibaly S, Levine AC, Grais R, Diaz J, Lane HC, Muyembe-Tamfum JJ, Group PW, Sivahera B, Camara M, Kojan R, Walker R, Dighero-Kemp B, Cao H, Mukumbayi P, Mbala-Kingebeni P, Ahuka S, Albert S, Bonnett T, Crozier I, Duvenhage M, Proffitt C, Teitelbaum M, Moench T, Aboulhab J, Barrett K, Cahill K, Cone K, Eckes R, Hensley L, Herpin B, Higgs E, Ledgerwood J, Pierson J, Smolskis M, Sow Y, Tierney J, Sivapalasingam S, Holman W, Gettinger N, Vallee D, Nordwall J, Team PCS. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. N Engl J Med. 2019;381(24):2293-303. Epub 2019/11/28. doi: 10.1056/NEJMoa1910993. PubMed PMID: 31774950.
99. Group PIW, Multi-National PIIST, Davey RT, Jr., Dodd L, Proschan MA, Neaton J, Neuhaus Nordwall J, Koopmeiners JS, Beigel J, Tierney J, Lane HC, Fauci AS, Massaquoi MBF, Sahr F, Malvy D. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. N Engl J Med. 2016;375(15):1448-56. doi: 10.1056/NEJMoa1604330. PubMed PMID: 27732819; PMCID: PMC5086427.
100. Politch JA, Cu-Uvin S, Moench TR, Tashima KT, Marathe JG, Guthrie KM, Cabral H, Nyhuis T, Brennan M, Zeitlin L, Spiegel HML, Mayer KH, Whaley KJ, Anderson DJ. Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial. PLoS Med. 2021;18(2):e1003495. Epub 2021/02/04. doi: 10.1371/journal.pmed.1003495. PubMed PMID: 33534791.

CENTER OVERVIEW: *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center – (PABVAX) RESEARCH STRATEGY

Introduction. Emerging and periodically reemerging pathogenic enveloped RNA viruses are continuous pandemic threats. Among viral taxon associated with human zoonoses, members of the family ***Paramyxoviridae*** and order ***Bunyavirales*** stand out for their capacity to cause debilitating and life-threatening disease**.** Humans can become infected via excreta from animal reservoir hosts such as rodents or bats, through intermediate amplifying hosts such as small or domestic animals, or by arthropod vectors such as mosquitos and ticks[1-4]. Humans often represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur. During this process, RNA viral pathogens may mutate and adapt to more efficiently replicate and shed in the host, posing an epidemic threat[5-8]. The viral order ***Bunyavirales*** is composed of several viral families containing high priority human pathogens[9]. Notably, the ***Arenaviridae*** and ***Nairoviridae*** families contain viruses that cause severe hemorrhagic fever diseases in humans globally with mortality rates up to 60%[10] and significant, long-term sequelae in survivors[11]. Many bunyaviruses are included on the World Health Organization's (WHO) List of Priority Pathogens[12,13]. Six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHAPV, GTOV, JUNV, MACV) and one nairovirus, Crimean-Congo Hemorrhagic Fever Virus (CCHFV), are also identified as NIAID Category A pathogens[14] due to their 1) high associated morbidity and mortality rates, 2) ease of dissemination and/or ability to transmit from person-to-person, 3) potential to cause public panic and social disruption, and 4) requirement for special actions in the context of public health preparedness. The ***Paramyxoviridae*** also includes members in the WHO Priority Pathogens, namely Nipah virus (NiV) and Hendra virus (HeV). These henipaviruses (HNVs) are the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease[15]. The genus now includes nine additional reported HNVs, but only HeV and NiV are associated with severe and often fatal henipaviral disease in humans and a number of animal species[16]. Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illness in humans[17].

These collective factors plus the absence of approved vaccines or therapeutics make bunyaviruses and paramyxoviruses a significant threat to global public health. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for developing vaccine and monoclonal antibody (mAb) countermeasures to prototype bunyavirus and paramyxovirus pathogens (**Table 1**). In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old World) Machupo (New World)	Lujo (Old World) Chapare (New World)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Table 1. PABVAX selected prototype and test case viral agents

Arenaviridae: The *Arenaviridae* is composed of five genera: *Mammarenavirus*, *Reptarenavirus*, *Innmovirus*, *Antennavirus*, and *Hartmanivirus*[18]. The genus *Mammarenavirus* contains a large number of rodent-borne viruses, some of which cause severe hemorrhagic fever, neurological damage, death, and permanent sequelae; hearing loss, vision impairment, and balance issues in survivors[19]. While these genera exhibit a global distribution, two geographically distinct serogroups exist that diverged[20]. Old World complex mammarenaviruses (OWAV) in the Eastern Hemisphere include LASV, Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered LUJV; New World complex mammarenaviruses (NWAV) in the Western Hemisphere include JUNV, MACV, Sabia Virus (SABV), and CHAPV. There are no FDA approved vaccines or therapeutics for mammarenaviruses, though the attenuated JUNV "Candid-1" vaccine is approved for use in Argentina.

Disease severity and case fatality rates (CFR) vary across mammarenaviruses even within the same clades and species. Up to 500,000 cases of LASV infection occur per year with CFRs sometimes approaching 70% in hospitalized cases[21]. Before the introduction of the Candid-1 vaccine, it is believed that an upwards of 60,000 cases of Argentine Hemorrhagic Fever (AHF) from infection with JUNV occurred per year with CFRs ranging from 15-30%. Convalescent plasma from JUNV survivors is still the primary means to treat acute AHF in Argentina[22]. Over 1000 cases were reported for the initial outbreak of MACV with CFRs ranging from 5-30%; enhanced rodent abatement practices and potentially cross-protective immunity in populations that have taken the JUNV Candid-1 vaccine may have reduced recent outbreak frequency[23]. All mammarenaviruses share a common genomic organization consisting of two ambisense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix) and L protein (polymerase), and the glycoprotein precursor (GPC) and nucleoprotein (NP)[19]. Despite general

similarity of their genomic architecture, mammarenaviruses exhibit marked differences in their replication life cycles including attachment factors, entry mechanisms, and immune evasion; nonetheless, the dominant antigens driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Despite the abundant genetic diversity of LASV, high levels of cross reactivity have been seen with antigen-based diagnostics across seven known lineages, suggesting the generation of cross-protective immunogens and antibodies against mammarenaviruses is achievable[24]. Monoclonal antibodies derived from human LASV survivors were shown to protect non-human primates (NHP) against lethal challenge[25]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[26]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, indicating this domain could serve as an antigenic target. Investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccines to test this approach and have demonstrated protection against lethal LASV lineage IV Josiah challenge. Specifically, a stabilized prefusion trimeric GPe, an immunogen containing a highly conserved NP NTD epitope, or a combination of these two approaches conferred protection in a lethal outbred guinea pig model. Nevertheless, even with the considerable molecular diversity in LASV strains, conservation at key antigenic sites within the GP and NP suggest a pan-LASV vaccine may be possible[24,27,28]. **RP1** and **Core E** investigators have demonstrated that a recombinant vesicular stomatitis virus (rVSV)-based vaccine expressing the LASV-Josiah GPC is protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[29], and against heterologous challenge with Lineage II[30], III, V[31], and VI viruses (Cross and Geisbert, unpublished data).

In **Phase I of RP1**, the ability of an optimized LASV GPe/NP subunit vaccine delivered intramuscularly (IM) will be assessed in guinea pig and NHP models across multiple viral lineages. We will also assess a microneedle skin patch (MNP) formulation optimized in **Core C** to determine if a needle-free vaccination route affords more potent vaccine-mediated responses and protection. Similarly, we have generated MACV GPe immunogens and will design a MACV NP-NTD subunit antigen to test the same approach across multiple MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing both OWAV (LUJV) and NWAV (CHAPV) complexes, for which there are no vaccines available. A unique advantage of the PABVAX Center is its access to biosafety level (BSL)-4 containment, allowing us to work with authentic versus surrogate arenaviruses. The BSL-4 facilities described in **Core E** are capable of evaluating countermeasures in small animal models and NHPs. The cynomolgus macaque is the current "gold standard" animal model for LASV, JUNV, and MACV research as disease recapitulates human infection and there are a multitude of immunological reagents available[32-34]. The evaluation of prototype OWAV and NWAV vaccines and therapies in the NHP model is likely pivotal for IND applications and for future licensure of medical countermeasures for human use.

<u>Nairoviridae:</u> Unique to most bunyaviruses, orthonairoviruses are transmitted primarily by ticks[2]. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[35], Issyk-Kul virus[36], Songling virus[37]), CCHFV is the most recognized public health threat. Outbreaks of CCHFV have occurred across Europe, Africa, the Middle East, and Asia. There is growing concern for expansion of the CCHFV endemic region due to increased range of its tick host (*Hyalomma* spp.) tied to bird migration changes, livestock trade, and global warming. For example, CCHFV cases have been reported in Spain[38] , a previously non-endemic region, and *Hyalomma* ticks have been found in Sweden[39]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to a severe hemorrhagic fever and death in 30% of cases[40]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[41]. Ribavirin has been used to treat infections with limited success[42].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and GPC [40]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[40]. Conversely, the CCHFV GPC is considerably less conserved (< 75% amino acid identity)[40]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location. Thus, creating a pan-CCHFV vaccine or therapeutic based solely on one clade poses a challenge. Nevertheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a rVSV vectored vaccine expressing the full GPC of CCHFV[43].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP immunogens with the highest level of homology across clades. We will assess their capacity to protect against lethal challenge with isolates from multiple CCHFV clades in established mouse[43,44] and NHP[45] models with **Core E**. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best formulation of CCHFV GP/NP MNP vaccine against the emerging test case nairovirus, Kasokero virus, for which there are no vaccines available.

Paramyxoviridae: The HNVs NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality[4,46]. HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality[47]. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs, which served as amplifying hosts[48]. NiV and HeV can infect at least 18 animal species across 6 orders of mammals, and among these they cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans[16,49]. The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission[5]. Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and Southeast Asia and Australia. Their broad host tropism and ability to infect livestock also causes increased risk of human infections. HNVs are single-stranded, negative sense, enveloped RNA viruses and their biology lends themselves to be synthetically produced. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS). The Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents[50] because the virus could be engineered for mass dissemination and it possesses the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the R&D Blueprint list of priority pathogens with epidemic potential[51].

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb exist. These postexposure treatments conferred protecion against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP)[52-55]. The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia[56]. In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs, and several vaccine platforms have clear potential for use in people[16]. The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies[16]. Such preventive vaccines would have utility for lab workers, first responders or individuals at high-risk exposure, and in mitigating the spread of an outbreak. In the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent[57]. It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection[49,58]. The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished)[59]. The divergent LayV will be the focus of the test case studies. The choice is justifiable for several important reasons: 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; and 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including rescuing recombinant LayV (rLayV), exploring animal model development, constructing additional LayV and AngV reporter virus tools, and then translating and testing the MNP vaccine "plug-and-play" approach. Finally, we will evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME. A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center's RPs and Core leaders currently have active and productive collaborative research programs in related areas, most spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

The pandemic preparedness research gaps addressed by PABVAX are diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **arenaviruses** and **nairoviruses**, and **RP2** will target the prototype **HNVs**. Both RP1 and RP2 will develop new prototype virus tools (recombinant prototype viruses) along with existing tools, to analyze vaccine responses and evaluate new mAbs and nabs from the other RPs. A third RP will develop a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant *Cedar henipavirus* (rCedV) *in vivo* and a bioluminescence detection method that can longitudinally trace the dynamics and anatomical progression of infection with a luciferase-encoding virus in individual animals (**RP3**). There will also be two RPs that focus on the development of broadly reactive human mAbs and nanobodies to the PABVAX selected viral prototypes. **RP4** will isolate human mAbs derived from NiV-infected survivors with a specific focus on F glycoprotein-targeting mAbs. Additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will engage in the manufacture, quality control, and testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**). Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core (**Core A**) will provide organizational and programmatic structure to promote research productivity and scientific interactions along with compliance with regulations concerning select agent, vertebrate animal, and human subject use. **Core A** will coordinate all activities with the Scientific Advisory Board (**SAB**) and make data available through the Data Management Core (**Core B**).

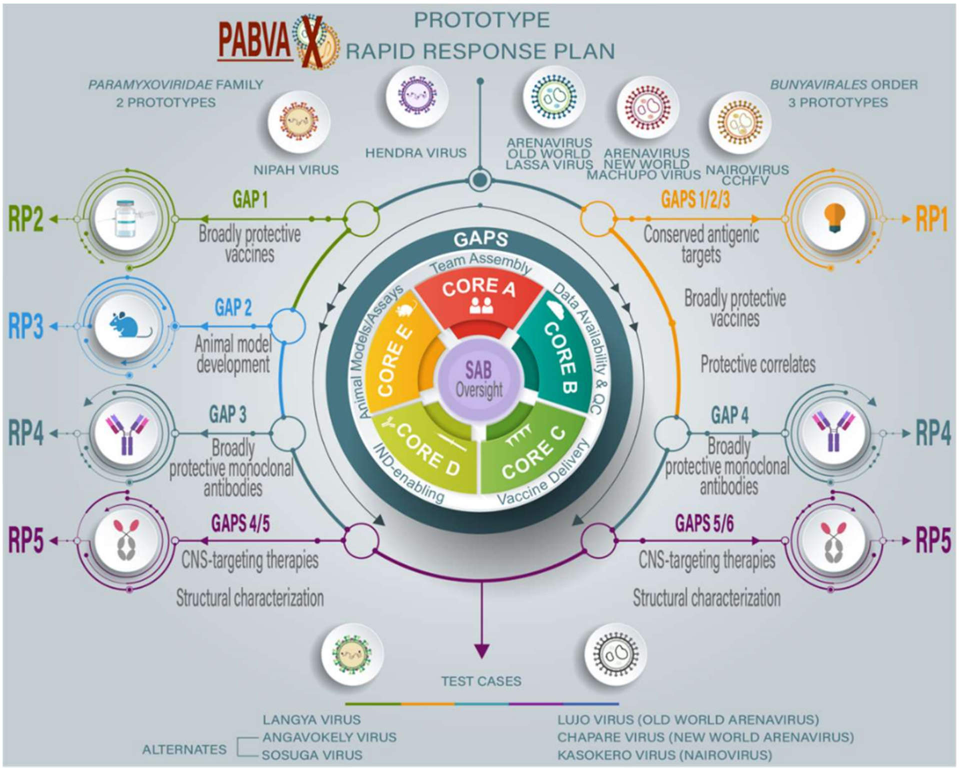


Figure 1: Research gaps addressed by the *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center- (PABVAX).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform. The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**). This project tested a dissolvable MNP-based vaccine technology ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine[60]. The MNP vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable microneedles that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants[61] to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as to facilitate improved vaccine storage and distribution due to their improved thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, Scientific **Core C** (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ)-adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (*Paramyxoviridae*). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro*, along with analysis of polyfunctional T-cell responses by antigenic peptide stimulation, followed by intracellular cytokine staining (ICS) and flow cytometry.

Discover effective antibody-based prophylactics and therapies. Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah[62], Hendra[63], Ebola[64,65], Marburg[66] and Lassa[67]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc*.), a feature to be exploited in **Core D** by Dr. Gunn. In addition to therapeutic applications, mAbs with Fc mutations dramatically extend serum half-life[68-70] and offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g*. rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization, and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures. Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal Model Development and Preclinical Evaluation Scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective efficacy of antivirals and vaccines, as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation, including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within

RP1. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they are generally less laborious and can be adapted to higher throughput platforms and/or integrated with other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput fluorescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic NiV and HeV vaccine-induced immune sera, mAbs (**RP4**), nbs (**RP5**), and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either HeV or NiV-B envelope GPs) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals to explore nab based therapeutics to CNS resident HNVs[71].

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the HNVs (*Paramyxoviridae*) and the *Bunyavirales* (*Arenaviridae* and *Nairoviridae*) that will bolster vaccine endurance to storage and transportation; we also anticipate improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology, and mAb and vaccine translational drug development along with Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

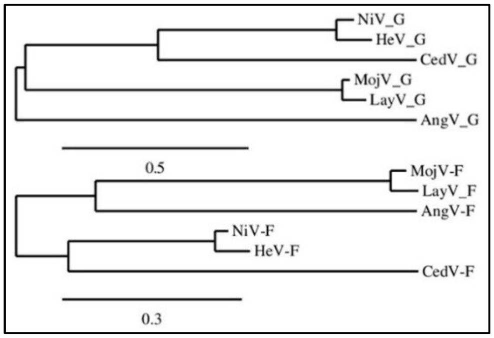


Figure 2: Phylogenetic analysis of select HNV G and F glycoproteins. Trees were constructed for using the PhyML program's maximum likelihood method and aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Paramyxoviruses: NiV and HeV are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis[4,46]. HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals. These viruses are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of CedV, Gamak virus, Daeryong virus, and (LayV)[72] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[73-76]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and

AngV, are old world pteropid fruit bats[73,77]. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province)[17]. The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**. Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal HNV disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters[49], and AGMs (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P). The P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists[78-82]. The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections[83,84]. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature[85]. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2[86] and CedV is now recognized as a BSL-2 restricted agent[87]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II "plug and play" research plan (**RP2** and **PR5** with **Cores C, D,** and **E**).

Arenaviruses (old world): For **phase 1**, **RP1** will utilize **LASV as the prototype virus** due its significant public health burden throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[88]. LASV is the most imported viral hemorrhagic fever (VHF) next to Dengue virus and has been documented in several countries outside of Africa over 35 times[89]. CFRs approaching 70% in hospitalized cases and marked long term sequelae are the result of the lack of approved vaccines or therapeutics, although several live attenuated virus candidates are currently being evaluated with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI)[90]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize only LASV GP as the target immunogen.

Investigators in **RP1**, **Core D**, and **Core E** have already demonstrated protective efficacy against challenge with LASV in outbred guinea pigs vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These immunogens have been engineered to express homologous antigenic regions to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach suggests a similar strategy could be implemented against other arenaviruses. The **OWAV test pathogen, LUJV,** was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia, which quickly spread nosocomially[91]. The CFR for this infectious virus was 80% in this small outbreak of 5 people. There are currently no approved vaccines or therapeutics available for this virus and its genetic divergence from suggest current LASV countermeasures may be of limited use. LUJV will serve as an ideal "test case" to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Arenaviruses (new world): The live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[92], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[93,94]. Furthermore, there is the potential for pathogenic reversion. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest exploring other JUNV countermeasures are warranted. Building on the success of the LASV GPe/NP-NTD, investigators in **RP1 and Cores D and E** will engineer broadly protective MACV GPe and NP-NTD antigens and perform efficacy testing in established guinea pig and NHP models. We will extend this plug-and-play approach to the **NWAV test case, CHAPV**. First reported in 2003 in Bolivia, CHAPV is a rodent-borne mammarenavirus associated with the development of severe hemorrhagic fever with case fatality rates up to 60%[95,96]. Recent studies have demonstrated clear human-to-human transmission during the acute phase evidenced by several nosocomial infections; isolation of viable virus from semen at 86 days after symptom onset demonstrates a clear need to implement specialized management

and treatment [96]. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Nairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as it represents the largest global public health burden within the orthonairovirus family with outbreaks annually across Europe, Africa, and Asia. There are no FDA-approved vaccines for CCHFV; however, a suckling mouse brain-derived, adjuvanted whole virus vaccine for CCHFV has been approved for use in Bulgaria. This vaccine has numerous safety concerns and has limited demonstrated efficacy. Several experimental vaccine approaches have been evaluated ranging from viral vectored approaches to DNA-based vaccines with varying efficacy, advantages, and disadvantages[97]; however, only a Modified Vaccinia Ankara virus vaccine (MVA-CCHFV) has begun to advance towards clinical trials[98]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test these for protective efficacy from challenge with 6 different CCHFV lineages using established mouse and NHP models. Next, we will deploy the same approach to the **test case nairovirus, Kasokero virus (KASV),** for which no vaccine or treatment exists.

In 1977, KASV was first isolated from Ugandan bats and caused several cases of acute febrile illness of laboratory workers from the same lab that processed the infected bats[99]. Surveys of 109 laboratory workers from the same facility demonstrated 13% seropositivity and clinical symptoms in individuals 4 to 15 months after the virus was isolated. These findings suggest either continued laboratory-associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the tick reservoir, *Ornithodoros faini,* to participate in enzootic maintenance of KASV[100]. While most chiropteran ticks exhibit host specificity, humans entering caves these bats inhabit have reported bites from *O.faini* ticks. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics suggest investment in countermeasure development against this virus is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Management Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**; arenavirus and nairovirus projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine active and passive immunization approaches to further improve the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

An additional and outstanding advantage of the PABVAX Center is the **Animal Model Development and Preclinical Evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL . Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for the Center RPs will be performed by the same BSL-4 staff under the

same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for members of the *Bunyavirales* and *Paramyxoviridae*.**
2. **Develop new tools and animal models for cell-based and *in vivo* therapeutic evaluation.**
3. **Develop antibodies and nanobodies to the selected prototype and test viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

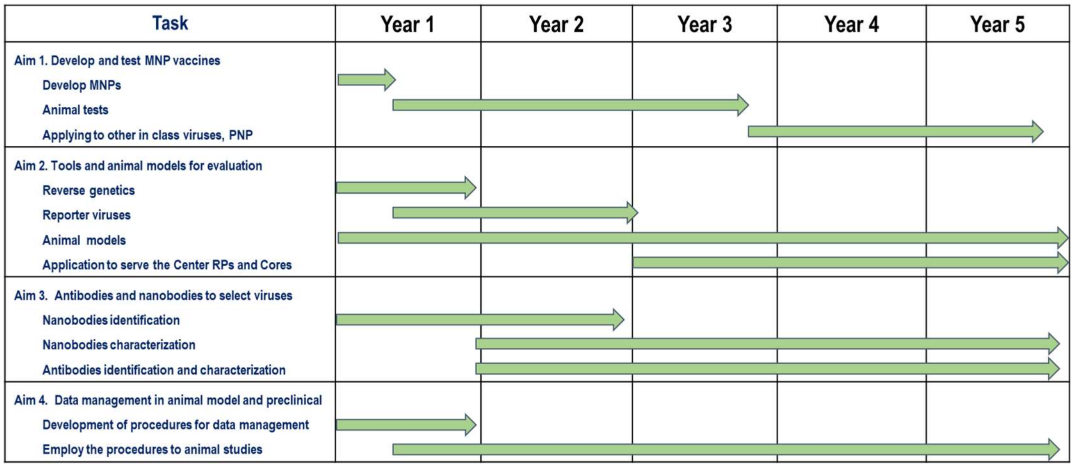


Figure 3: Projected PABVAX timeline to achieve the 4 overarching tasks.

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**. Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (details are specified within the Administration **Core A** Research Strategy). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommending new directions as appropriate, and providing Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

<u>**Overall Objectives**</u>

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

The first milestone will address whether formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made (**Figure 4**). The determination of MNP vaccine potential success will first be established using immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune

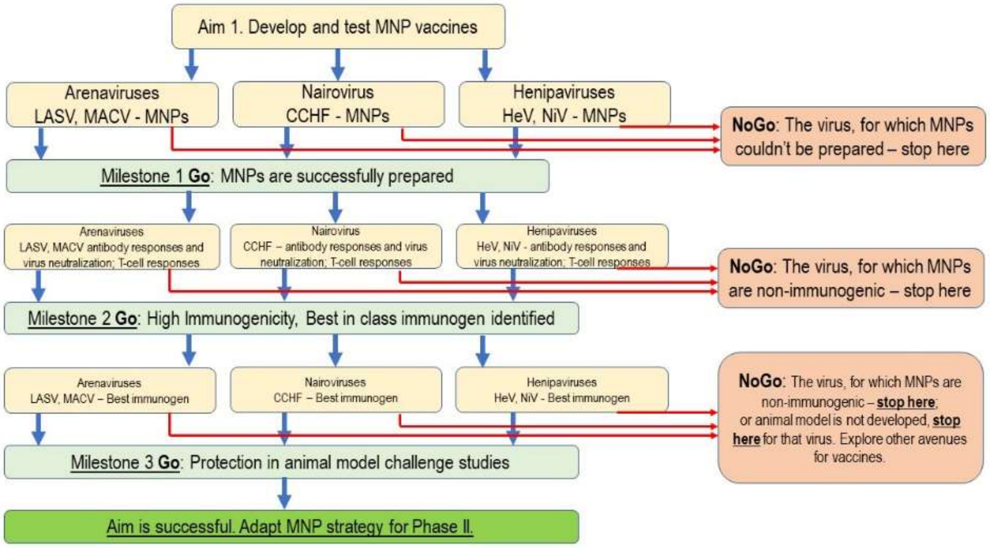


Figure 4. Milestones and criteria for **Aim 1** of the PABVAX Center's overall objectives.

responses will be assessed (antigen-specific antibody induction, virus neutralization, and polyfunctional T-cell responses by *in vitro* stimulation of isolated cells). Go/no go decisions will be made based on outcomes of these first-tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as a success. Comparisons will also be made such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of the best-in-class immunogens, or immunogen combinations, will also be explored to down-select vaccines. The third milestone will determine whether MNP vaccination affords protection in animal model challenge experiments. In the case of NiV and HeV prototypes, the animal models chosen (ferrets and African green monkeys (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales*, there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASV, and guinea pig models exist for some but not all of these lineages. For MACV, there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is the limitations of cost and availability of rhesus and cynomolgus monkeys. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be developing and evaluating animal models within the PABVAX program, including further development of AGM models for arenaviruses and possibly CCHFV as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for cell-based and in vivo therapeutics evaluation.

The second overall objective of PABVAX is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation (**Figure 5**). These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed because genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If successful, a large battery of new materials will be available to share with the ReVAMPP network and be key milestones for execution of Phase II of the PABVAX Center program.

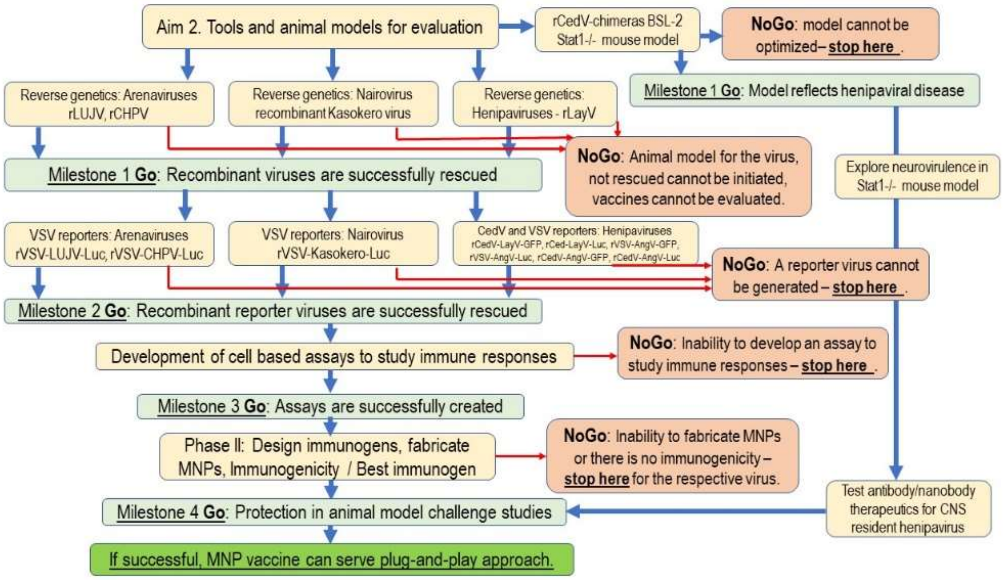


Figure 5. Milestones and criteria for **Aim 2** of the PABVAX Center's overall objectives.

The translation of the MNP vaccine plug-and-play approach will be in Phase II (**Table 1**). Milestones will be similar to those in **Figure 4**. New virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new *in vivo* bioluminescence-based immune deficient mouse model developed by the PIs of **PR2** and **RP3** that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease *in vivo* safely and expediently at BSL-2 containment. The model

will be deployed to test a critically important therapeutic need against CNS resident HNV infection, and **RP3** will collaborate with **RP5** in the testing of engineered nabs.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

New human mAbs and nbs as tools and potential therapeutics will be developed by **RP4** and **RP5** in collaboration with **RP2** and **Core D** (**Figure 6**). NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs, and in collaboration with **RP2**, neutralization potencies will be rapidly assessed (as well as evaluating synergistic cell-based mAb neutralization) that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

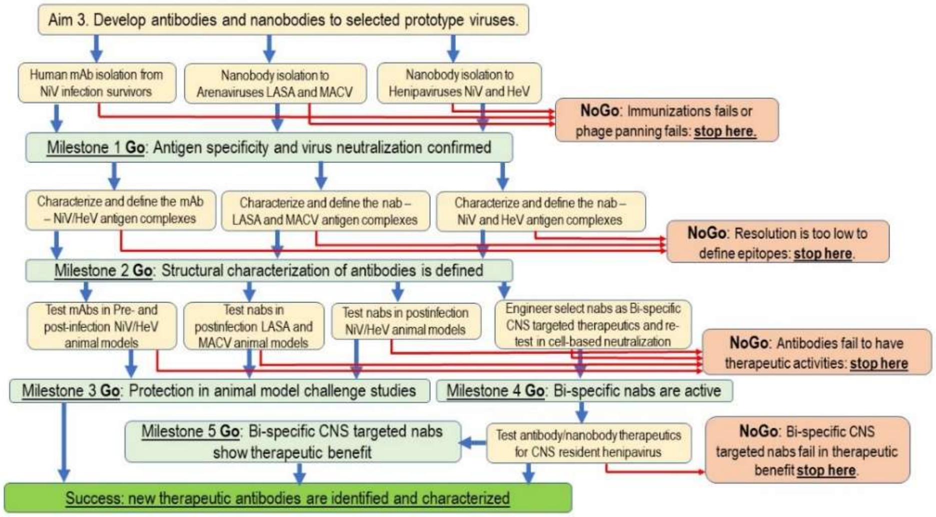


Figure 6. Milestones and criteria for **Aim 3** of the PABVAX Center's overall objectives.

Nbs, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nbs can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nbs targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus), and select animal models of LASV and MACV by **Core E**. Success will be measured by the 1) identification of potent virus-neutralizing nbs, 2) discovery of more broadly reactive nbs particularly to the multiple LASV clades, 3) evidence of therapeutic benefit in animal models, and 4) the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

***Industry Partnerships**.*

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience in developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and

make an ideal partner for PABVAX. Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

SPECIAL FEATURES OF THE PABVAX

Galveston National Laboratory (GNL). The GNL is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics, and therapeutics. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370 NHP studies employing some 2,772 animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses have been performed. These studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder, and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP facility and a staff of full-time veterinarians. **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above, another special feature is the location of the WRCEVA virus collection within the GNL.

Core E (Animal model development and preclinical evaluation). **Core E** operations will be conducted at the GNL, and in conjunction with **Core A**, will also include a regulatory expert from the UTMB Institutional Office of Regulated Nonclinical Studies (ORNcS). The ORNcS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL, and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses, arenaviruses, and henipaviruses. He has published over 250 peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI), was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs). Mapp Biopharmaceutical has deep experience with antigens and mAbs against these antigens. Their early development and research group has expertise in immunology, infectious diseases, and the production of antigens, antibodies, and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [101] [102]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1[103]. Dr. Gunn and Genovac will join the team to analyze antibody Fc effector functions and to provide antigen/antibody discovery resources, respectively. Dr. Geisbert will contribute his experience with high containment animal models and vaccine development. As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

BIBLIOGRAPHY AND REFERENCES CITED

1. Lamb RA, Parks GD. Paramyxoviridae: the viruses and their replication. In Fields BN, Knipe DM, Howley PM, editors, Fields Virology: Sixth Edition. Vol. 1. Lippincott, Williams, and Wilkins. 2013. p. 957-995.
2. Boshra H. An Overview of the Infectious Cycle of Bunyaviruses. *Viruses*. Sep 28 2022;14(10)doi:10.3390/v14102139
3. Horne KM, Vanlandingham DL. Bunyavirus-vector interactions. *Viruses*. Nov 13 2014;6(11):4373-97. doi:10.3390/v6114373
4. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia), p 1070-1085.
5. Luby SP. The pandemic potential of Nipah virus. *Antiviral research*. Oct 2013;100(1):38-43. doi:10.1016/j.antiviral.2013.07.011
6. Skowron K, Bauza-Kaszewska J, Grudlewska-Buda K, et al. Nipah Virus-Another Threat From the World of Zoonotic Viruses. *Front Microbiol*. 2021;12:811157. doi:10.3389/fmicb.2021.811157
7. Kummer S, Kranz DC. Henipaviruses-A constant threat to livestock and humans. *PLoS Negl Trop Dis*. Feb 2022;16(2):e0010157. doi:10.1371/journal.pntd.0010157
8. Gouglas D, Christodoulou M, Plotkin SA, Hatchett R. CEPI: Driving Progress Toward Epidemic Preparedness and Response. *Epidemiol Rev*. Jan 31 2019;41(1):28-33. doi:10.1093/epirev/mxz012
9. Maes P, Adkins S, Alkhovsky SV, et al. Taxonomy of the order Bunyavirales: second update 2018. *Arch Virol*. Mar 2019;164(3):927-941. doi:10.1007/s00705-018-04127-3
10. Silva-Ramos CR, Montoya-Ruíz C, Faccini-Martínez Á A, Rodas JD. An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever. *Arch Virol*. Sep 2022;167(9):1727-1738. doi:10.1007/s00705-022-05453-3
11. Garry RF. Lassa fever - the road ahead. *Nat Rev Microbiol*. Feb 2023;21(2):87-96. doi:10.1038/s41579-022-00789-8
12. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research*. Nov 2018;159:63-67. doi:10.1016/j.antiviral.2018.09.009
13. WHO. List of Blueprint Priority Diseases. Accessed November 14, 2018. http://www.who.int/blueprint/priority-diseases/en/
14. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens. Accessed 04 Jun 2023, https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens
15. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol*. Jan 2006;4(1):23-35. doi:10.1038/nrmicro1323
16. Amaya M, Broder CC. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol*. Sep 29 2020;7(1):447-473. doi:10.1146/annurev-virology-021920-113833
17. Zhang XA, Li H, Jiang FC, et al. A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med*. Aug 4 2022;387(5):470-472. doi:10.1056/NEJMc2202705
18. Radoshitzky SR, Buchmeier MJ, Charrel RN, et al. ICTV Virus Taxonomy Profile: Arenaviridae. *J Gen Virol*. Aug 2019;100(8):1200-1201. doi:10.1099/jgv.0.001280
19. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. *Front Microbiol*. 2018;9:1751. doi:10.3389/fmicb.2018.01751
20. Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient Evolution of Mammarenaviruses: Adaptation via Changes in the L Protein and No Evidence for Host-Virus Codivergence. *Genome Biol Evol*. 2018/03// 2018;10(3):863-874. doi:10.1093/gbe/evy050
21. Shaffer JG, Grant DS, Schieffelin JS, et al. Lassa Fever in Post-Conflict Sierra Leone. *PLOS Neglected Tropical Diseases*. 2014;8(3):e2748. doi:10.1371/journal.pntd.0002748
22. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Review. *Antiviral research*. Apr 2008;78(1):132-9. doi:10.1016/j.antiviral.2007.10.010
23. Kilgore PE, Peters CJ, Mills JN, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. Jul-Sep 1995;1(3):97-100. doi:10.3201/eid0103.950308
24. Heinrich ML, Boisen ML, Nelson DKS, et al. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. *Scientific Reports*. 2020/09/29 2020;10(1):16030. doi:10.1038/s41598-020-72539-w

25. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nature Medicine*. 2017/10/01 2017;23(10):1146-1149. doi:10.1038/nm.4396
26. Hastie KM, Zandonatti MA, Kleinfelter LM, et al. Structural basis for antibody-mediated neutralization of Lassa virus. *Science*. Jun 2 2017;356(6341):923-928. doi:10.1126/science.aam7260
27. Sullivan BM, Sakabe S, Hartnett JN, et al. High crossreactivity of human T cell responses between Lassa virus lineages. *PLoS Pathog*. Mar 2020;16(3):e1008352. doi:10.1371/journal.ppat.1008352
28. Ugwu C, Olumade T, Nwakpakpa E, et al. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. *Scientific Reports*. 2022/12/25 2022;12(1):22330. doi:10.1038/s41598-022-26045-w
29. Geisbert TW, Jones S, Fritz EA, et al. Development of a New Vaccine for the Prevention of Lassa Fever. *PLOS Medicine*. 2005;2(6):e183. doi:10.1371/journal.pmed.0020183
30. Cross RW, Woolsey C, Prasad AN, et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep*. Jul 19 2022;40(3):111094. doi:10.1016/j.celrep.2022.111094
31. Safronetz D, Mire C, Rosenke K, et al. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. *PLOS Neglected Tropical Diseases*. 2015;9(4):e0003736. doi:10.1371/journal.pntd.0003736
32. Hensley LE, Smith MA, Geisbert JB, et al. Pathogenesis of Lassa fever in cynomolgus macaques. *Virol J*. May 6 2011;8:205. doi:10.1186/1743-422x-8-205
33. Zeitlin L, Cross RW, Geisbert JB, et al. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. *Proceedings of the National Academy of Sciences*. 2021;118(11):e2023332118. doi:doi:10.1073/pnas.2023332118
34. Bell TM, Shaia CI, Bunton TE, et al. Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure. *Veterinary Pathology*. 2015;52(1):26-37. doi:10.1177/0300985814540544
35. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. *Epidemiol Infect*. Jun 1996;116(3):353-61. doi:10.1017/s0950268800052687
36. Brinkmann A, Kohl C, Radonić A, et al. First detection of bat-borne Issyk-Kul virus in Europe. *Sci Rep*. Dec 24 2020;10(1):22384. doi:10.1038/s41598-020-79468-8
37. Ma J, Lv XL, Zhang X, et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat Med*. Mar 2021;27(3):434-439. doi:10.1038/s41591-020-01228-y
38. Lorenzo Juanes HM, Carbonell C, Sendra BF, et al. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. *Emerg Infect Dis*. Feb 2023;29(2):252-259. doi:10.3201/eid2902.220677
39. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, et al. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. *Ticks Tick Borne Dis*. May 2020;11(3):101403. doi:10.1016/j.ttbdis.2020.101403
40. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology*. 2023/03/14 2023;doi:10.1038/s41579-023-00871-9
41. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral research*. May 2011;90(2):85-92. doi:10.1016/j.antiviral.2011.02.010
42. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. *BMC Infectious Diseases*. 2010/07/13 2010;10(1):207. doi:10.1186/1471-2334-10-207
43. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep*. May 23 2019;9(1):7755. doi:10.1038/s41598-019-44210-6
44. Bente DA, Alimonti JB, Shieh WJ, et al. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol*. Nov 2010;84(21):11089-100. doi:10.1128/jvi.01383-10
45. Cross RW, Prasad AN, Borisevich V, et al. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. *PLoS Negl Trop Dis*. Aug 2020;14(8):e0008637. doi:10.1371/journal.pntd.0008637

46. Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York, p 519-536.
47. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science*. Apr 7 1995;268(5207):94-7. doi:10.1126/science.7701348
48. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science (New York, NY)*. 2000/05// 2000;288(5470):1432-1435. doi:10.1126/science.288.5470.1432
49. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol*. 2012;359:153-77. doi:10.1007/82_2012_208
50. *Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087*.
51. Sweileh WM. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health*. Feb 8 2017;13(1):9. doi:10.1186/s12992-017-0233-9
52. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Science translational medicine*. 2011/10// 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901
53. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine*. 2014/06// 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929
54. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep*. Aug 3 2016;6:30916. doi:10.1038/srep30916
55. Mire CE, Chan YP, Borisevich V, et al. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis*. May 11 2020;221(Suppl 4):S471-s479. doi:10.1093/infdis/jiz515
56. Playford EG, Munro T, Mahler SM, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*. Apr 2020;20(4):445-454. doi:10.1016/s1473-3099(19)30634-6
57. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerging infectious diseases*. 2014/03// 2014;20(3):372-379. doi:10.3201/eid2003.131159
58. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One*. May 18 2010;5(5):e10690. doi:10.1371/journal.pone.0010690
59. Cheliout Da Silva S, Yan L, Dang HV, et al. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses*. Mar 22 2021;13(3)doi:10.3390/v13030517
60. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo LD, Jr. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. *Expert Opin Drug Deliv*. Feb 2021;18(2):151-167. doi:10.1080/17425247.2021.1823964
61. Andrianov AK, DeCollibus DP, Gillis HA, et al. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A*. Nov 10 2009;106(45):18936-41. doi:10.1073/pnas.0908842106
62. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med*. Jun 25 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929
63. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med*. Oct 19 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901
64. Pettitt J, Zeitlin L, Kim do H, et al. Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. *Sci Transl Med*. Aug 21 2013;5(199):199ra113. doi:10.1126/scitranslmed.3006608
65. Qiu X, Wong G, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature*. Oct 2 2014;514(7520):47-53. doi:10.1038/nature13777

66. Mire CE, Geisbert JB, Borisevich V, et al. Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci Transl Med*. Apr 5 2017;9(384)doi:10.1126/scitranslmed.aai8711

67. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat Med*. Oct 2017;23(10):1146-1149. doi:10.1038/nm.4396

68. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. *mAbs*. Sep-Oct 2011;3(5):422-30. doi:10.4161/mabs.3.5.16983

69. Robbie GJ, Criste R, Dall'acqua WF, et al. A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study. *Antimicrobial agents and chemotherapy*. Sep 30 2013;doi:10.1128/aac.01285-13

70. Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. *PLoS Med*. Jan 2018;15(1):e1002493. doi:10.1371/journal.pmed.1002493

71. Mastraccio KE, Huaman C, Laing ED, Broder CC, Schaefer BC. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. *Methods Mol Biol*. 2022;2524:369-394. doi:10.1007/978-1-0716-2453-1_30

72. Rima B, Balkema-Buschmann A, Dundon WG, et al. ICTV Virus Taxonomy Profile: Paramyxoviridae. *J Gen Virol*. Dec 2019;100(12):1593-1594. doi:10.1099/jgv.0.001328

73. Madera S, Kistler A, Ranaivoson HC, et al. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol*. Sep 28 2022;96(18):e0092122. doi:10.1128/jvi.00921-22

74. Vanmechelen B, Meurs S, Horemans M, et al. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol*. 2022;8(2):veac061. doi:10.1093/ve/veac061

75. Drexler JF, Corman VM, Müller MA, et al. Bats host major mammalian paramyxoviruses. *Nat Commun*. Apr 24 2012;3:796. doi:10.1038/ncomms1796

76. Wu Z, Yang L, Yang F, et al. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis*. Jun 2014;20(6):1064-6. doi:10.3201/eid2006.131022

77. Voigt K, Hoffmann M, Drexler JF, et al. Fusogenicity of the Ghana Virus (Henipavirus: Ghanaian bat henipavirus) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. *Viruses*. Aug 29 2019;11(9)doi:10.3390/v11090800

78. Childs K, Randall R, Goodbourn S. Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction. *J Virol*. Apr 2012;86(7):3411-21. doi:10.1128/jvi.06405-11

79. Davis ME, Wang MK, Rennick LJ, et al. Antagonism of the phosphatase PP1 by the measles virus V protein is required for innate immune escape of MDA5. *Cell Host Microbe*. Jul 9 2014;16(1):19-30. doi:10.1016/j.chom.2014.06.007

80. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol*. Nov 2002;76(22):11476-83. doi:10.1128/jvi.76.22.11476-11483.2002

81. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol*. May 2005;79(10):6078-88. doi:10.1128/jvi.79.10.6078-6088.2005

82. Basler CF. Nipah and hendra virus interactions with the innate immune system. *Curr Top Microbiol Immunol*. 2012;359:123-52. doi:10.1007/82_2012_209

83. Yoneda M, Guillaume V, Sato H, et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One*. Sep 15 2010;5(9):e12709. doi:10.1371/journal.pone.0012709

84. Satterfield BA, Cross RW, Fenton KA, et al. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J Virol*. Jul 15 2016;90(14):6326-6343. doi:10.1128/jvi.00215-16

85. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog*. 2012;8(8):e1002836. doi:10.1371/journal.ppat.1002836

86. Amaya M, Cheng H, Borisevich V, et al. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research*. Sep 2021;193:105084. doi:10.1016/j.antiviral.2021.105084

87. CDC. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. . Updated June 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

88. Organization WH. Lassa fever fact sheet. *World Health Organization*. 2017;

89. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. *J Travel Med*. Jul 14 2020;27(4)doi:10.1093/jtm/taaa035

90. Purushotham J, Lambe T, Gilbert SC. Vaccine platforms for the prevention of Lassa fever. *Immunol Lett*. Nov 2019;215:1-11. doi:10.1016/j.imlet.2019.03.008

91. Sewlall NH, Richards G, Duse A, et al. Clinical features and patient management of Lujo hemorrhagic fever. *PLoS Negl Trop Dis*. 2014;8(11):e3233. doi:10.1371/journal.pntd.0003233

92. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. *Vaccines*. 2023;11(3):635.

93. Koma T, Patterson M, Huang C, et al. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. *J Virol*. Feb 1 2016;90(3):1290-7. doi:10.1128/jvi.02615-15

94. Jahrling P TR, Barrero O. . Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 1988:53.

95. Delgado S, Erickson BR, Agudo R, et al. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. *PLOS Pathogens*. 2008;4(4):e1000047. doi:10.1371/journal.ppat.1000047

96. Loayza Mafayle R, Morales-Betoulle ME, Romero C, et al. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. *New England Journal of Medicine*. 2022;386(24):2283-2294. doi:10.1056/NEJMoa2110339

97. Tipih T, Burt FJ. Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development. *Biores Open Access*. 2020;9(1):137-150. doi:10.1089/biores.2019.0057

98. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease. 2023. Accessed 03/06, 2023. https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease

99. Kalunda M, Mukwaya LG, Mukuye A, et al. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. *The American Journal of Tropical Medicine and Hygiene*. 01 Mar. 1986 1986;35(2):387-392. doi:10.4269/ajtmh.1986.35.387

100. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. *Emerg Infect Dis*. Dec 2020;26(12):2944-2950. doi:10.3201/eid2612.202411

101. Mulangu S, Dodd LE, Davey RT, Jr., et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. *N Engl J Med*. Dec 12 2019;381(24):2293-2303. doi:10.1056/NEJMoa1910993

102. Group PIW, Multi-National PIIST, Davey RT, Jr., et al. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. *N Engl J Med*. Oct 13 2016;375(15):1448-1456. doi:10.1056/NEJMoa1604330

103. Politch JA, Cu-Uvin S, Moench TR, et al. Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial. *PLoS Med*. Feb 2021;18(2):e1003495. doi:10.1371/journal.pmed.1003495

CENTER OVERVIEW: *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction. Emerging and periodically reemerging pathogenic enveloped RNA viruses are continuous pandemic threats. Among viral taxon associated with human zoonoses, members of the family ***Paramyxoviridae*** and order ***Bunyavirales*** stand out for their capacity to cause debilitating and life-threatening disease**.** Humans can become infected via excreta from animal reservoir hosts such as rodents or bats, through intermediate amplifying hosts such as small or domestic animals, or by arthropod vectors such as mosquitos and ticks[1-4]. Humans often represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur. During this process, RNA viral pathogens may mutate and adapt to more efficiently replicate and shed in the host, posing an epidemic threat[5-8]. The viral order *Bunyavirales* is composed of several viral families containing high priority human pathogens[9]. Notably, the ***Arenaviridae*** and ***Nairoviridae*** families contain viruses that cause severe hemorrhagic fever diseases in humans globally with mortality rates up to 60%[10] and significant, long-term sequelae in survivors[11]. Many bunyaviruses are included on the World Health Organization's (WHO) List of Priority Pathogens[12,13]. Six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHAPV, GTOV, JUNV, MACV) and one nairovirus, Crimean-Congo Hemorrhagic Fever Virus (CCHFV), are also identified as NIAID Category A pathogens[14] due to their 1) high associated morbidity and mortality rates, 2) ease of dissemination and/or ability to transmit from person-to-person, 3) potential to cause public panic and social disruption, and 4) requirement for special actions in the context of public health preparedness. The ***Paramyxoviridae*** also includes members in the WHO Priority Pathogens, namely Nipah virus (NiV) and Hendra virus (HeV). These henipaviruses (HNVs) are the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease[15]. The genus now includes nine additional reported HNVs, but only HeV and NiV are associated with severe and often fatal henipaviral disease in humans and a number of animal species[16]. Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illness in humans[17].

These collective factors plus the absence of approved vaccines or therapeutics make bunyaviruses and paramyxoviruses a significant threat to global public health. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for developing vaccine and monoclonal antibody (mAb) countermeasures to prototype bunyavirus and paramyxovirus pathogens (**Table 1**). In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old World) Machupo (New World)	Lujo (Old World) Chapare (New World)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Table 1. PABVAX selected prototype and test case viral agents

***Arenaviridae**:* The *Arenaviridae* is composed of five genera: *Mammarenavirus*, *Reptarenavirus*, *Innmovirus*, *Antennavirus*, and *Hartmanivirus*[18]. The genus *Mammarenavirus* contains a large number of rodent-borne viruses, some of which cause severe hemorrhagic fever, neurological damage, death, and permanent sequelae; hearing loss, vision impairment, and balance issues in survivors[19]. While these genera exhibit a global distribution, two geographically distinct serogroups exist that diverged[20]. Old World complex mammarenaviruses (OWAV) in the Eastern Hemisphere include LASV, Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered LUJV; New World complex mammarenaviruses (NWAV) in the Western Hemisphere include JUNV, MACV, Sabia Virus (SABV), and CHAPV. There are no FDA approved vaccines or therapeutics for mammarenaviruses, though the attenuated JUNV "Candid-1" vaccine is approved for use in Argentina.

Disease severity and case fatality rates (CFR) vary across mammarenaviruses even within the same clades and species. Up to 500,000 cases of LASV infection occur per year with CFRs sometimes approaching 70% in hospitalized cases[21]. Before the introduction of the Candid-1 vaccine, it is believed that an upwards of 60,000 cases of Argentine Hemorrhagic Fever (AHF) from infection with JUNV occurred per year with CFRs ranging from 15-30%. Convalescent plasma from JUNV survivors is still the primary means to treat acute AHF in Argentina[22]. Over 1000 cases were reported for the initial outbreak of MACV with CFRs ranging from 5-30%; enhanced rodent abatement practices and potentially cross-protective immunity in populations that have taken the JUNV Candid-1 vaccine may have reduced recent outbreak frequency[23]. All mammarenaviruses share a common genomic organization consisting of two ambisense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix) and L protein (polymerase), and the glycoprotein precursor (GPC) and nucleoprotein (NP)[19]. Despite general similarity of their genomic architecture, mammarenaviruses exhibit marked differences in their replication life cycles including attachment factors, entry mechanisms, and immune evasion; nonetheless, the dominant antigens driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Despite the abundant genetic diversity of LASV, high levels of cross reactivity have been seen with antigen-based diagnostics across seven known lineages, suggesting the generation of cross-protective immunogens and antibodies against mammarenaviruses is achievable[24]. Monoclonal antibodies derived from human LASV survivors were shown to protect non-human primates (NHP) against lethal challenge[25]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[26]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, indicating this domain could serve as an antigenic target. Investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccines to test this approach and have demonstrated protection against lethal LASV-Josiah challenge. Specifically, a stabilized prefusion trimeric GPe, an immunogen containing a highly conserved NP NTD epitope, or a combination of these two approaches conferred protection in a lethal outbred guinea pig model. Nevertheless, even with the considerable molecular diversity in LASV strains, conservation at key antigenic sites within the GP and NP suggest a pan-LASV vaccine may be possible[24,27,28]. **RP1** and **Core E** investigators have demonstrated that a Vesicular stomatitis virus (rVSV)-based vaccine expressing the LASV-Josiah GPC is protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[29], and against heterologous challenge with Lineage II[30], III, V[31], and VI viruses (Cross and Geisbert Unpublished).

In **Phase I of RP1**, the ability of an optimized LASV GPe/NP subunit vaccine delivered intramuscularly (IM) will be assessed in guinea pig and NHP models across multiple viral lineages. We will also assess a microneedle skin patch (MNP) formulation optimized in **Core C** to determine if a needle-free vaccination route affords more potent vaccine-mediated responses and protection. Similarly, we have generated MACV GPe immunogens and will design a MACV NP-NTD subunit antigen to test the same approach across multiple MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing both OWAV (LUJV) and NWAV (CHAPV) complexes, for which there are no vaccines available. A unique advantage of the PABVAX Center is its access to biosafety level (BSL)-4 containment, allowing us to work with authentic versus surrogate arenaviruses. The BSL4 facilities described in **Core E** are capable of evaluating countermeasures in small animal models and NHPs. The cynomolgus macaque is the current “gold standard” animal model for LASV, JUNV, and MACV research as disease recapitulates human infection and there are a multitude of immunological reagents available[32-34]. The evaluation of prototype OWAV and NWAV vaccines and therapies in the NHP model is likely pivotal for IND applications and for future licensure of medical countermeasures for human use.

<u>Nairoviridae:</u> Unique to most bunyaviruses, orthonairoviruses are transmitted primarily by ticks[2]. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[35], Issyk-Kul virus[36], Songling virus[37]), CCHFV is the most recognized public health threat. Outbreaks of CCHFV have occurred across Europe, Africa, the Middle East, and Asia. There is growing concern for expansion of the CCHFV endemic region due to increased range of its tick host (*Hyalomma* spp.) tied to bird migration changes, livestock trade, and global warming. For example, CCHFV cases have been reported in Spain[38] , a previously non-endemic region, and *Hyalomma* ticks have been found in Sweden[39]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to a severe hemorrhagic fever and death in 30% of cases[40]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[41]. Ribavirin has been used to treat infections with limited success[42].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and GPC [40]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[40]. Conversely, the CCHFV GPC is considerably less conserved (< 75% amino acid identity)[40]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location. Thus, creating a pan-CCHFV vaccine or therapeutic based solely on one clade poses a challenge. Nevertheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a rVSV vectored vaccine expressing the full GPC of CCHFV[43].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP immunogens with the highest level of homology across clades. We will assess their capacity to protect against lethal challenge with isolates from multiple CCHFV clades in established mouse[43,44] and NHP[45] models with **Core E**. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best formulation of CCHFV GP/NP MNP vaccine against the emerging test case nairovirus, Kasokero virus, for which there are no vaccines available.

Paramyxoviridae: The HNVs NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality[4,46]. HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality[47]. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs, which served as amplifying hosts[48]. NiV and HeV can infect at least 18 animal species across 6 orders of mammals, and among these they cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans[16,49]. The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission[5]. Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and Southeast Asia and Australia. Their broad host tropism and ability to infect livestock also causes increased risk of human infections. HNVs are single-stranded, negative sense, enveloped RNA viruses and their biology lends themselves to be synthetically produced. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS). The Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents[50] because the virus could be engineered for mass dissemination and it possesses the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the R&D Blueprint list of priority pathogens with epidemic potential[51].

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb exist. These postexposure treatments conferred protecion against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP)[52-55]. The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia[56]. In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs, and several vaccine platforms have clear potential for use in people[16]. The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies[16]. Such preventive vaccines would have utility for lab workers, first responders or individuals at high-risk exposure, and in mitigating the spread of an outbreak. In the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent[57]. It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection[49,58]. The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished)[59]. The divergent LayV will be the focus of the test case studies. The choice is justifiable for several important reasons: 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; and 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including rescuing recombinant LayV (rLayV), exploring animal model development, constructing additional LayV and AngV reporter virus tools, and then translating and testing the MNP vaccine 'plug-and-play' approach. Finally, we will evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME. A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center's RPs and Core leaders currently have active and productive collaborative research programs in related areas, most spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

The pandemic preparedness research gaps addressed by PABVAX are diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **arenaviruses** and **nairoviruses**, and **RP2** will target the prototype **HNVs**. Both RP1 and RP2 will develop new prototype virus tools (recombinant prototype viruses) along with existing tools, to analyze vaccine responses and evaluate new mAbs and nabs from the other RPs. A third RP will develop a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant *Cedar henipavirus* (rCedV) in vivo and a bioluminescence detection method that can longitudinally trace the dynamics and anatomical progression of infection with a luciferase-encoding virus in individual animals (**RP3**). There will also be two RPs that focus on the development of broadly reactive human mAbs and nanobodies to the PABVAX selected viral prototypes. **RP4** will isolate human mAbs derived from NiV-infected survivors with a specific focus on F glycoprotein-targeting mAbs. Additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will engage in the manufacture, quality control, and testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**). Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core (**Core A**) will provide organizational and programmatic structure to promote research productivity and scientific interactions along with compliance with regulations concerning select agent, vertebrate animal, and human subject use. **Core A** will coordinate all activities with the Scientific Advisory Board (**SAB**) and make data available through the Data Management Core (**Core B**).

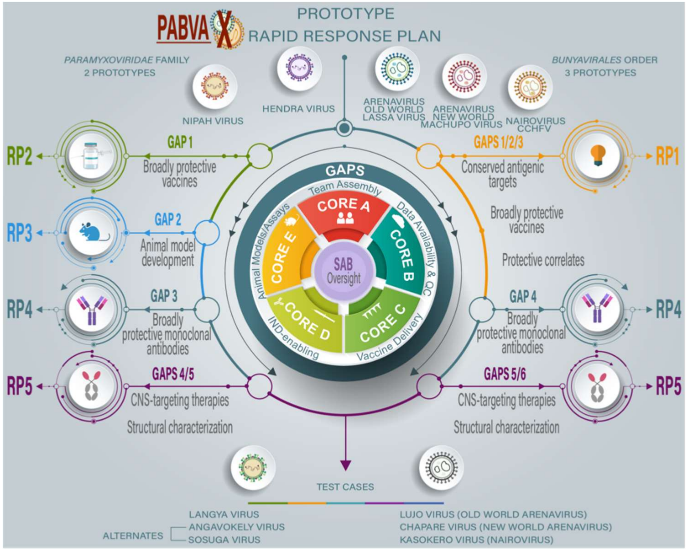


Figure 1: Research gaps addressed by the *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center- (PABVAX).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform. The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**). This project tested a dissolvable MNP-based vaccine technology ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine[60]. The MNP vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable microneedles that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants[61] to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as to facilitate improved vaccine storage and distribution due to their improved thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, Scientific **Core C** (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ)-adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (*Paramyxoviridae*). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro*, along with analysis of polyfunctional T-cell responses by antigenic peptide stimulation, followed by intracellular cytokine staining (ICS) and flow cytometry.

Discover effective antibody-based prophylactics and therapies. Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah[62], Hendra[63], Ebola[64,65], Marburg[66] and Lassa[67]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc*.), a feature to be exploited in **Core D** by Dr. Gunn. In addition to therapeutic applications, mAbs with Fc mutations dramatically extend serum half-life[68-70] and offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g*. rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization, and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures. Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal Model Development and Preclinical Evaluation Scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective efficacy of antivirals and vaccines, as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation, including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within

RP1. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they are generally less laborious and can be adapted to higher throughput platforms and/or integrated with other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput fluorescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic NiV and HeV vaccine-induced immune sera, mAbs (**RP4**), nbs (**RP5**), and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either HeV or NiV-B envelope GPs) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals to explore nab based therapeutics to CNS resident HNVs[71].

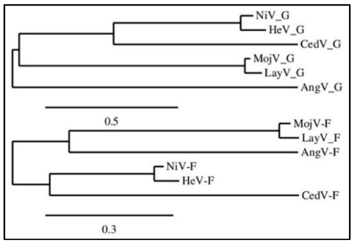
The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the HNVs (*Paramyxoviridae*) and the *Bunyavirales* (*Arenaviridae* and *Nairoviridae*) that will bolster vaccine endurance to storage and transportation; we also anticipate improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology, and mAb and vaccine translational drug development along with Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Paramyxoviruses: NiV and HeV are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis[4,46]. HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals. These viruses are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of CedV, Gamak virus, Daeryong virus, and (LayV)[72] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[73,74]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV, are old world pteropid fruit bats[73,75]. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of



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Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

apparent rodent origin from Southeast China (Yunnan province)[17]. The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**. Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal HNV disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters[49], and AGMs (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P). The P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists[76-80]. The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections[81,82]. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature[83]. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2[84] and CedV is now recognized as a BSL-2 restricted agent[85]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D,** and **E**).

Figure 2: Phylogenetic analysis of select HNV G and F glycoproteins. Trees were constructed for using the PhyML program's maximum likelihood method and aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Arenaviruses (old world): For **phase 1**, **RP1** will utilize **LASV as the prototype virus** due its significant public health burden throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[86]. LASV is the most imported viral hemorrhagic fever (VHF) next to Dengue virus and has been documented in several countries outside of Africa over 35 times[87]. CFRs approaching 70% in hospitalized cases and marked long term sequelae are the result of the lack of approved vaccines or therapeutics, although several live attenuated virus candidates are currently being evaluated with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI)[88]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize only LASV GP as the target immunogen.

Investigators in **RP1**, **Core D**, and **Core E** have already demonstrated protective efficacy against challenge with LASV in outbred guinea pigs vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These immunogens have been engineered to express homologous antigenic regions to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach suggests a similar strategy could be implemented against other arenaviruses. The **OWAV test pathogen, LUJV,** was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia, which quickly spread nosocomially[89]. The CFR for this infectious virus was 80% in this small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from suggest current LASV countermeasures may be of limited use. LUJV will serve as an ideal "test case" to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Arenaviruses (new world): The live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[90], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[91,92]. Furthermore, there is the potential for pathogenic reversion. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest exploring other JUNV countermeasures are warranted. Building on the success of the LASV GPe/NP-NTD, investigators in **RP1 and Cores D and E** will engineer broadly protective MACV GPe and NP-NTD antigens and perform efficacy testing in established guinea pig and NHP models. We will extend this plug-and-play approach to the **NWAV test case, CHAPV**. First reported in 2003 in Bolivia, CHAPV is a rodent-borne mammarenavirus associated with the development of severe hemorrhagic fever with case fatality rates up to 60%[93,94]. Recent studies have demonstrated clear human-to-human transmission during the acute phase evidenced by several nosocomial infections; isolation of viable virus from semen at 86 days after symptom onset demonstrates a clear need to implement specialized management and treatment [94]. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Nairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as it represents the largest global public health burden within the orthonairovirus family with outbreaks annually across Europe, Africa, and Asia. There are no FDA-approved vaccines for CCHFV; however, a suckling mouse brain-derived, adjuvanted whole virus vaccine for CCHFV has been approved for use in Bulgaria. This vaccine has numerous safety concerns and has limited demonstrated efficacy. Several experimental vaccine approaches have been evaluated ranging from viral vectored approaches to DNA-based vaccines with varying efficacy, advantages, and disadvantages[95]; however, only a Modified Vaccinia Ankara virus vaccine (MVA-CCHFV) has begun to advance towards clinical trials[96]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test these for protective efficacy from challenge with 6 different CCHFV lineages using established mouse and NHP models. Next, we will deploy the same approach to the **test case nairovirus, Kasokero virus (KASV),** for which no vaccine exists.

In 1977, KASV was first isolated from Ugandan bats and caused several cases of acute febrile illness of laboratory workers from the same lab that processed the infected bats[97]. Surveys of 109 laboratory workers from the same facility demonstrated 13% seropositivity and clinical symptoms in individuals 4 to 15 months after the virus was isolated. These findings suggest either continued laboratory-associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the tick reservoir, *Ornithodoros faini,* to participate in enzootic maintenance of KASV[98]. While most chiropteran ticks exhibit host specificity, humans entering caves these bats inhabit have reported bites from *O.faini* ticks. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics suggest investment in countermeasure development against this virus is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**; arenavirus and nairovirus projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine active and passive immunization approaches to further improve the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

An additional and outstanding advantage of the PABVAX Center is the **Animal Model Development and Preclinical Evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for members of the *Bunyavirales* and *Paramyxoviridae*.**
2. **Develop new tools and animal models for cell-based and *in vivo* therapeutic evaluation.**
3. **Develop antibodies and nanobodies to the selected prototype and test viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

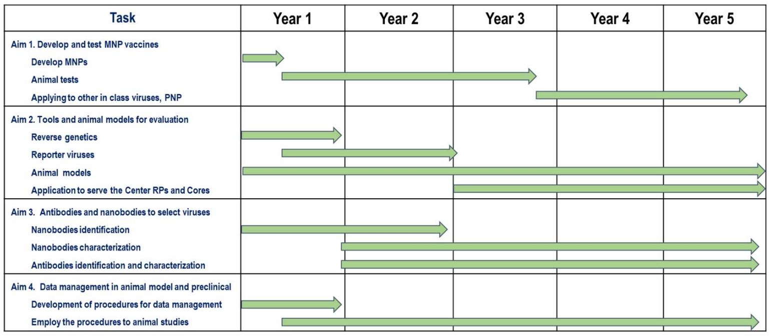

Figure 3: Projected PABVAX timeline to achieve the 4 overarching tasks.

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**. Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (details are specified within the Administration **Core A** Research Strategy). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommending new directions as appropriate, and providing Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

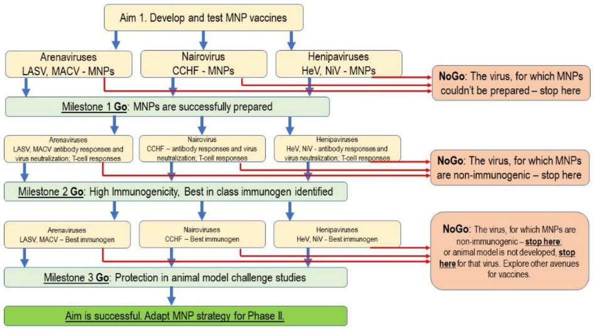

Figure 4. Milestones and criteria for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will address whether formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be established using immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen-specific antibody induction, virus neutralization, and polyfunctional T-cell responses by *in vitro* stimulation of isolated cells). Go/no go decisions will be made based on outcomes of these first-tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as a success. Comparisons will also be made such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of the best-in-class immunogens, or immunogen combinations, will also be explored to down-select vaccines. The third milestone will determine whether MNP vaccination affords protection in animal model challenge experiments. In the case of NiV and HeV prototypes, the animal models chosen (ferrets and AGMs) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales*, there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASV and guinea pig models exist for some but not all of these lineages. For MACV, there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is the limitations of cost and availability of rhesus and cynomolgus monkeys. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be developing and evaluating animal models within the PABVAX program, including further development of AGM models for arenaviruses and possibly CCHFV as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for cell-based and in vivo therapeutics evaluation.

The second overall objective of PABVAX is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed because genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If successful, a large battery of new materials will be available to share with the ReVAMPP network, and be key milestones for execution of Phase II of the PABVAX Center program.

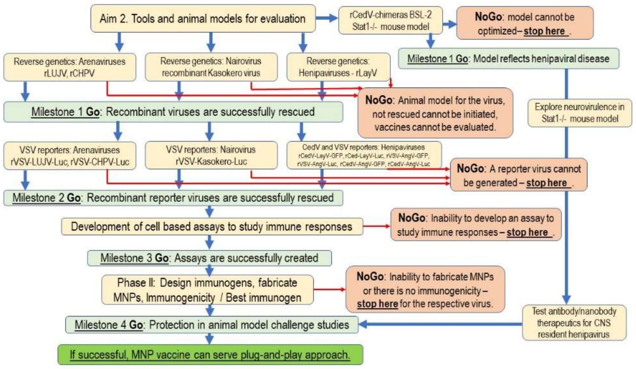

Figure 5. Milestones and criteria for **Aim 2** of the PABVAX Center's overall objectives.

The translation of the MNP vaccine plug-and-play approach will be in Phase II (**Table 1**). Milestones will be similar to those in **Figure 4**. New virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new *in vivo* bioluminescence-based immune deficient mouse model developed by the PIs of **PR2** and **RP3** that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease *in vivo* safely and expediently at BSL-2 containment. The model will be deployed to test a critically important therapeutic need against CNS resident HNV infection, and **RP3** will collaborate with **RP5** in the testing of engineered nabs.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

New human mAbs and nbs as tools and potential therapeutics will be developed by **RP4** and **RP5** in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs, and in collaboration with **RP2**, neutralization potencies will be rapidly assessed (as well as evaluating synergistic cell-based mAb neutralization) that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

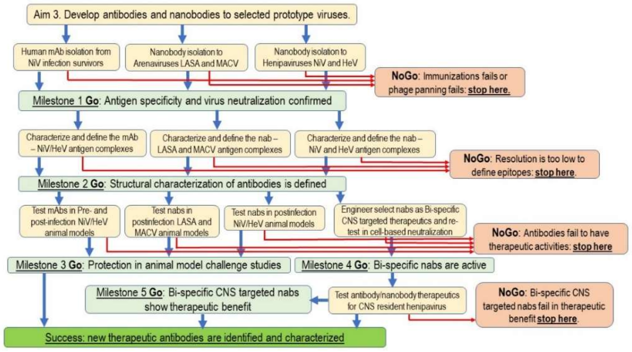

Figure 5. Milestones and criteria for **Aim 3** of the PABVAX Center's overall objectives.

Nbs, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nbs can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nbs targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus), and select animal models of LASV and MACV by **Core E**. Success will be measured by the 1) identification of potent virus-neutralizing nbs, 2) discovery of more broadly reactive nbs particularly to the multiple LASV clades, 3) evidence of therapeutic benefit in animal models, and 4) the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

Industry Partnerships. ~~*Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner that will provide access to vaccine expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.*~~

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience in developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX. Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

SPECIAL FEATURES OF THE PABVAX

Galveston National Laboratory (GNL). The GNL is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The

GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics, and therapeutics. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370 NHP studies employing some 2,772 animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses have been performed. These studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder, and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP facility and a staff of full-time veterinarians. **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of the WRCEVA virus collection within the GNL.

***Core E (Animal model development and preclinical evaluation)*. Core E** operations will be conducted at the GNL, and in conjunction with **Core A**, will also include the UTMB Institutional Office of Regulated Nonclinical Studies (ORNcS). The ORNcS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a “well documented” quality system program. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL, and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses, arenaviruses, and henipaviruses. He has published over 250 peer-reviewed articles in these areas. Dr. Geisbert’s research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI), was the first to develop a NHP model of NiV/HeV infection and disease, and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs). Mapp Biopharmaceutical has deep experience with antigens and mAbs against these antigens. Their early development and research group has expertise in immunology, infectious diseases, and the production of antigens, antibodies, and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [99] [100]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1[101]. Dr. Gunn and Genovac will join the team to analyze antibody Fc effector functions and to provide antigen/antibody discovery resources, respectively. Dr. Geisbert will contribute his experience with high containment animal models and vaccine development.

As per NIAID’s expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID’s preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID’s preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

1. Lamb RA, Parks GD. Paramyxoviridae: the viruses and their replication. In Fields BN, Knipe DM, Howley PM, editors, Fields Virology: Sixth Edition. Vol. 1. Lippincott, Williams, and Wilkins. 2013. p. 957-995.
2. Boshra H. An Overview of the Infectious Cycle of Bunyaviruses. *Viruses*. Sep 28 2022;14(10)doi:10.3390/v14102139
3. Horne KM, Vanlandingham DL. Bunyavirus-vector interactions. *Viruses*. Nov 13 2014;6(11):4373-97. doi:10.3390/v6114373
4. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia), p 1070-1085.
5. Luby SP. The pandemic potential of Nipah virus. *Antiviral research*. Oct 2013;100(1):38-43. doi:10.1016/j.antiviral.2013.07.011
6. Skowron K, Bauza-Kaszewska J, Grudlewska-Buda K, et al. Nipah Virus-Another Threat From the World of Zoonotic Viruses. *Front Microbiol*. 2021;12:811157. doi:10.3389/fmicb.2021.811157
7. Kummer S, Kranz DC. Henipaviruses-A constant threat to livestock and humans. *PLoS Negl Trop Dis*. Feb 2022;16(2):e0010157. doi:10.1371/journal.pntd.0010157
8. Gouglas D, Christodoulou M, Plotkin SA, Hatchett R. CEPI: Driving Progress Toward Epidemic Preparedness and Response. *Epidemiol Rev*. Jan 31 2019;41(1):28-33. doi:10.1093/epirev/mxz012
9. Maes P, Adkins S, Alkhovsky SV, et al. Taxonomy of the order Bunyavirales: second update 2018. *Arch Virol*. Mar 2019;164(3):927-941. doi:10.1007/s00705-018-04127-3
10. Silva-Ramos CR, Montoya-Ruíz C, Faccini-Martínez Á A, Rodas JD. An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever. *Arch Virol*. Sep 2022;167(9):1727-1738. doi:10.1007/s00705-022-05453-3
11. Garry RF. Lassa fever - the road ahead. *Nat Rev Microbiol*. Feb 2023;21(2):87-96. doi:10.1038/s41579-022-00789-8
12. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research*. Nov 2018;159:63-67. doi:10.1016/j.antiviral.2018.09.009
13. WHO. List of Blueprint Priority Diseases. Accessed November 14, 2018. http://www.who.int/blueprint/priority-diseases/en/
14. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens. Accessed 04 Jun 2023, https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens
15. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol*. Jan 2006;4(1):23-35. doi:10.1038/nrmicro1323
16. Amaya M, Broder CC. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol*. Sep 29 2020;7(1):447-473. doi:10.1146/annurev-virology-021920-113833
17. Zhang XA, Li H, Jiang FC, et al. A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med*. Aug 4 2022;387(5):470-472. doi:10.1056/NEJMc2202705
18. Radoshitzky SR, Buchmeier MJ, Charrel RN, et al. ICTV Virus Taxonomy Profile: Arenaviridae. *J Gen Virol*. Aug 2019;100(8):1200-1201. doi:10.1099/jgv.0.001280
19. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. *Front Microbiol*. 2018;9:1751. doi:10.3389/fmicb.2018.01751
20. Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient Evolution of Mammarenaviruses: Adaptation via Changes in the L Protein and No Evidence for Host-Virus Codivergence. *Genome Biol Evol*. 2018/03// 2018;10(3):863-874. doi:10.1093/gbe/evy050
21. Shaffer JG, Grant DS, Schieffelin JS, et al. Lassa Fever in Post-Conflict Sierra Leone. *PLOS Neglected Tropical Diseases*. 2014;8(3):e2748. doi:10.1371/journal.pntd.0002748
22. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Review. *Antiviral research*. Apr 2008;78(1):132-9. doi:10.1016/j.antiviral.2007.10.010
23. Kilgore PE, Peters CJ, Mills JN, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. Jul-Sep 1995;1(3):97-100. doi:10.3201/eid0103.950308

24. Heinrich ML, Boisen ML, Nelson DKS, et al. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. *Scientific Reports*. 2020/09/29 2020;10(1):16030. doi:10.1038/s41598-020-72539-w
25. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nature Medicine*. 2017/10/01 2017;23(10):1146-1149. doi:10.1038/nm.4396
26. Hastie KM, Zandonatti MA, Kleinfelter LM, et al. Structural basis for antibody-mediated neutralization of Lassa virus. *Science*. Jun 2 2017;356(6341):923-928. doi:10.1126/science.aam7260
27. Sullivan BM, Sakabe S, Hartnett JN, et al. High crossreactivity of human T cell responses between Lassa virus lineages. *PLoS Pathog*. Mar 2020;16(3):e1008352. doi:10.1371/journal.ppat.1008352
28. Ugwu C, Olumade T, Nwakpakpa E, et al. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. *Scientific Reports*. 2022/12/25 2022;12(1):22330. doi:10.1038/s41598-022-26045-w
29. Geisbert TW, Jones S, Fritz EA, et al. Development of a New Vaccine for the Prevention of Lassa Fever. *PLOS Medicine*. 2005;2(6):e183. doi:10.1371/journal.pmed.0020183
30. Cross RW, Woolsey C, Prasad AN, et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep*. Jul 19 2022;40(3):111094. doi:10.1016/j.celrep.2022.111094
31. Safronetz D, Mire C, Rosenke K, et al. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. *PLOS Neglected Tropical Diseases*. 2015;9(4):e0003736. doi:10.1371/journal.pntd.0003736
32. Hensley LE, Smith MA, Geisbert JB, et al. Pathogenesis of Lassa fever in cynomolgus macaques. *Virol J*. May 6 2011;8:205. doi:10.1186/1743-422x-8-205
33. Zeitlin L, Cross RW, Geisbert JB, et al. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. *Proceedings of the National Academy of Sciences*. 2021;118(11):e2023332118. doi:doi:10.1073/pnas.2023332118
34. Bell TM, Shaia CI, Bunton TE, et al. Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure. *Veterinary Pathology*. 2015;52(1):26-37. doi:10.1177/0300985814540544
35. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. *Epidemiol Infect*. Jun 1996;116(3):353-61. doi:10.1017/s0950268800052687
36. Brinkmann A, Kohl C, Radonić A, et al. First detection of bat-borne Issyk-Kul virus in Europe. *Sci Rep*. Dec 24 2020;10(1):22384. doi:10.1038/s41598-020-79468-8
37. Ma J, Lv XL, Zhang X, et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat Med*. Mar 2021;27(3):434-439. doi:10.1038/s41591-020-01228-y
38. Lorenzo Juanes HM, Carbonell C, Sendra BF, et al. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. *Emerg Infect Dis*. Feb 2023;29(2):252-259. doi:10.3201/eid2902.220677
39. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, et al. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. *Ticks Tick Borne Dis*. May 2020;11(3):101403. doi:10.1016/j.ttbdis.2020.101403
40. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology*. 2023/03/14 2023;doi:10.1038/s41579-023-00871-9
41. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral research*. May 2011;90(2):85-92. doi:10.1016/j.antiviral.2011.02.010
42. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. *BMC Infectious Diseases*. 2010/07/13 2010;10(1):207. doi:10.1186/1471-2334-10-207
43. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep*. May 23 2019;9(1):7755. doi:10.1038/s41598-019-44210-6
44. Bente DA, Alimonti JB, Shieh WJ, et al. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol*. Nov 2010;84(21):11089-100. doi:10.1128/jvi.01383-10
45. Cross RW, Prasad AN, Borisevich V, et al. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. *PLoS Negl Trop Dis*. Aug 2020;14(8):e0008637. doi:10.1371/journal.pntd.0008637

46. Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York, p 519-536.
47. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science*. Apr 7 1995;268(5207):94-7. doi:10.1126/science.7701348
48. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science (New York, NY)*. 2000/05// 2000;288(5470):1432-1435. doi:10.1126/science.288.5470.1432
49. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol*. 2012;359:153-77. doi:10.1007/82_2012_208
50. *Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087*.
51. Sweileh WM. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health*. Feb 8 2017;13(1):9. doi:10.1186/s12992-017-0233-9
52. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Science translational medicine*. 2011/10// 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901
53. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine*. 2014/06// 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929
54. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep*. Aug 3 2016;6:30916. doi:10.1038/srep30916
55. Mire CE, Chan YP, Borisevich V, et al. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis*. May 11 2020;221(Suppl 4):S471-s479. doi:10.1093/infdis/jiz515
56. Playford EG, Munro T, Mahler SM, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*. Apr 2020;20(4):445-454. doi:10.1016/s1473-3099(19)30634-6
57. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerging infectious diseases*. 2014/03// 2014;20(3):372-379. doi:10.3201/eid2003.131159
58. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One*. May 18 2010;5(5):e10690. doi:10.1371/journal.pone.0010690
59. Cheliout Da Silva S, Yan L, Dang HV, et al. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses*. Mar 22 2021;13(3)doi:10.3390/v13030517
60. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo LD, Jr. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. *Expert Opin Drug Deliv*. Feb 2021;18(2):151-167. doi:10.1080/17425247.2021.1823964
61. Andrianov AK, DeCollibus DP, Gillis HA, et al. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A*. Nov 10 2009;106(45):18936-41. doi:10.1073/pnas.0908842106
62. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med*. Jun 25 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929
63. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med*. Oct 19 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901
64. Pettitt J, Zeitlin L, Kim do H, et al. Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. *Sci Transl Med*. Aug 21 2013;5(199):199ra113. doi:10.1126/scitranslmed.3006608
65. Qiu X, Wong G, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature*. Oct 2 2014;514(7520):47-53. doi:10.1038/nature13777
66. Mire CE, Geisbert JB, Borisevich V, et al. Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci Transl Med*. Apr 5 2017;9(384)doi:10.1126/scitranslmed.aai8711
67. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat Med*. Oct 2017;23(10):1146-1149. doi:10.1038/nm.4396

68. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. *mAbs*. Sep-Oct 2011;3(5):422-30. doi:10.4161/mabs.3.5.16983

69. Robbie GJ, Criste R, Dall'acqua WF, et al. A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study. *Antimicrobial agents and chemotherapy*. Sep 30 2013;doi:10.1128/aac.01285-13

70. Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. *PLoS Med*. Jan 2018;15(1):e1002493. doi:10.1371/journal.pmed.1002493

71. Mastraccio KE, Huaman C, Laing ED, Broder CC, Schaefer BC. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. *Methods Mol Biol*. 2022;2524:369-394. doi:10.1007/978-1-0716-2453-1_30

72. Rima B, Balkema-Buschmann A, Dundon WG, et al. ICTV Virus Taxonomy Profile: Paramyxoviridae. *J Gen Virol*. Dec 2019;100(12):1593-1594. doi:10.1099/jgv.0.001328

73. Madera S, Kistler A, Ranaivoson HC, et al. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol*. Sep 28 2022;96(18):e0092122. doi:10.1128/jvi.00921-22

74. Vanmechelen B, Meurs S, Horemans M, et al. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol*. 2022;8(2):veac061. doi:10.1093/ve/veac061

75. Voigt K, Hoffmann M, Drexler JF, et al. Fusogenicity of the Ghana Virus (Henipavirus: Ghanaian bat henipavirus) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. *Viruses*. Aug 29 2019;11(9)doi:10.3390/v11090800

76. Childs K, Randall R, Goodbourn S. Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction. *J Virol*. Apr 2012;86(7):3411-21. doi:10.1128/jvi.06405-11

77. Davis ME, Wang MK, Rennick LJ, et al. Antagonism of the phosphatase PP1 by the measles virus V protein is required for innate immune escape of MDA5. *Cell Host Microbe*. Jul 9 2014;16(1):19-30. doi:10.1016/j.chom.2014.06.007

78. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol*. Nov 2002;76(22):11476-83. doi:10.1128/jvi.76.22.11476-11483.2002

79. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol*. May 2005;79(10):6078-88. doi:10.1128/jvi.79.10.6078-6088.2005

80. Basler CF. Nipah and hendra virus interactions with the innate immune system. *Curr Top Microbiol Immunol*. 2012;359:123-52. doi:10.1007/82_2012_209

81. Yoneda M, Guillaume V, Sato H, et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One*. Sep 15 2010;5(9):e12709. doi:10.1371/journal.pone.0012709

82. Satterfield BA, Cross RW, Fenton KA, et al. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J Virol*. Jul 15 2016;90(14):6326-6343. doi:10.1128/jvi.00215-16

83. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog*. 2012;8(8):e1002836. doi:10.1371/journal.ppat.1002836

84. Amaya M, Cheng H, Borisevich V, et al. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research*. Sep 2021;193:105084. doi:10.1016/j.antiviral.2021.105084

85. CDC. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. . Updated June 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

86. Organization WH. Lassa fever fact sheet. *World Health Organization*. 2017;

87. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. *J Travel Med*. Jul 14 2020;27(4)doi:10.1093/jtm/taaa035

88. Purushotham J, Lambe T, Gilbert SC. Vaccine platforms for the prevention of Lassa fever. *Immunol Lett*. Nov 2019;215:1-11. doi:10.1016/j.imlet.2019.03.008

89. Sewlall NH, Richards G, Duse A, et al. Clinical features and patient management of Lujo hemorrhagic fever. *PLoS Negl Trop Dis*. 2014;8(11):e3233. doi:10.1371/journal.pntd.0003233

90. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. *Vaccines*. 2023;11(3):635.

91. Koma T, Patterson M, Huang C, et al. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. *J Virol*. Feb 1 2016;90(3):1290-7. doi:10.1128/jvi.02615-15

92. Jahrling P TR, Barrero O. . Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 1988:53.

93. Delgado S, Erickson BR, Agudo R, et al. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. *PLOS Pathogens*. 2008;4(4):e1000047. doi:10.1371/journal.ppat.1000047

94. Loayza Mafayle R, Morales-Betoulle ME, Romero C, et al. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. *New England Journal of Medicine*. 2022;386(24):2283-2294. doi:10.1056/NEJMoa2110339

95. Tipih T, Burt FJ. Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development. *Biores Open Access*. 2020;9(1):137-150. doi:10.1089/biores.2019.0057

96. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease. 2023. Accessed 03/06, 2023. https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease

97. Kalunda M, Mukwaya LG, Mukuye A, et al. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. *The American Journal of Tropical Medicine and Hygiene*. 01 Mar. 1986 1986;35(2):387-392. doi:10.4269/ajtmh.1986.35.387

98. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. *Emerg Infect Dis*. Dec 2020;26(12):2944-2950. doi:10.3201/eid2612.202411

99. Mulangu S, Dodd LE, Davey RT, Jr., et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. *N Engl J Med*. Dec 12 2019;381(24):2293-2303. doi:10.1056/NEJMoa1910993

100. Group PIW, Multi-National PIIST, Davey RT, Jr., et al. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. *N Engl J Med*. Oct 13 2016;375(15):1448-1456. doi:10.1056/NEJMoa1604330

101. Politch JA, Cu-Uvin S, Moench TR, et al. Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial. *PLoS Med*. Feb 2021;18(2):e1003495. doi:10.1371/journal.pmed.1003495

CENTER OVERVIEW: *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction. Emerging and periodically reemerging pathogenic enveloped RNA viruses are continuous pandemic threats. Among viral taxon associated with human zoonoses, members of the family ***Paramyxoviridae*** and order ***Bunyavirales*** stand out for their capacity to cause debilitating and life-threatening disease**.** Humans can become infected via excreta from animal reservoir hosts such as rodents or bats, through intermediate amplifying hosts such as small or domestic animals, or by arthropod vectors such as mosquitos and ticks[1-4]. Humans often represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur. During this process, RNA viral pathogens may mutate and adapt to more efficiently replicate and shed in the host, posing an epidemic threat[5-8]. The viral order *Bunyavirales* is composed of several viral families containing high priority human pathogens[9]. Notably, the ***Arenaviridae*** and ***Nairoviridae*** families contain viruses that cause severe hemorrhagic fever diseases in humans globally with mortality rates up to 60%[10] and significant, long-term sequelae in survivors[11]. Many bunyaviruses are included on the World Health Organization's (WHO) List of Priority Pathogens[12,13]. Six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHAPV, GTOV, JUNV, MACV) and one nairovirus, Crimean-Congo Hemorrhagic Fever Virus (CCHFV), are also identified as NIAID Category A pathogens[14] due to their 1) high associated morbidity and mortality rates, 2) ease of dissemination and/or ability to transmit from person-to-person, 3) potential to cause public panic and social disruption, and 4) requirement for special actions in the context of public health preparedness. The ***Paramyxoviridae*** also includes members in the WHO Priority Pathogens, namely Nipah virus (NiV) and Hendra virus (HeV). These henipaviruses (HNVs) are the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease[15]. The genus now includes nine additional reported HNVs, but only HeV and NiV are associated with severe and often fatal henipaviral disease in humans and a number of animal species[16]. Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illness in humans[17].

These collective factors plus the absence of approved vaccines or therapeutics make bunyaviruses and paramyxoviruses a significant threat to global public health. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for developing vaccine and monoclonal antibody (mAb) countermeasures to prototype bunyavirus and paramyxovirus pathogens (**Table 1**). In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old World) Machupo (New World)	Lujo (Old World) Chapare (New World)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Table 1. PABVAX selected prototype and test case viral agents

Arenaviridae*:* The *Arenaviridae* is composed of five genera: *Mammarenavirus*, *Reptarenavirus*, *Innmovirus*, *Antennavirus*, and *Hartmanivirus*[18]. The genus *Mammarenavirus* contains a large number of rodent-borne viruses, some of which cause severe hemorrhagic fever, neurological damage, death, and permanent sequelae; hearing loss, vision impairment, and balance issues in survivors[19]. While these genera exhibit a global distribution, two geographically distinct serogroups exist that diverged[20]. Old World complex mammarenaviruses (OWAV) in the Eastern Hemisphere include LASV, Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered LUJV; New World complex mammarenaviruses (NWAV) in the Western Hemisphere include JUNV, MACV, Sabia Virus (SABV), and CHAPV. There are no FDA approved vaccines or therapeutics for mammarenaviruses, though the attenuated JUNV "Candid-1" vaccine is approved for use in Argentina.

Disease severity and case fatality rates (CFR) vary across mammarenaviruses even within the same clades and species. Up to 500,000 cases of LASV infection occur per year with CFRs sometimes approaching 70% in hospitalized cases[21]. Before the introduction of the Candid-1 vaccine, it is believed that an upwards of 60,000 cases of Argentine Hemorrhagic Fever (AHF) from infection with JUNV occurred per year with CFRs ranging from 15-30%. Convalescent plasma from JUNV survivors is still the primary means to treat acute AHF in Argentina[22]. Over 1000 cases were reported for the initial outbreak of MACV with CFRs ranging from 5-30%; enhanced rodent abatement practices and potentially cross-protective immunity in populations that have taken the JUNV Candid-1 vaccine may have reduced recent outbreak frequency[23]. All mammarenaviruses share a common genomic organization consisting of two ambisense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix) and L protein (polymerase), and the glycoprotein precursor (GPC) and nucleoprotein (NP)[19]. Despite general similarity of their genomic architecture, mammarenaviruses exhibit marked differences in their replication life cycles including attachment factors, entry mechanisms, and immune evasion; nonetheless, the dominant antigens driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Despite the abundant genetic diversity of LASV, high levels of cross reactivity have been seen with antigen-based diagnostics across seven known lineages, suggesting the generation of cross-protective immunogens and antibodies against mammarenaviruses is achievable[24]. Monoclonal antibodies derived from human LASV survivors were shown to protect non-human primates (NHP) against lethal challenge[25]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[26]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, indicating this domain could serve as an antigenic target. Investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccines to test this approach and have demonstrated protection against lethal LASV-Josiah challenge. Specifically, a stabilized prefusion trimeric GPe, an immunogen containing a highly conserved NP NTD epitope, or a combination of these two approaches conferred protection in a lethal outbred guinea pig model. Nevertheless, even with the considerable molecular diversity in LASV strains, conservation at key antigenic sites within the GP and NP suggest a pan-LASV vaccine may be possible[24,27,28]. **RP1** and **Core E** investigators have demonstrated that a Vesicular stomatitis virus (rVSV)-based vaccine expressing the LASV-Josiah GPC is protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[29], and against heterologous challenge with Lineage II[30], III, V[31], and VI viruses (Cross and Geisbert Unpublished).

In **Phase I of RP1**, the ability of an optimized LASV GPe/NP subunit vaccine delivered intramuscularly (IM) will be assessed in guinea pig and NHP models across multiple viral lineages. We will also assess a microneedle skin patch (MNP) formulation optimized in **Core C** to determine if a needle-free vaccination route affords more potent vaccine-mediated responses and protection. Similarly, we have generated MACV GPe immunogens and will design a MACV NP-NTD subunit antigen to test the same approach across multiple MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing both OWAV (LUJV) and NWAV (CHAPV) complexes, for which there are no vaccines available. A unique advantage of the PABVAX Center is its access to biosafety level (BSL)-4 containment, allowing us to work with authentic versus surrogate arenaviruses. The BSL4 facilities described in **Core E** are capable of evaluating countermeasures in small animal models and NHPs. The cynomolgus macaque is the current "gold standard" animal model for LASV, JUNV, and MACV research as disease recapitulates human infection and there are a multitude of immunological reagents available[32-34]. The evaluation of prototype OWAV and NWAV vaccines and therapies in the NHP model is likely pivotal for IND applications and for future licensure of medical countermeasures for human use.

__Nairoviridae:__ Unique to most bunyaviruses, orthonairoviruses are transmitted primarily by ticks[2]. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[35], Issyk-Kul virus[36], Songling virus[37]), CCHFV is the most recognized public health threat. Outbreaks of CCHFV have occurred across Europe, Africa, the Middle East, and Asia. There is growing concern for expansion of the CCHFV endemic region due to increased range of its tick host (*Hyalomma* spp.) tied to bird migration changes, livestock trade, and global warming. For example, CCHFV cases have been reported in Spain[38] , a previously non-endemic region, and *Hyalomma* ticks have been found in Sweden[39]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to a severe hemorrhagic fever and death in 30% of cases[40]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[41]. Ribavirin has been used to treat infections with limited success[42].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and GPC [40]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[40]. Conversely, the CCHFV GPC is considerably less conserved (< 75% amino acid identity)[40]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location. Thus, creating a pan-CCHFV vaccine or therapeutic based solely on one clade poses a challenge. Nevertheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a rVSV vectored vaccine expressing the full GPC of CCHFV[43].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP immunogens with the highest level of homology across clades. We will assess their capacity to protect against lethal challenge with isolates from multiple CCHFV clades in established mouse[43,44] and NHP[45] models with **Core E**. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best formulation of CCHFV GP/NP MNP vaccine against the emerging test case nairovirus, Kasokero virus, for which there are no vaccines available.

<u>Paramyxoviridae:</u> The HNVs NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality[4,46]. HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality[47]. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs, which served as amplifying hosts[48]. NiV and HeV can infect at least 18 animal species across 6 orders of mammals, and among these they cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans[16,49]. The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission[5]. Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and Southeast Asia and Australia. Their broad host tropism and ability to infect livestock also causes increased risk of human infections. HNVs are single-stranded, negative sense, enveloped RNA viruses and their biology lends themselves to be synthetically produced. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS). The Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents[50] because the virus could be engineered for mass dissemination and it possesses the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the R&D Blueprint list of priority pathogens with epidemic potential[51].

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb exist. These postexposure treatments conferred protecion against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP)[52-55]. The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia[56]. In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs, and several vaccine platforms have clear potential for use in people[16]. The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies[16]. Such preventive vaccines would have utility for lab workers, first responders or individuals at high-risk exposure, and in mitigating the spread of an outbreak. In the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent[57]. It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection[49,58]. The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished)[59]. The divergent LayV will be the focus of the test case studies. The choice is justifiable for several important reasons: 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; and 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including rescuing recombinant LayV (rLayV), exploring animal model development, constructing additional LayV and AngV reporter virus tools, and then translating and testing the MNP vaccine 'plug-and-play' approach. Finally, we will evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME. A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center's RPs and Core leaders currently have active and productive collaborative research programs in related areas, most spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

The pandemic preparedness research gaps addressed by PABVAX are diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **arenaviruses** and **nairoviruses**, and **RP2** will target the prototype **HNVs**. Both RP1 and RP2 will develop new prototype virus tools (recombinant prototype viruses) along with existing tools, to analyze vaccine responses and evaluate new mAbs and nabs from the other RPs. A third RP will develop a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant *Cedar henipavirus* (rCedV) in vivo and a bioluminescence detection method that can longitudinally trace the dynamics and anatomical progression of infection with a luciferase-encoding virus in individual animals (**RP3**). There will also be two RPs that focus on the development of broadly reactive human mAbs and nanobodies to the PABVAX selected viral prototypes. **RP4** will isolate human mAbs derived from NiV-infected survivors with a specific focus on F glycoprotein-targeting mAbs. Additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will engage in the manufacture, quality control, and testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**). Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core (**Core A**) will provide organizational and programmatic structure

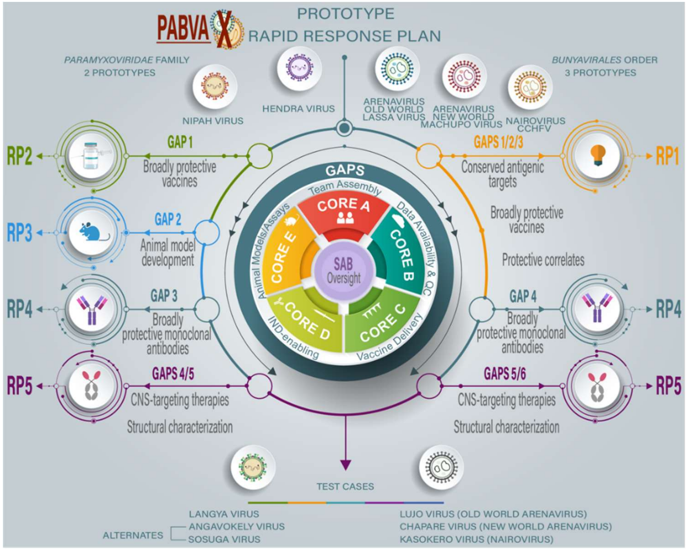

Figure 1: Research gaps addressed by the *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center- (PABVAX).

to promote research productivity and scientific interactions along with compliance with regulations concerning select agent, vertebrate animal, and human subject use. **Core A** will coordinate all activities with the Scientific Advisory Board (**SAB**) and make data available through the Data Management Core (**Core B**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform. The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**). This project tested a dissolvable MNP-based vaccine technology ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine[60]. The MNP vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable microneedles that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants[61] to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as to facilitate improved vaccine storage and distribution due to their improved thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, Scientific **Core C** (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ)-adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (*Paramyxoviridae*). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro*, along with analysis of polyfunctional T-cell responses by antigenic peptide stimulation, followed by intracellular cytokine staining (ICS) and flow cytometry.

Discover effective antibody-based prophylactics and therapies. Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah[62], Hendra[63], Ebola[64,65], Marburg[66] and Lassa[67]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc*.), a feature to be exploited in **Core D** by Dr. Gunn. In addition to therapeutic applications, mAbs with Fc mutations dramatically extend serum half-life[68-70] and offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g*. rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization, and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures. Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal Model Development and Preclinical Evaluation Scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective efficacy of antivirals and vaccines, as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation, including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they are generally less laborious and can be adapted to higher throughput platforms and/or integrated with other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput fluorescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic NiV and HeV vaccine-induced immune sera, mAbs (**RP4**), nbs (**RP5**), and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either HeV or NiV-B envelope GPs) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals to explore nab based therapeutics to CNS resident HNVs[71].

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the HNVs (*Paramyxoviridae*) and the *Bunyavirales* (*Arenaviridae* and *Nairoviridae*) that will bolster vaccine endurance to storage and transportation; we also anticipate improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology, and mAb and vaccine translational drug development along with Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Paramyxoviruses: NiV and HeV are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis[4,46]. HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals. These viruses are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of CedV, Gamak virus, Daeryong virus, and (LayV)[72] and five additional species known only from nucleic acid sequence information;

Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[73,74]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV, are old world pteropid fruit bats[73,75]. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province)[17]. The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**. Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal HNV disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters[49], and AGMs (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P). The P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists[76-80]. The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections[81,82]. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature[83]. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2[84] and CedV is now recognized as a BSL-2 restricted agent[85]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D,** and **E**).

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

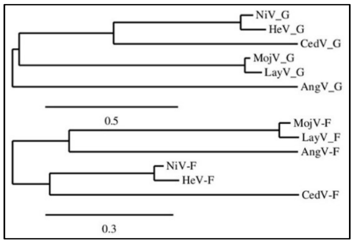

Figure 2: Phylogenetic analysis of select HNV G and F glycoproteins. Trees were constructed for using the PhyML program's maximum likelihood method and aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Arenaviruses (old world): For **phase 1**, **RP1** will utilize **LASV as the prototype virus** due its significant public health burden throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[86]. LASV is the most imported viral hemorrhagic fever (VHF) next to Dengue virus and has been documented in several countries outside of Africa over 35 times[87]. CFRs approaching 70% in hospitalized cases and marked long term sequelae are the result of the lack of approved vaccines or therapeutics, although several live attenuated virus candidates are currently being evaluated with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI)[88]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize only LASV GP as the target immunogen.

Investigators in **RP1**, **Core D**, and **Core E** have already demonstrated protective efficacy against challenge with LASV in outbred guinea pigs vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These immunogens have been engineered to express homologous antigenic regions to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach suggests a similar strategy could be implemented against other arenaviruses. The **OWAV test pathogen, LUJV,** was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia, which quickly spread nosocomially[89]. The CFR for this infectious virus was 80% in this small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from suggest current LASV countermeasures may be of limited use. LUJV will serve as an ideal "test case" to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Arenaviruses (new world): The live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[90], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[91,92]. Furthermore, there is the potential for pathogenic reversion. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest exploring other JUNV countermeasures are warranted.

Building on the success of the LASV GPe/NP-NTD, investigators in **RP1 and Cores D and E** will engineer broadly protective MACV GPe and NP-NTD antigens and perform efficacy testing in established guinea pig and NHP models. We will extend this plug-and-play approach to the **NWAV test case, CHAPV**. First reported in 2003 in Bolivia, CHAPV is a rodent-borne mammarenavirus associated with the development of severe hemorrhagic fever with case fatality rates up to 60%[93,94]. Recent studies have demonstrated clear human-to-human transmission during the acute phase evidenced by several nosocomial infections; isolation of viable virus from semen at 86 days after symptom onset demonstrates a clear need to implement specialized management and treatment [94]. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Nairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as it represents the largest global public health burden within the orthonairovirus family with outbreaks annually across Europe, Africa, and Asia. There are no FDA-approved vaccines for CCHFV; however, a suckling mouse brain-derived, adjuvanted whole virus vaccine for CCHFV has been approved for use in Bulgaria. This vaccine has numerous safety concerns and has limited demonstrated efficacy. Several experimental vaccine approaches have been evaluated ranging from viral vectored approaches to DNA-based vaccines with varying efficacy, advantages, and disadvantages[95]; however, only a Modified Vaccinia Ankara virus vaccine (MVA-CCHFV) has begun to advance towards clinical trials[96]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test these for protective efficacy from challenge with 6 different CCHFV lineages using established mouse and NHP models. Next, we will deploy the same approach to the **test case nairovirus, Kasokero virus (KASV),** for which no vaccine exists.

In 1977, KASV was first isolated from Ugandan bats and caused several cases of acute febrile illness of laboratory workers from the same lab that processed the infected bats[97]. Surveys of 109 laboratory workers from the same facility demonstrated 13% seropositivity and clinical symptoms in individuals 4 to 15 months after the virus was isolated. These findings suggest either continued laboratory-associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the tick reservoir, *Ornithodoros faini,* to participate in enzootic maintenance of KASV[98]. While most chiropteran ticks exhibit host specificity, humans entering caves these bats inhabit have reported bites from *O.faini* ticks. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics suggest investment in countermeasure development against this virus is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**; arenavirus and nairovirus projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine active and passive immunization approaches to further improve the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

An additional and outstanding advantage of the PABVAX Center is the **Animal Model Development and Preclinical Evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for members of the *Bunyavirales* and *Paramyxoviridae*.**
2. **Develop new tools and animal models for cell-based and *in vivo* therapeutic evaluation.**
3. **Develop antibodies and nanobodies to the selected prototype and test viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

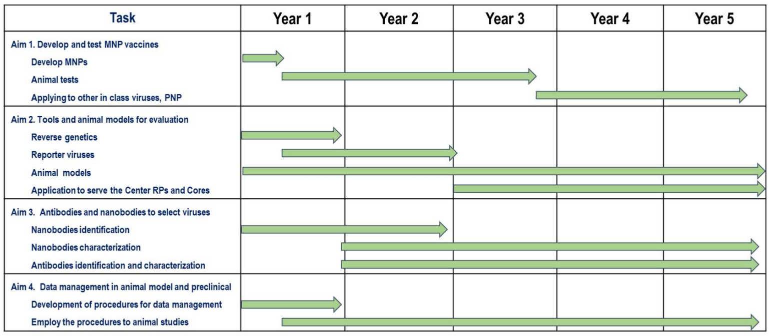

Figure 3: Projected PABVAX timeline to achieve the 4 overarching tasks.

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**. Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (details are specified within the Administration **Core A** Research Strategy). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommending new directions as appropriate, and providing Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

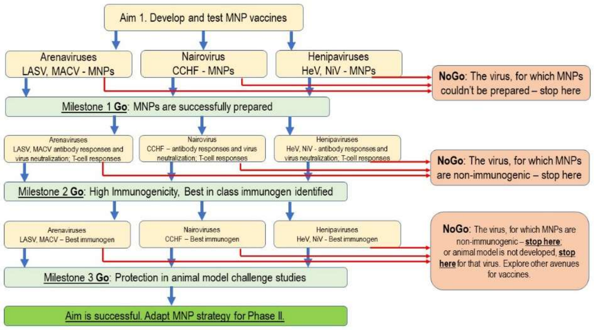

Figure 4. Milestones and criteria for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will address whether formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be established using immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen-specific antibody induction, virus neutralization, and polyfunctional T-cell responses by *in vitro* stimulation of isolated cells). Go/no go decisions will be made based on outcomes of these first-tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as a success. Comparisons will also be made such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of the best-in-class immunogens, or immunogen combinations, will also be explored to down-select vaccines. The third milestone will determine whether MNP vaccination affords protection in animal model challenge experiments. In the case of NiV and HeV prototypes, the animal models chosen (ferrets and AGMs) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales*, there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASV and guinea pig models exist for some but not all of these lineages. For MACV, there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is the limitations of cost and availability of rhesus and cynomolgus monkeys. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be developing and evaluating animal models within the PABVAX program, including further development of AGM models for arenaviruses and possibly CCHFV as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for cell-based and in vivo therapeutics evaluation.

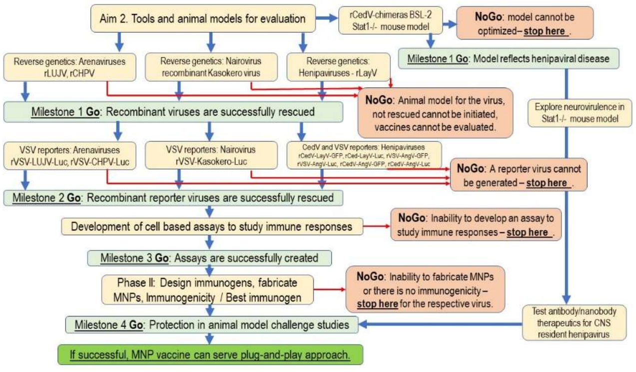

Figure 5. Milestones and criteria for **Aim 2** of the PABVAX Center's overall objectives.

The second overall objective of PABVAX is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed because genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If successful, a large battery of new materials will be available to share with the ReVAMPP network, and be key milestones for execution of Phase II of the PABVAX Center program.

The translation of the MNP vaccine plug-and-play approach will be in Phase II (**Table 1**). Milestones will be similar to those in **Figure 4**. New virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new *in vivo* bioluminescence-based immune deficient mouse model developed by the PIs of **PR2** and **RP3** that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease *in vivo* safely and expediently at BSL-2 containment. The model will be deployed to test a critically important therapeutic need against CNS resident HNV infection, and **RP3** will collaborate with **RP5** in the testing of engineered nabs.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

New human mAbs and nbs as tools and potential therapeutics will be developed by **RP4** and **RP5** in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs, and in collaboration with **RP2**, neutralization potencies will be rapidly assessed (as well as evaluating synergistic cell-based mAb neutralization) that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

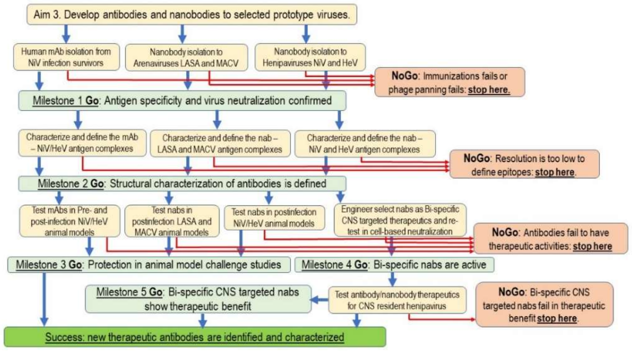

Figure 5. Milestones and Criteria for **Aim 3** of the PABVAX Center's overall objectives.

Nbs, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nbs can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nbs targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus), and select animal models of LASV and MACV by **Core E**. Success will be measured by the 1) identification of potent virus-neutralizing nbs, 2) discovery of more broadly reactive nbs particularly to the multiple LASV clades, 3) evidence of therapeutic benefit in animal models, and 4) the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

Industry Partnerships*. Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner that will provide access to vaccine expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.*

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience in developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX. Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

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SPECIAL FEATURES OF THE PABVAX

Galveston National Laboratory (GNL). The GNL is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics, and therapeutics. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370 NHP studies employing some 2,772 animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses have been performed. These studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder, and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP facility and a staff of full-time veterinarians. **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of the WRCEVA virus collection within the GNL.

***Core E (Animal model development and preclinical evaluation)*. Core E** operations will be conducted at the GNL, and in conjunction with **Core A**, will also include the UTMB Institutional Office of Regulated Nonclinical Studies (ORNcS). The ORNcS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL, and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses, arenaviruses, and henipaviruses. He has published over 250 peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI), was the first to develop a NHP model of NiV/HeV infection and disease, and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs). Mapp Biopharmaceutical has deep experience with antigens and mAbs against these antigens. Their early development and research group has expertise in immunology, infectious diseases, and the production of antigens, antibodies, and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [99] [100]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-

Ebolavirus therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1[101]. Dr. Gunn and Genovac will join the team to analyze antibody Fc effector functions and to provide antigen/antibody discovery resources, respectively.

As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

1. Lamb RA, Parks GD. Paramyxoviridae: the viruses and their replication. In Fields BN, Knipe DM, Howley PM, editors, Fields Virology: Sixth Edition. Vol. 1. Lippincott, Williams, and Wilkins. 2013. p. 957-995.
2. Boshra H. An Overview of the Infectious Cycle of Bunyaviruses. *Viruses*. Sep 28 2022;14(10)doi:10.3390/v14102139
3. Horne KM, Vanlandingham DL. Bunyavirus-vector interactions. *Viruses*. Nov 13 2014;6(11):4373-97. doi:10.3390/v6114373
4. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia), p 1070-1085.
5. Luby SP. The pandemic potential of Nipah virus. *Antiviral research*. Oct 2013;100(1):38-43. doi:10.1016/j.antiviral.2013.07.011
6. Skowron K, Bauza-Kaszewska J, Grudlewska-Buda K, et al. Nipah Virus-Another Threat From the World of Zoonotic Viruses. *Front Microbiol*. 2021;12:811157. doi:10.3389/fmicb.2021.811157
7. Kummer S, Kranz DC. Henipaviruses-A constant threat to livestock and humans. *PLoS Negl Trop Dis*. Feb 2022;16(2):e0010157. doi:10.1371/journal.pntd.0010157
8. Gouglas D, Christodoulou M, Plotkin SA, Hatchett R. CEPI: Driving Progress Toward Epidemic Preparedness and Response. *Epidemiol Rev*. Jan 31 2019;41(1):28-33. doi:10.1093/epirev/mxz012
9. Maes P, Adkins S, Alkhovsky SV, et al. Taxonomy of the order Bunyavirales: second update 2018. *Arch Virol*. Mar 2019;164(3):927-941. doi:10.1007/s00705-018-04127-3
10. Silva-Ramos CR, Montoya-Ruíz C, Faccini-Martínez Á A, Rodas JD. An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever. *Arch Virol*. Sep 2022;167(9):1727-1738. doi:10.1007/s00705-022-05453-3
11. Garry RF. Lassa fever - the road ahead. *Nat Rev Microbiol*. Feb 2023;21(2):87-96. doi:10.1038/s41579-022-00789-8
12. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research*. Nov 2018;159:63-67. doi:10.1016/j.antiviral.2018.09.009
13. WHO. List of Blueprint Priority Diseases. Accessed November 14, 2018. http://www.who.int/blueprint/priority-diseases/en/
14. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens. Accessed 04 Jun 2023, https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens

15. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol*. Jan 2006;4(1):23-35. doi:10.1038/nrmicro1323
16. Amaya M, Broder CC. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol*. Sep 29 2020;7(1):447-473. doi:10.1146/annurev-virology-021920-113833
17. Zhang XA, Li H, Jiang FC, et al. A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med*. Aug 4 2022;387(5):470-472. doi:10.1056/NEJMc2202705
18. Radoshitzky SR, Buchmeier MJ, Charrel RN, et al. ICTV Virus Taxonomy Profile: Arenaviridae. *J Gen Virol*. Aug 2019;100(8):1200-1201. doi:10.1099/jgv.0.001280
19. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. *Front Microbiol*. 2018;9:1751. doi:10.3389/fmicb.2018.01751
20. Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient Evolution of Mammarenaviruses: Adaptation via Changes in the L Protein and No Evidence for Host-Virus Codivergence. *Genome Biol Evol*. 2018/03// 2018;10(3):863-874. doi:10.1093/gbe/evy050
21. Shaffer JG, Grant DS, Schieffelin JS, et al. Lassa Fever in Post-Conflict Sierra Leone. *PLOS Neglected Tropical Diseases*. 2014;8(3):e2748. doi:10.1371/journal.pntd.0002748
22. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Review. *Antiviral research*. Apr 2008;78(1):132-9. doi:10.1016/j.antiviral.2007.10.010
23. Kilgore PE, Peters CJ, Mills JN, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. Jul-Sep 1995;1(3):97-100. doi:10.3201/eid0103.950308
24. Heinrich ML, Boisen ML, Nelson DKS, et al. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. *Scientific Reports*. 2020/09/29 2020;10(1):16030. doi:10.1038/s41598-020-72539-w
25. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nature Medicine*. 2017/10/01 2017;23(10):1146-1149. doi:10.1038/nm.4396
26. Hastie KM, Zandonatti MA, Kleinfelter LM, et al. Structural basis for antibody-mediated neutralization of Lassa virus. *Science*. Jun 2 2017;356(6341):923-928. doi:10.1126/science.aam7260
27. Sullivan BM, Sakabe S, Hartnett JN, et al. High crossreactivity of human T cell responses between Lassa virus lineages. *PLoS Pathog*. Mar 2020;16(3):e1008352. doi:10.1371/journal.ppat.1008352
28. Ugwu C, Olumade T, Nwakpakpa E, et al. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. *Scientific Reports*. 2022/12/25 2022;12(1):22330. doi:10.1038/s41598-022-26045-w
29. Geisbert TW, Jones S, Fritz EA, et al. Development of a New Vaccine for the Prevention of Lassa Fever. *PLOS Medicine*. 2005;2(6):e183. doi:10.1371/journal.pmed.0020183
30. Cross RW, Woolsey C, Prasad AN, et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep*. Jul 19 2022;40(3):111094. doi:10.1016/j.celrep.2022.111094
31. Safronetz D, Mire C, Rosenke K, et al. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. *PLOS Neglected Tropical Diseases*. 2015;9(4):e0003736. doi:10.1371/journal.pntd.0003736
32. Hensley LE, Smith MA, Geisbert JB, et al. Pathogenesis of Lassa fever in cynomolgus macaques. *Virol J*. May 6 2011;8:205. doi:10.1186/1743-422x-8-205
33. Zeitlin L, Cross RW, Geisbert JB, et al. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. *Proceedings of the National Academy of Sciences*. 2021;118(11):e2023332118. doi:doi:10.1073/pnas.2023332118
34. Bell TM, Shaia CI, Bunton TE, et al. Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure. *Veterinary Pathology*. 2015;52(1):26-37. doi:10.1177/0300985814540544
35. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. *Epidemiol Infect*. Jun 1996;116(3):353-61. doi:10.1017/s0950268800052687
36. Brinkmann A, Kohl C, Radonić A, et al. First detection of bat-borne Issyk-Kul virus in Europe. *Sci Rep*. Dec 24 2020;10(1):22384. doi:10.1038/s41598-020-79468-8
37. Ma J, Lv XL, Zhang X, et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat Med*. Mar 2021;27(3):434-439. doi:10.1038/s41591-020-01228-y

38. Lorenzo Juanes HM, Carbonell C, Sendra BF, et al. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. *Emerg Infect Dis*. Feb 2023;29(2):252-259. doi:10.3201/eid2902.220677

39. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, et al. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. *Ticks Tick Borne Dis*. May 2020;11(3):101403. doi:10.1016/j.ttbdis.2020.101403

40. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology*. 2023/03/14 2023;doi:10.1038/s41579-023-00871-9

41. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral research*. May 2011;90(2):85-92. doi:10.1016/j.antiviral.2011.02.010

42. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. *BMC Infectious Diseases*. 2010/07/13 2010;10(1):207. doi:10.1186/1471-2334-10-207

43. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep*. May 23 2019;9(1):7755. doi:10.1038/s41598-019-44210-6

44. Bente DA, Alimonti JB, Shieh WJ, et al. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol*. Nov 2010;84(21):11089-100. doi:10.1128/jvi.01383-10

45. Cross RW, Prasad AN, Borisevich V, et al. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. *PLoS Negl Trop Dis*. Aug 2020;14(8):e0008637. doi:10.1371/journal.pntd.0008637

46. Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York, p 519-536.

47. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science*. Apr 7 1995;268(5207):94-7. doi:10.1126/science.7701348

48. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science (New York, NY)*. 2000/05// 2000;288(5470):1432-1435. doi:10.1126/science.288.5470.1432

49. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol*. 2012;359:153-77. doi:10.1007/82_2012_208

50. *Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087*.

51. Sweileh WM. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health*. Feb 8 2017;13(1):9. doi:10.1186/s12992-017-0233-9

52. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Science translational medicine*. 2011/10// 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901

53. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine*. 2014/06// 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929

54. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep*. Aug 3 2016;6:30916. doi:10.1038/srep30916

55. Mire CE, Chan YP, Borisevich V, et al. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis*. May 11 2020;221(Suppl 4):S471-s479. doi:10.1093/infdis/jiz515

56. Playford EG, Munro T, Mahler SM, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*. Apr 2020;20(4):445-454. doi:10.1016/s1473-3099(19)30634-6

57. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerging infectious diseases*. 2014/03// 2014;20(3):372-379. doi:10.3201/eid2003.131159

58. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One*. May 18 2010;5(5):e10690. doi:10.1371/journal.pone.0010690

59. Cheliout Da Silva S, Yan L, Dang HV, et al. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses*. Mar 22 2021;13(3)doi:10.3390/v13030517

60. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo LD, Jr. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. *Expert Opin Drug Deliv*. Feb 2021;18(2):151-167. doi:10.1080/17425247.2021.1823964

61. Andrianov AK, DeCollibus DP, Gillis HA, et al. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A*. Nov 10 2009;106(45):18936-41. doi:10.1073/pnas.0908842106

62. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med*. Jun 25 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929

63. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med*. Oct 19 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901

64. Pettitt J, Zeitlin L, Kim do H, et al. Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. *Sci Transl Med*. Aug 21 2013;5(199):199ra113. doi:10.1126/scitranslmed.3006608

65. Qiu X, Wong G, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature*. Oct 2 2014;514(7520):47-53. doi:10.1038/nature13777

66. Mire CE, Geisbert JB, Borisevich V, et al. Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci Transl Med*. Apr 5 2017;9(384)doi:10.1126/scitranslmed.aai8711

67. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat Med*. Oct 2017;23(10):1146-1149. doi:10.1038/nm.4396

68. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. *mAbs*. Sep-Oct 2011;3(5):422-30. doi:10.4161/mabs.3.5.16983

69. Robbie GJ, Criste R, Dall'acqua WF, et al. A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study. *Antimicrobial agents and chemotherapy*. Sep 30 2013;doi:10.1128/aac.01285-13

70. Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. *PLoS Med*. Jan 2018;15(1):e1002493. doi:10.1371/journal.pmed.1002493

71. Mastraccio KE, Huaman C, Laing ED, Broder CC, Schaefer BC. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. *Methods Mol Biol*. 2022;2524:369-394. doi:10.1007/978-1-0716-2453-1_30

72. Rima B, Balkema-Buschmann A, Dundon WG, et al. ICTV Virus Taxonomy Profile: Paramyxoviridae. *J Gen Virol*. Dec 2019;100(12):1593-1594. doi:10.1099/jgv.0.001328

73. Madera S, Kistler A, Ranaivoson HC, et al. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol*. Sep 28 2022;96(18):e0092122. doi:10.1128/jvi.00921-22

74. Vanmechelen B, Meurs S, Horemans M, et al. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol*. 2022;8(2):veac061. doi:10.1093/ve/veac061

75. Voigt K, Hoffmann M, Drexler JF, et al. Fusogenicity of the Ghana Virus (Henipavirus: Ghanaian bat henipavirus) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. *Viruses*. Aug 29 2019;11(9)doi:10.3390/v11090800

76. Childs K, Randall R, Goodbourn S. Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction. *J Virol*. Apr 2012;86(7):3411-21. doi:10.1128/jvi.06405-11

77. Davis ME, Wang MK, Rennick LJ, et al. Antagonism of the phosphatase PP1 by the measles virus V protein is required for innate immune escape of MDA5. *Cell Host Microbe*. Jul 9 2014;16(1):19-30. doi:10.1016/j.chom.2014.06.007

78. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol*. Nov 2002;76(22):11476-83. doi:10.1128/jvi.76.22.11476-11483.2002

79. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol*. May 2005;79(10):6078-88. doi:10.1128/jvi.79.10.6078-6088.2005

80. Basler CF. Nipah and hendra virus interactions with the innate immune system. *Curr Top Microbiol Immunol*. 2012;359:123-52. doi:10.1007/82_2012_209

81. Yoneda M, Guillaume V, Sato H, et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One*. Sep 15 2010;5(9):e12709. doi:10.1371/journal.pone.0012709

82. Satterfield BA, Cross RW, Fenton KA, et al. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J Virol*. Jul 15 2016;90(14):6326-6343. doi:10.1128/jvi.00215-16
83. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog*. 2012;8(8):e1002836. doi:10.1371/journal.ppat.1002836
84. Amaya M, Cheng H, Borisevich V, et al. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research*. Sep 2021;193:105084. doi:10.1016/j.antiviral.2021.105084
85. CDC. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. . Updated June 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.
86. Organization WH. Lassa fever fact sheet. *World Health Organization*. 2017;
87. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. *J Travel Med*. Jul 14 2020;27(4)doi:10.1093/jtm/taaa035
88. Purushotham J, Lambe T, Gilbert SC. Vaccine platforms for the prevention of Lassa fever. *Immunol Lett*. Nov 2019;215:1-11. doi:10.1016/j.imlet.2019.03.008
89. Sewlall NH, Richards G, Duse A, et al. Clinical features and patient management of Lujo hemorrhagic fever. *PLoS Negl Trop Dis*. 2014;8(11):e3233. doi:10.1371/journal.pntd.0003233
90. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. *Vaccines*. 2023;11(3):635.
91. Koma T, Patterson M, Huang C, et al. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. *J Virol*. Feb 1 2016;90(3):1290-7. doi:10.1128/jvi.02615-15
92. Jahrling P TR, Barrero O. . Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 1988:53.
93. Delgado S, Erickson BR, Agudo R, et al. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. *PLOS Pathogens*. 2008;4(4):e1000047. doi:10.1371/journal.ppat.1000047
94. Loayza Mafayle R, Morales-Betoulle ME, Romero C, et al. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. *New England Journal of Medicine*. 2022;386(24):2283-2294. doi:10.1056/NEJMoa2110339
95. Tipih T, Burt FJ. Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development. *Biores Open Access*. 2020;9(1):137-150. doi:10.1089/biores.2019.0057
96. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease. 2023. Accessed 03/06, 2023. https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease
97. Kalunda M, Mukwaya LG, Mukuye A, et al. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. *The American Journal of Tropical Medicine and Hygiene*. 01 Mar. 1986 1986;35(2):387-392. doi:10.4269/ajtmh.1986.35.387
98. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. *Emerg Infect Dis*. Dec 2020;26(12):2944-2950. doi:10.3201/eid2612.202411
99. Mulangu S, Dodd LE, Davey RT, Jr., et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. *N Engl J Med*. Dec 12 2019;381(24):2293-2303. doi:10.1056/NEJMoa1910993
100. Group PIW, Multi-National PIIST, Davey RT, Jr., et al. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. *N Engl J Med*. Oct 13 2016;375(15):1448-1456. doi:10.1056/NEJMoa1604330
101. Politch JA, Cu-Uvin S, Moench TR, et al. Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial. *PLoS Med*. Feb 2021;18(2):e1003495. doi:10.1371/journal.pmed.1003495

CENTER OVERVIEW: *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction. Emerging and periodically reemerging pathogenic enveloped RNA viruses are continuous pandemic threats. Among viral taxon associated with human zoonoses, members of the family ***Paramyxoviridae*** and order ***Bunyavirales*** stand out for their capacity to cause debilitating and life-threatening disease**.** Humans can become infected via excreta from animal reservoir hosts such as rodents or bats, through intermediate amplifying hosts such as small or domestic animals, or by arthropod vectors such as mosquitos and ticks[1-4]. Humans often represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur. During this process, RNA viral pathogens may mutate and adapt to more efficiently replicate and shed in the host, posing an epidemic threat[5-8]. The viral order *Bunyavirales* is composed of several viral families containing high priority human pathogens[9]. Notably, the ***Arenaviridae*** and ***Nairoviridae*** families contain viruses that cause severe hemorrhagic fever diseases in humans globally with mortality rates up to 60%[10] and significant, long-term sequelae in survivors[11]. Many bunyaviruses are included on the World Health Organization's (WHO) List of Priority Pathogens[12,13]. Six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHAPV, GTOV, JUNV, MACV) and one nairovirus, Crimean-Congo Hemorrhagic Fever Virus (CCHFV), are also identified as NIAID Category A pathogens[14] due to their 1) high associated morbidity and mortality rates, 2) ease of dissemination and/or ability to transmit from person-to-person, 3) potential to cause public panic and social disruption, and 4) requirement for special actions in the context of public health preparedness. The family ***Paramyxoviridae*** also includes members that are WHO Priority Pathogens, namely Nipah virus (NiV) and Hendra virus (HeV). These zoonotic henipaviruses (HNVs) are considered prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease[15]. The genus now includes nine additional reported HNVs, but only HeV and NiV are associated with severe and often fatal henipaviral disease in humans and a number of animal species[16]. Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illness in humans[17].

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These collective factors plus the absence of approved vaccines or therapeutics make bunyaviruses and paramyxoviruses a significant threat to global public health. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for developing vaccine and monoclonal antibody (mAb) countermeasures to prototype bunyavirus and paramyxovirus pathogens (**Table 1**). In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old World) Machupo (New World)	Lujo (Old World) Chapare (New World)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Table 1. PABVAX selected prototype and test case viral agents

Arenaviridae*:* The family *Arenaviridae* is composed of five genera: *Mammarenavirus*, *Reptarenavirus*, *Innmovirus*, *Antennavirus*, and *Hartmanivirus*[18]. The genus *Mammarenavirus* contains a large number of rodent-borne viruses, some of which cause severe hemorrhagic fever, neurological damage, death, and permanent sequelae such as hearing loss, vision impairment, and balance issues in survivors[19]. While these genera exhibit a global distribution, two geographically distinct serogroups exist that diverged ~45,000 years ago[20]. Old World complex mammarenaviruses (OWAV) in the Eastern Hemisphere include LASV, Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered LUJV; New World complex mammarenaviruses (NWAV) in the Western Hemisphere include JUNV, MACV, Sabia Virus (SABV), and CHAPV. There are currently no US FDA approved vaccines or therapeutics for mammarenaviruses, though the attenuated JUNV "Candid-1" vaccine is approved for use in Argentina.

Disease severity and case fatality rates (CFR) vary across mammarenaviruses even within the same clades and species. Up to 500,000 cases of LASV infection occur per year with CFRs sometimes approaching 70% in hospitalized cases[21]. Before the introduction of the Candid-1 vaccine, it is believed that an upwards of 60,000 cases of Argentine Hemorrhagic Fever (AHF) from infection with JUNV occurred per year with CFRs ranging from 15-30%. Convalescent plasma from JUNV survivors is still the primary means to treat acute AHF in Argentina[22]. Over 1000 cases were reported for the initial outbreak of MACV with CFRs ranging from 5-30%;

enhanced rodent abatement practices and potentially cross-protective immunity in populations that have taken the JUNV Candid-1 vaccine may have reduced recent outbreak frequency[23].

All mammarenaviruses share a common genomic organization consisting of two ambisense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix) and L protein (polymerase), and the glycoprotein precursor (GPC) and nucleoprotein (NP)[19]. Despite general similarity of their genomic architecture, mammarenaviruses exhibit marked differences in their replication life cycles including attachment factors, entry mechanisms, and immune evasion; nonetheless, the dominant antigens driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Despite the abundant genetic diversity of LASV, high levels of cross reactivity have been seen with antigen-based diagnostics across seven known lineages, suggesting the generation of cross-protective immunogens and antibodies against mammarenaviruses is achievable[24]. Monoclonal antibodies derived from human LASV survivors were shown to protect non-human primates (NHP) against lethal challenge[25]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[26]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, indicating this domain could serve as an antigenic target. Investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccines to test this approach and have demonstrated protection against lethal LASV-Josiah challenge. Specifically, a stabilized prefusion trimeric GPe, an immunogen containing a highly conserved NP NTD epitope, or a combination of these two approaches conferred protection in a lethal outbred guinea pig model.

Despite the considerable molecular diversity in LASV strains, conservation at key antigenic sites within the GP and NP suggest the feasibility of generating a pan-LASV vaccine[24,27,28]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a recombinant Vesicular stomatitis virus (rVSV)-based vector expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[29], but also protects against heterologous challenge with Lineage II[30], III (Cross, Geisbert unpublished), V[31], and VI viruses (Cross and Geisbert Unpublished).

In **Phase I of RP1**, the ability of an optimized LASV GPe/NP subunit vaccine delivered intramuscularly (IM) will be assessed in guinea pig and NHP models across multiple viral lineages. We will also assess a microneedle skin patch (MNP) formulation optimized in **Core C** to determine if a needle-free vaccination route affords more potent vaccine-mediated responses and protection. Similarly, we have generated MACV GPe immunogens and will design a MACV NP-NTD subunit antigen to test the same approach across multiple MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing both OWAV (LUJV) and NWAV (CHAPV) complexes, for which there are no vaccines available. A unique advantage of the PABVAX Center is its access to biosafety level (BSL)-4 containment, allowing us to work with authentic versus surrogate arenaviruses. The BSL4 facilities described in **Core E** are capable of evaluating countermeasures in small animal models and NHPs. The cynomolgus macaque is the current "gold standard" animal model for LASV, JUNV, and MACV research as disease recapitulates human infection and there are a multitude of immunological reagents available[32-34]. The evaluation of prototype OWAV and NWAV vaccines and therapies in the NHP model is likely pivotal for IND applications and for future licensure of medical countermeasures for human use.

<u>Nairoviridae:</u> Unique to most other bunyaviruses, orthonairoviruses are transmitted primarily by ticks[2]. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[35], Issyk-Kul virus[36], Songling virus[37]), CCHFV is by far the most recognized public health threat. Outbreaks of CCHFV have occurred across Europe, Africa, the Middle East, and Asia. There is growing concern for expansion of the CCHFV endemic region due to increased range of its tick reservoir host (*Hyalomma* spp.) tied to bird migration changes, livestock trade, and global warming. For example, CCHFV cases have been reported in Spain[38] , a previously non-endemic region, and *Hyalomma* ticks have been found as north as Sweden[39]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to a severe hemorrhagic fever progressing to death in 30% of cases in some regions[40]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[41]. Ribavirin has been used to treat infections with limited success[42].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and GPC [40]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[40]. Conversely, the CCHFV GPC is considerably less conserved (< 75% amino acid identity)[40]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location. Thus, creating a pan-CCHFV vaccine or therapeutic based solely on one clade poses a challenge. Nevertheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a rVSV vectored vaccine expressing the full GPC of CCHFV[43].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP immunogens with the highest level of homology across clades. We will assess their capacity to protect against lethal challenge with isolates from multiple CCHFV clades in established mouse[43,44] and NHP[45] models with **Core E**. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best formulation of CCHFV GP/NP MNP vaccine against the emerging test case nairovirus, Kasokero virus, for which there are no vaccines available.

Paramyxoviridae: The HNVs NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality[4,46]. HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality[47]. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs, which served as amplifying hosts[48]. NiV and HeV can infect at least 18 animal species across 6 orders of mammals, and among these they cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans[16,49]. The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission[5]. Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and Southeast Asia and Australia. Their broad host tropism and ability to infect livestock offers an amplification opportunity for increased risk of human spillover. The HNVs are single-stranded, negative sense, enveloped RNA viruses and their biology lends themselves to be synthetically produced as agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS). The Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents[50] because the virus could be engineered for mass dissemination and it possesses the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the R&D Blueprint list of priority pathogens with epidemic potential[51].

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb exist. These postexposure treatments conferred protecion against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP)[52-55]. The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia[56]. In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs, and several vaccine platforms have clear potential for use in people[16]. The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies[16]. Such preventive vaccines would have utility for lab workers, first responders or individuals at high-risk exposure, and in mitigating the spread of an outbreak. In the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent[57]. It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection[49,58]. The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished)[59]. The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons: 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; and 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including rescuing recombinant LayV (rLayV), exploring animal model development, constructing additional LayV and AngV reporter virus tools, and then translating and testing the MNP vaccine ‘plug-and-play’ approach. Finally, we will evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

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PABVAX THEME. A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center’s RPs and Core leaders currently have active and productive collaborative research programs in related areas, most spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

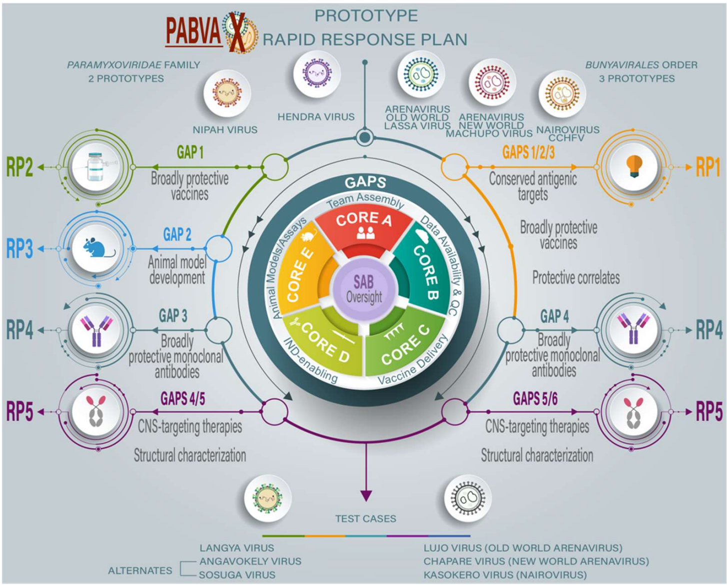

Figure 1: Research gaps addressed by the *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center (PABVAX).

The pandemic preparedness research gaps addressed by PABVAX are diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **arenaviruses** and **nairoviruses**, and **RP2** will target the prototype **HNVs**. Both RP1 and RP2 will develop new prototype virus tools (recombinant prototype viruses) along with existing tools, to analyze vaccine responses and evaluate new mAbs and nabs from the other RPs. A third RP will develop a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant *Cedar henipavirus* (rCedV) in vivo and a bioluminescence detection method that can longitudinally trace the dynamics and anatomical progression of infection with a luciferase-encoding virus in individual animals (**RP3**). There will also be two RPs that focus on the development of broadly reactive human mAbs and nanobodies to the PABVAX selected viral prototypes. **RP4** will isolate human mAbs derived from NiV-infected survivors with a specific focus on F glycoprotein-targeting mAbs. Additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will engage in the manufacture, quality control, and testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**). Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core (**Core A**) will provide organizational and programmatic structure to promote research productivity and scientific interactions along with compliance with regulations concerning select agent, vertebrate animal, and human subject use. **Core A** will coordinate all activities with the Scientific Advisory Board (**SAB**) and make data available through the Data Management Core (**Core B**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform. The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**). This project tested a dissolvable MNP-based vaccine technology ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine[60]. The MNP vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable microneedles that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants[61] to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as to facilitate improved vaccine storage and distribution due to their improved thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, Scientific **Core C** (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ)-adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (*Paramyxoviridae*). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro*, along with analysis of polyfunctional T-cell responses by antigenic peptide stimulation, followed by intracellular cytokine staining (ICS) and flow cytometry.

Discover effective antibody-based prophylactics and therapies. Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah[62], Hendra[63], Ebola[64,65], Marburg[66] and Lassa[67]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc*.), a feature to be exploited in **Core D** by Dr. Gunn. In addition to therapeutic applications, mAbs with Fc mutations dramatically extend serum half-life[68-70] and offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g*. rapid response personnel). Members of

PABVAX have extensive experience with the discovery, characterization, and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures. Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal Model Development and Preclinical Evaluation Scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective efficacy of antivirals and vaccines, as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation, including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they are generally less laborious and can be adapted to higher throughput platforms and/or integrated with other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput fluorescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic NiV and HeV vaccine-induced immune sera, mAbs (**RP4**), nbs (**RP5**), and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either HeV or NiV-B envelope GPs) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals to explore nab based therapeutics to CNS resident HNVs[71].

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the HNVs (*Paramyxoviridae*) and the *Bunyavirales* (*Arenaviridae* and *Nairoviridae*) that will bolster vaccine endurance to storage and transportation; we also anticipate improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology, and mAb and vaccine translational drug development along with Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Paramyxoviruses: NiV and HeV are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis[4,46]. HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals. These viruses are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of CedV, Gamak virus, Daeryong virus, and (LayV)[72] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[73,74]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV, are old world pteropid fruit bats[73,75]. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province)[17]. The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**. Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal HNV disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters[49], and AGMs (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P). The P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists[76-80]. The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections[81,82]. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature[83]. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2[84] and CedV is now recognized as a BSL-2 restricted agent[85]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D,** and **E**).

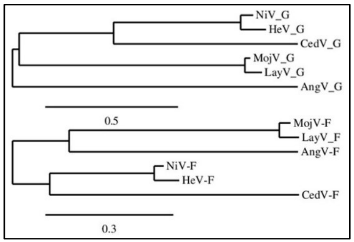

Figure 2: Phylogenetic analysis of select HNV G and F glycoproteins. Trees were constructed for using the PhyML program's maximum likelihood method and aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

Arenaviruses (old world): For **phase 1**, **RP1** will utilize **LASV as the prototype virus** due its significant public health burden throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[86]. LASV is the most imported viral hemorrhagic fever (VHF) next to Dengue virus and has been documented in several countries outside of Africa over 35 times[87]. CFRs approaching 70% in hospitalized cases and marked long term sequelae are the result of the lack of approved vaccines or therapeutics, although several live attenuated virus candidates are currently being evaluated with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI)[88]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize only LASV GP as the target immunogen.

Investigators in **RP1**, **Core D**, and **Core E** have already demonstrated protective efficacy against challenge with LASV in outbred guinea pigs vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These immunogens have been engineered to express homologous antigenic regions to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach suggests a similar strategy could be implemented against other arenaviruses. The **OWAV test pathogen, LUJV,** was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia, which quickly spread nosocomially[89]. The CFR for this infectious virus was 80% in this small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from suggest current LASV countermeasures may be of limited use. LUJV will serve as an ideal "test case" to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Arenaviruses (new world): The live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[90], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[91,92]. Furthermore, there is the potential for pathogenic reversion. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest exploring other JUNV countermeasures are warranted. Building on the success of the LASV GPe/NP-NTD, investigators in **RP1 and Cores D and E** will engineer broadly protective MACV GPe and NP-NTD antigens and perform efficacy testing in established guinea pig and NHP models. We will extend this plug-and-play approach to the **NWAV test case, CHAPV**. First reported in 2003 in Bolivia, CHAPV is a rodent-borne mammarenavirus associated with the development of severe hemorrhagic fever with case fatality rates up to 60%[93,94]. Recent studies have demonstrated clear human-to-human transmission during the acute phase evidenced by several nosocomial infections; isolation of viable virus from semen at 86 days after symptom onset demonstrates a clear need to implement specialized management and treatment [94]. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Nairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as it represents the largest global public health burden within the orthonairovirus family with outbreaks annually across Europe, Africa, and Asia. There are no FDA-approved vaccines for CCHFV; however, a suckling mouse brain-derived, adjuvanted whole virus vaccine for CCHFV has been approved for use in Bulgaria. This vaccine has numerous safety concerns and has limited demonstrated efficacy. Several experimental vaccine approaches have been evaluated ranging from viral vectored approaches to DNA-based vaccines with varying efficacy, advantages, and disadvantages[95]; however, only a Modified Vaccinia Ankara virus vaccine (MVA-CCHFV) has begun to advance towards clinical trials[96]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test these for protective efficacy from challenge with 6 different CCHFV lineages using established mouse and NHP models. Next, we will deploy the same approach to the **test case nairovirus, Kasokero virus (KASV),** for which no vaccine exists.

In 1977, KASV was first isolated from Ugandan bats and caused several cases of acute febrile illness of laboratory workers from the same lab that processed the infected bats[97]. Surveys of 109 laboratory workers from the same facility demonstrated 13% seropositivity and clinical symptoms in individuals 4 to 15 months after the virus was isolated. These findings suggest either continued laboratory-associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the tick reservoir, *Ornithodoros faini,* to participate in enzootic maintenance of KASV[98]. While most chiropteran ticks exhibit host specificity, humans entering caves these bats inhabit have reported bites from *O.faini* ticks. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics suggest investment in countermeasure development against this virus is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**; arenavirus and nairovirus projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine active and passive immunization approaches to further improve the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

An additional and outstanding advantage of the PABVAX Center is the **Animal Model Development and Preclinical Evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for members of the *Bunyavirales* and *Paramyxoviridae*.**
2. **Develop new tools and animal models for the conduct of cell-based and *in vivo* therapeutic evaluation.**
3. **Develop antibodies and nanobodies to the selected prototype and test viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

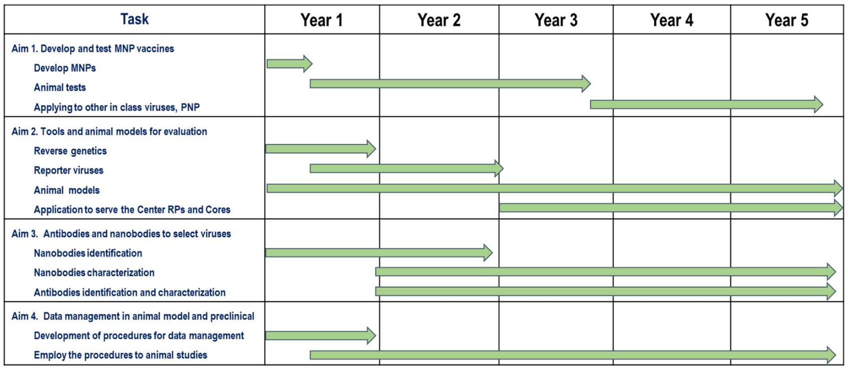

Figure 3: Projected PABVAX timeline to achieve the 4 overarching tasks.

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**. Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (details are specified within the Administration **Core A** Research Strategy). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommending new directions as appropriate, and providing Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

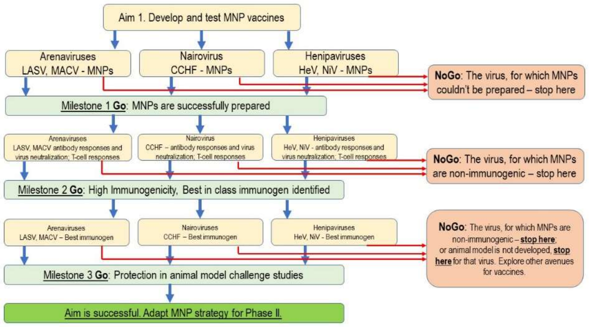

Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will address whether formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be established using immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen-specific antibody induction, virus neutralization, and polyfunctional T-cell responses by *in vitro* stimulation of isolated cells). Go/no go decisions will be made based on outcomes of these first-tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as a success. Comparisons will also be made such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of the best-in-class immunogens, or immunogen combinations, will also be explored to down-select vaccines. The third milestone will determine whether MNP vaccination affords protection in animal model challenge experiments. In the case of NiV and HeV prototypes, the animal models chosen (ferrets and AGMs) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales*, there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASV and guinea pig models exist for some but not all of these lineages. For MACV, there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys now exceed costs of $35K each. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of AGM models for arenaviruses and possibly CCHFV as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.

The second overall objective of PABVAX is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will

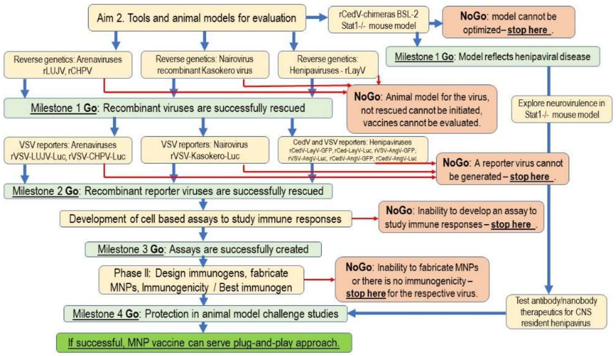

Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center's overall objectives.

be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing of the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**). The milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new *in vivo* bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease *in vivo* safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**), it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and **RP3** will collaborate with **RP5** in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

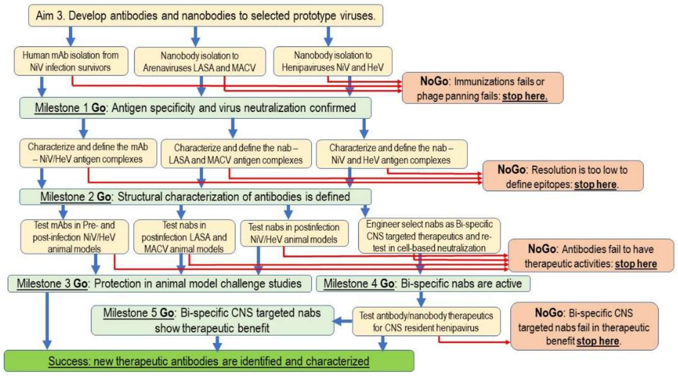

Figure 5. Milestones and criteria for progress for **Aim 3** of the PABVAX Center's overall objectives.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

New human mAbs and nbs as tools and potential therapeutics will be developed by **RP4** and **RP5** in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs, and in collaboration with **RP2**, neutralization potencies will be rapidly assessed (as well as evaluating synergistic cell-based mAb neutralization) that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

Nbs, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nbs can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nbs targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus), and select animal models of LASV and MACV by **Core E**. Success will be measured by the 1) identification of potent virus-neutralizing nbs, 2) discovery of more broadly reactive nbs particularly to the multiple LASV clades, 3) evidence of therapeutic benefit in animal models, and 4) the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

Industry Partnerships*. Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner that will provide access to vaccine expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.*

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience in developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX. Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

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Galveston National Laboratory (GNL). The GNL is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics, and therapeutics. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370 separate NHP studies employing a total of 2,772 animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses have been successfully performed at the GNL. These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder, and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP facility and a staff of full-time veterinarians. In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of the WRCEVA virus collection within the GNL.

Core E (Animal model development and preclinical evaluation)**. Core E** operations will be conducted at the GNL, and in conjunction with **Core A**, will also include the UTMB Institutional Office of Regulated Nonclinical Studies (ORNcS). The ORNcS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL, and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses, arenaviruses, and henipaviruses. He has published over 250 peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI), was the first to develop a NHP model of NiV/HeV infection and disease, and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs). Mapp Biopharmaceutical has deep experience with antigens and mAbs against these antigens. Their early development and research group has expertise in immunology, infectious diseases, and the production of antigens, antibodies, and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [99] [100]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1[101]. Dr. Gunn and Genovac will join the team to analyze antibody Fc effector functions and to provide antigen/antibody discovery resources, respectively.

Core A (Administrative Core)

The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews, and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills, and his experience in translational research activities, qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe's (**RP4**) research focuses on the molecular basis for the development of effective B cell responses to viruses in humans. He has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently HNVs NiV and HeV (owing to the collaboration between the Crowe, Broder and Geisbert laboratories). Perhaps of greatest import, the SARS-CoV-2 mAbs generated by Dr. Crowe's laboratory were used in thousands of people during the COVID-19 pandemic. RP4 will work closely with Cores D and E in advancing mAb prototypes.

As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

1. Lamb RA, Parks GD. Paramyxoviridae: the viruses and their replication. In Fields BN, Knipe DM, Howley PM, editors, Fields Virology: Sixth Edition. Vol. 1. Lippincott, Williams, and Wilkins. 2013. p. 957-995.
2. Boshra H. An Overview of the Infectious Cycle of Bunyaviruses. *Viruses*. Sep 28 2022;14(10)doi:10.3390/v14102139
3. Horne KM, Vanlandingham DL. Bunyavirus-vector interactions. *Viruses*. Nov 13 2014;6(11):4373-97. doi:10.3390/v6114373
4. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia), p 1070-1085.
5. Luby SP. The pandemic potential of Nipah virus. *Antiviral research*. Oct 2013;100(1):38-43. doi:10.1016/j.antiviral.2013.07.011
6. Skowron K, Bauza-Kaszewska J, Grudlewska-Buda K, et al. Nipah Virus-Another Threat From the World of Zoonotic Viruses. *Front Microbiol*. 2021;12:811157. doi:10.3389/fmicb.2021.811157
7. Kummer S, Kranz DC. Henipaviruses-A constant threat to livestock and humans. *PLoS Negl Trop Dis*. Feb 2022;16(2):e0010157. doi:10.1371/journal.pntd.0010157
8. Gouglas D, Christodoulou M, Plotkin SA, Hatchett R. CEPI: Driving Progress Toward Epidemic Preparedness and Response. *Epidemiol Rev*. Jan 31 2019;41(1):28-33. doi:10.1093/epirev/mxz012
9. Maes P, Adkins S, Alkhovsky SV, et al. Taxonomy of the order Bunyavirales: second update 2018. *Arch Virol*. Mar 2019;164(3):927-941. doi:10.1007/s00705-018-04127-3
10. Silva-Ramos CR, Montoya-Ruíz C, Faccini-Martínez Á A, Rodas JD. An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever. *Arch Virol*. Sep 2022;167(9):1727-1738. doi:10.1007/s00705-022-05453-3
11. Garry RF. Lassa fever - the road ahead. *Nat Rev Microbiol*. Feb 2023;21(2):87-96. doi:10.1038/s41579-022-00789-8
12. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research*. Nov 2018;159:63-67. doi:10.1016/j.antiviral.2018.09.009
13. WHO. List of Blueprint Priority Diseases. Accessed November 14, 2018. http://www.who.int/blueprint/priority-diseases/en/
14. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens. Accessed 04 Jun 2023, https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens
15. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol*. Jan 2006;4(1):23-35. doi:10.1038/nrmicro1323
16. Amaya M, Broder CC. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol*. Sep 29 2020;7(1):447-473. doi:10.1146/annurev-virology-021920-113833
17. Zhang XA, Li H, Jiang FC, et al. A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med*. Aug 4 2022;387(5):470-472. doi:10.1056/NEJMc2202705
18. Radoshitzky SR, Buchmeier MJ, Charrel RN, et al. ICTV Virus Taxonomy Profile: Arenaviridae. *J Gen Virol*. Aug 2019;100(8):1200-1201. doi:10.1099/jgv.0.001280
19. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. *Front Microbiol*. 2018;9:1751. doi:10.3389/fmicb.2018.01751
20. Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient Evolution of Mammarenaviruses: Adaptation via Changes in the L Protein and No Evidence for Host-Virus Codivergence. *Genome Biol Evol*. 2018/03// 2018;10(3):863-874. doi:10.1093/gbe/evy050
21. Shaffer JG, Grant DS, Schieffelin JS, et al. Lassa Fever in Post-Conflict Sierra Leone. *PLOS Neglected Tropical Diseases*. 2014;8(3):e2748. doi:10.1371/journal.pntd.0002748
22. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Review. *Antiviral research*. Apr 2008;78(1):132-9. doi:10.1016/j.antiviral.2007.10.010
23. Kilgore PE, Peters CJ, Mills JN, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. Jul-Sep 1995;1(3):97-100. doi:10.3201/eid0103.950308

24. Heinrich ML, Boisen ML, Nelson DKS, et al. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. *Scientific Reports*. 2020/09/29 2020;10(1):16030. doi:10.1038/s41598-020-72539-w
25. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nature Medicine*. 2017/10/01 2017;23(10):1146-1149. doi:10.1038/nm.4396
26. Hastie KM, Zandonatti MA, Kleinfelter LM, et al. Structural basis for antibody-mediated neutralization of Lassa virus. *Science*. Jun 2 2017;356(6341):923-928. doi:10.1126/science.aam7260
27. Sullivan BM, Sakabe S, Hartnett JN, et al. High crossreactivity of human T cell responses between Lassa virus lineages. *PLoS Pathog*. Mar 2020;16(3):e1008352. doi:10.1371/journal.ppat.1008352
28. Ugwu C, Olumade T, Nwakpakpa E, et al. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. *Scientific Reports*. 2022/12/25 2022;12(1):22330. doi:10.1038/s41598-022-26045-w
29. Geisbert TW, Jones S, Fritz EA, et al. Development of a New Vaccine for the Prevention of Lassa Fever. *PLOS Medicine*. 2005;2(6):e183. doi:10.1371/journal.pmed.0020183
30. Cross RW, Woolsey C, Prasad AN, et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep*. Jul 19 2022;40(3):111094. doi:10.1016/j.celrep.2022.111094
31. Safronetz D, Mire C, Rosenke K, et al. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. *PLOS Neglected Tropical Diseases*. 2015;9(4):e0003736. doi:10.1371/journal.pntd.0003736
32. Hensley LE, Smith MA, Geisbert JB, et al. Pathogenesis of Lassa fever in cynomolgus macaques. *Virol J*. May 6 2011;8:205. doi:10.1186/1743-422x-8-205
33. Zeitlin L, Cross RW, Geisbert JB, et al. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. *Proceedings of the National Academy of Sciences*. 2021;118(11):e2023332118. doi:doi:10.1073/pnas.2023332118
34. Bell TM, Shaia CI, Bunton TE, et al. Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure. *Veterinary Pathology*. 2015;52(1):26-37. doi:10.1177/0300985814540544
35. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. *Epidemiol Infect*. Jun 1996;116(3):353-61. doi:10.1017/s0950268800052687
36. Brinkmann A, Kohl C, Radonić A, et al. First detection of bat-borne Issyk-Kul virus in Europe. *Sci Rep*. Dec 24 2020;10(1):22384. doi:10.1038/s41598-020-79468-8
37. Ma J, Lv XL, Zhang X, et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat Med*. Mar 2021;27(3):434-439. doi:10.1038/s41591-020-01228-y
38. Lorenzo Juanes HM, Carbonell C, Sendra BF, et al. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. *Emerg Infect Dis*. Feb 2023;29(2):252-259. doi:10.3201/eid2902.220677
39. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, et al. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. *Ticks Tick Borne Dis*. May 2020;11(3):101403. doi:10.1016/j.ttbdis.2020.101403
40. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology*. 2023/03/14 2023;doi:10.1038/s41579-023-00871-9
41. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral research*. May 2011;90(2):85-92. doi:10.1016/j.antiviral.2011.02.010
42. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. *BMC Infectious Diseases*. 2010/07/13 2010;10(1):207. doi:10.1186/1471-2334-10-207
43. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep*. May 23 2019;9(1):7755. doi:10.1038/s41598-019-44210-6
44. Bente DA, Alimonti JB, Shieh WJ, et al. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol*. Nov 2010;84(21):11089-100. doi:10.1128/jvi.01383-10
45. Cross RW, Prasad AN, Borisevich V, et al. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. *PLoS Negl Trop Dis*. Aug 2020;14(8):e0008637. doi:10.1371/journal.pntd.0008637

46. Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York, p 519-536.
47. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science*. Apr 7 1995;268(5207):94-7. doi:10.1126/science.7701348
48. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science (New York, NY)*. 2000/05// 2000;288(5470):1432-1435. doi:10.1126/science.288.5470.1432
49. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol*. 2012;359:153-77. doi:10.1007/82_2012_208
50. *Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087*.
51. Sweileh WM. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health*. Feb 8 2017;13(1):9. doi:10.1186/s12992-017-0233-9
52. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Science translational medicine*. 2011/10// 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901
53. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine*. 2014/06// 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929
54. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep*. Aug 3 2016;6:30916. doi:10.1038/srep30916
55. Mire CE, Chan YP, Borisevich V, et al. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis*. May 11 2020;221(Suppl 4):S471-s479. doi:10.1093/infdis/jiz515
56. Playford EG, Munro T, Mahler SM, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*. Apr 2020;20(4):445-454. doi:10.1016/s1473-3099(19)30634-6
57. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerging infectious diseases*. 2014/03// 2014;20(3):372-379. doi:10.3201/eid2003.131159
58. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One*. May 18 2010;5(5):e10690. doi:10.1371/journal.pone.0010690
59. Cheliout Da Silva S, Yan L, Dang HV, et al. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses*. Mar 22 2021;13(3)doi:10.3390/v13030517
60. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo LD, Jr. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. *Expert Opin Drug Deliv*. Feb 2021;18(2):151-167. doi:10.1080/17425247.2021.1823964
61. Andrianov AK, DeCollibus DP, Gillis HA, et al. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A*. Nov 10 2009;106(45):18936-41. doi:10.1073/pnas.0908842106
62. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med*. Jun 25 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929
63. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med*. Oct 19 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901
64. Pettitt J, Zeitlin L, Kim do H, et al. Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. *Sci Transl Med*. Aug 21 2013;5(199):199ra113. doi:10.1126/scitranslmed.3006608
65. Qiu X, Wong G, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature*. Oct 2 2014;514(7520):47-53. doi:10.1038/nature13777
66. Mire CE, Geisbert JB, Borisevich V, et al. Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci Transl Med*. Apr 5 2017;9(384)doi:10.1126/scitranslmed.aai8711
67. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat Med*. Oct 2017;23(10):1146-1149. doi:10.1038/nm.4396

68. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. *mAbs*. Sep-Oct 2011;3(5):422-30. doi:10.4161/mabs.3.5.16983

69. Robbie GJ, Criste R, Dall'acqua WF, et al. A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study. *Antimicrobial agents and chemotherapy*. Sep 30 2013;doi:10.1128/aac.01285-13

70. Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. *PLoS Med*. Jan 2018;15(1):e1002493. doi:10.1371/journal.pmed.1002493

71. Mastraccio KE, Huaman C, Laing ED, Broder CC, Schaefer BC. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. *Methods Mol Biol*. 2022;2524:369-394. doi:10.1007/978-1-0716-2453-1_30

72. Rima B, Balkema-Buschmann A, Dundon WG, et al. ICTV Virus Taxonomy Profile: Paramyxoviridae. *J Gen Virol*. Dec 2019;100(12):1593-1594. doi:10.1099/jgv.0.001328

73. Madera S, Kistler A, Ranaivoson HC, et al. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol*. Sep 28 2022;96(18):e0092122. doi:10.1128/jvi.00921-22

74. Vanmechelen B, Meurs S, Horemans M, et al. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol*. 2022;8(2):veac061. doi:10.1093/ve/veac061

75. Voigt K, Hoffmann M, Drexler JF, et al. Fusogenicity of the Ghana Virus (Henipavirus: Ghanaian bat henipavirus) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. *Viruses*. Aug 29 2019;11(9)doi:10.3390/v11090800

76. Childs K, Randall R, Goodbourn S. Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction. *J Virol*. Apr 2012;86(7):3411-21. doi:10.1128/jvi.06405-11

77. Davis ME, Wang MK, Rennick LJ, et al. Antagonism of the phosphatase PP1 by the measles virus V protein is required for innate immune escape of MDA5. *Cell Host Microbe*. Jul 9 2014;16(1):19-30. doi:10.1016/j.chom.2014.06.007

78. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol*. Nov 2002;76(22):11476-83. doi:10.1128/jvi.76.22.11476-11483.2002

79. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol*. May 2005;79(10):6078-88. doi:10.1128/jvi.79.10.6078-6088.2005

80. Basler CF. Nipah and hendra virus interactions with the innate immune system. *Curr Top Microbiol Immunol*. 2012;359:123-52. doi:10.1007/82_2012_209

81. Yoneda M, Guillaume V, Sato H, et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One*. Sep 15 2010;5(9):e12709. doi:10.1371/journal.pone.0012709

82. Satterfield BA, Cross RW, Fenton KA, et al. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J Virol*. Jul 15 2016;90(14):6326-6343. doi:10.1128/jvi.00215-16

83. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog*. 2012;8(8):e1002836. doi:10.1371/journal.ppat.1002836

84. Amaya M, Cheng H, Borisevich V, et al. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research*. Sep 2021;193:105084. doi:10.1016/j.antiviral.2021.105084

85. CDC. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. . Updated June 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

86. Organization WH. Lassa fever fact sheet. *World Health Organization*. 2017;

87. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. *J Travel Med*. Jul 14 2020;27(4)doi:10.1093/jtm/taaa035

88. Purushotham J, Lambe T, Gilbert SC. Vaccine platforms for the prevention of Lassa fever. *Immunol Lett*. Nov 2019;215:1-11. doi:10.1016/j.imlet.2019.03.008

89. Sewlall NH, Richards G, Duse A, et al. Clinical features and patient management of Lujo hemorrhagic fever. *PLoS Negl Trop Dis*. 2014;8(11):e3233. doi:10.1371/journal.pntd.0003233

90. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. *Vaccines*. 2023;11(3):635.

91. Koma T, Patterson M, Huang C, et al. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. *J Virol*. Feb 1 2016;90(3):1290-7. doi:10.1128/jvi.02615-15

92. Jahrling P TR, Barrero O. . Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 1988:53.
93. Delgado S, Erickson BR, Agudo R, et al. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. *PLOS Pathogens*. 2008;4(4):e1000047. doi:10.1371/journal.ppat.1000047
94. Loayza Mafayle R, Morales-Betoulle ME, Romero C, et al. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. *New England Journal of Medicine*. 2022;386(24):2283-2294. doi:10.1056/NEJMoa2110339
95. Tipih T, Burt FJ. Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development. *Biores Open Access*. 2020;9(1):137-150. doi:10.1089/biores.2019.0057
96. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease. 2023. Accessed 03/06, 2023. https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease
97. Kalunda M, Mukwaya LG, Mukuye A, et al. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. *The American Journal of Tropical Medicine and Hygiene*. 01 Mar. 1986 1986;35(2):387-392. doi:10.4269/ajtmh.1986.35.387
98. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. *Emerg Infect Dis*. Dec 2020;26(12):2944-2950. doi:10.3201/eid2612.202411
99. Mulangu S, Dodd LE, Davey RT, Jr., et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. *N Engl J Med*. Dec 12 2019;381(24):2293-2303. doi:10.1056/NEJMoa1910993
100. Group PIW, Multi-National PIIST, Davey RT, Jr., et al. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. *N Engl J Med*. Oct 13 2016;375(15):1448-1456. doi:10.1056/NEJMoa1604330
101. Politch JA, Cu-Uvin S, Moench TR, et al. Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial. *PLoS Med*. Feb 2021;18(2):e1003495. doi:10.1371/journal.pmed.1003495

CENTER OVERVIEW: *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction

Emerging and periodically reemerging pathogenic enveloped RNA viruses are ~~a~~ continuous ~~infectious disease and potentially~~ pandemic threats ~~to global public health~~. Among ~~the many groups of these types of pathogens are viral~~viral taxon associated with human zoonoses ~~within~~ , members of the family ***Paramyxoviridae*** and order ***Bunyavirales*** stand out for their capacity to cause debilitating and life-threatening disease. Humans can become infected ~~where humans can acquire infections directly from~~via excreta from animal reservoir hosts such as rodents~~s~~ or ~~or~~ bats~~s,~~ through ~~through an~~ intermediate amplifying hosts such as ~~livestock~~small or domestic animals, or by ~~from~~ arthropod vectors such as mosquitos and ticks[1-4] ~~(Fields virology)~~. ~~More often than not,~~ H~~h~~umans often ~~-~~represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur~~, and should~~. During this process, RNA viral pathogens may mutate and adapt to more efficiently replicate~~ion~~ and shed~~ding~~ in the ~~human~~ host, posing an~~the~~ epidemic ~~potential of these viruses could~~threat ~~manifest~~[5-8] ~~(PMID: 23911335 -Nipah / others?)~~. The viral order *Bunyavirales* is composed of several viral families ~~known to contain~~ containing ~~multiple~~ high priority human pathogens[9]. Notably, the ***Arenaviridae*** and ***Nairoviridae*** families contain viruses ~~which~~ that cause severe hemorrhagic fever diseases in humans ~~across the world~~globally with mortality rates up to 60%[10] ~~XX~~ and significant, long-term sequelae in survivors[11]. Many bunya~~These~~ viruses are included on the World Health Organization's (WHO) List of Priority Pathogens[12,13] ~~where~~. S~~s~~ix arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHAPV, GTOV, JUNV, MACV) and one n~~N~~airovirus, ~~-~~Crimean-Congo Hemorrhagic Fever Virus (CCHFV), ~~and~~ are also identified as NIAID Category A pathogens[14] due to their 1) high associated morbidity and mortality rates, 2) ease of dissemination and/or ~~abilities~~ ability to transmit from person-to-person, 3) ~~, produce significant morbidity and mortality, have~~ potential ~~for~~ to cause public panic and social disruption~~major public health impact~~, and 4)~~,~~ requirement for ~~and require~~ special actions ~~for~~ in the context of public health preparedness. ~~-~~The family ***Paramyxoviridae*** also includes members that are ~~Also, within the~~ WHO Priority Pathogens, namely ~~s list is~~ Nipah virus (NiV) and ~~henipaviral disease which includes~~ Hendra virus (HeV). These ~~which are~~ zoonotic henipaviruses (HNVs) ~~in the family **Paramyxoviridae**. NiV and HeV have long been~~are considered ~~the~~ prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease[15] ~~(PMID: 16357858)~~. The genus now includes nine additional reported HNVs, but only HeV and NiV are ~~known to be~~ associated with severe and often fatal henipaviral disease in humans and a number of animal species[16] ~~(PMID: 32991264)~~. Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illness~~es~~ in humans[17] ~~[PMID: 35921459]~~.

~~Taken together, t~~These collective factors plus the absence of approved vaccines or therapeutics make bunyaviruses and paramyxoviruses ~~viral threats~~ a significant threat to global public health ~~are heightened due to the absence of approved vaccines or therapeutics~~. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for developing vaccine and monoclonal antibody (mAb) countermeasures ~~in developing~~to prototype ~~candidates~~ bunyavirus and paramyxovirus pathogens (**Table 1**) ~~against a selection of these viruses~~. In the out years of the Center effort, PABVAX will exploit these learned key principles in a “plug and play” demonstration against related viruses.

~~Family~~	~~Prototype~~	~~Plug and Play Test Case~~
~~Arenaviruses~~	~~Lassa (Old world), Machupo (New world)~~	~~Lujo (Old world), Chapare (New world)~~
~~Nairoviruses~~	~~CCHF~~	~~Kasokero~~

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old World) Machupo (New World)	Lujo (Old World) Chapare (New World)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Table 1. PABVAX selected prototype and test case viral agents

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~~Nairoviruses~~	~~CCHF~~	~~Kasokero~~
~~Paramyxoviruses~~	~~Nipah, Hendra~~	~~Langya~~

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~~**Bunyavirales:**~~

Arenaviridae: The family *Arenaviridae* is composed of ~~three~~five genera: *Mammarenavirus~~idae~~*, *Reptarenavirus*, *Innmovirus*, *Antennavirus*, and *Hartmanivirus*[18~~9~~]. The genus *Mammarenavirus*idae ~~are~~contains ~~composed of~~ a large number of rodent-~~-~~borne viruses, some of which ~~are demonstrated human pathogens responsible for~~ caus~~e~~ing severe hemorrhagic fever, neurological damage, death, and ~~if not fatal can result~~ permanent ~~neurological~~ sequelae ~~including~~such as hearing loss, vision impairment, and balance issues in survivors[19]~~derangement~~. While these genera exhibit a global distribution, two geographically distinct ~~sub-genera~~serogroups exist ~~have formed due to the separation across continents by the Atlantic and Pacific Ocean~~that diverged ~45,000 years ago[20]. ~~–~~Old World complex ~~m~~Mammarenaviruses (OWAV) in the Eastern Hemisphere include ~~(Lassa Virus (~~LASV~~)~~, Lymphocytic Choriomeningitis Virus (LCMV), and ~~and~~ the newly discovered ~~Lujo Virus (~~LUJV~~);)~~ ~~and the~~ New World complex mamma~~A~~renaviruses (NWAV) in the Western Hemisphere include ~~(Junin Virus (~~JUNV~~)~~, ~~Machupo Virus (~~MACV~~)~~, Sabia Virus (SABV), and ~~the newly emerging Chapare Virus (~~CHAPV~~))~~. ~~Many of these viruses are identified as NIAID Category A pathogens requiring biosafety level 4 (BSL4) containment[14,19]. This classification is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness. While several promising medical countermeasures are in development,~~T~~t~~here are currently no US FDA approved vaccines or therapeutics for ~~any~~ mammarenaviruses, though the attenuated JUNV “Candid-1” vaccine is approved for use in Argentina.

~~Infection in humans is often the result of exposure to rodent excreta from infected animals~~ D~~where d~~isease severity and case fatality rates (CFR) ~~can~~ vary across ~~the~~ mammarenaviruses ~~genera and~~ even within ~~genetic lineages as~~the same clades and species. Up to 500,000 cases of LASV infection occur per year with CFRs sometimes approaching 70% in hospitalized cases[21]. ~~can be the case for infections with LASV where genetic diversity can be high, even within clades. While this genetic variability creates significant challenges for LASV molecular diagnostics, antigen-based diagnostics have seen high levels of cross reactivity across the seven known lineages of LASV suggesting cross protective antigens may be possible[21]. Lassa virus, the causative agent of Lassa fever (LF), likely causes the largest number of annual cases per year of LF, where its understood up to 500,000 cases per year can occur where the CFR can approach 70% in hospitalized cases[22]. Ribivirin has been used to treat infections with uncertain success[23].~~ Before the introduction of the ~~JUNV~~ Candid-1 vaccine, it is believed that an upwards of 60,000 cases of ~~JUNV infection, also known as~~ Argentine Hemorrhagic Fever (AHF) from infection with JUNV occurred per year ~~occurred where~~ with CFRs rang~~ed~~ing from 15-30%. Convalescent plasma from JUNV survivors is still the primary means to treat acute ~~JUNV infections~~AHF in Argentina[22]. Over 1000 cases were reported for t~~T~~he initial outbreak of MACV ~~Machupo virus had over 1000 cases and~~with CFRs ~~have ranged from~~ranging from 5-30%~~; with a CFR~~ , ~~where outbreak frequency is thought to have been reduced through~~ enhanced rodent abatement practices and potentially cross-protective immunity in populations ~~who~~that have taken the JUNV Candid-1 vaccine may have reduced recent outbreak frequency[23].

All mammarenaviruses share a common genomic organization consisting of two ambi-sense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix)~~,~~ and ~~the~~ L protein (polymerase), and the glycoprotein precursor (GPC)~~,~~ and ~~the~~ nucleoprotein (NP)[19]. ~~–~~Despite general similarity of their genomic architecture, ~~these groups~~mammarenaviruses exhibit marked differences in their replication life cycles~~. This includes differences in~~ including attachment factors, entry mechanisms, and immune evasion; nonetheless, the dominant antigen~~ic protein~~s driving immune responses for both ~~OWAV and NWAV~~OWAV and NWAV are the glycoprotein (GP) and NP.

Despite the abundant genetic diversity of LASV, high levels of cross reactivity have been seen with antigen-based diagnostics across seven known lineages, suggesting the generation of cross-protective immunogens and antibodies against mammarenaviruses is achievable[24]. Monoclonal antibodies derived from human LASV survivors ~~have been demonstrated~~were shown to protect non-human primates (NHP)~~,~~ against lethal challenge[25]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe)[26]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, ~~suggesting~~indicating ~~using~~ this domain ~~as a vaccine antigen may also be possible~~could serve as an antigenic target. ~~To test these notions, i~~Investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccines ~~approaches~~ to test this ~~idea~~approach.

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and have demonstrated protection against lethal LASV-Josiah challenge. Specifically, ~~using~~ a stabilized prefusion~~,~~ trimeric GPe, an immunogen containing a highly conserved NP NTD epitope, or a combination of these two ~~antigen~~approaches conferred protection in ~~s using~~ a lethal outbred guinea pig model.

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Despite the considerable molecular diversity in LASV strains, ~~there is understood that there is some~~ conservation at~~in~~ key antigenic sites within ~~both~~ the GPe and ~~the~~ NP~~, suggesting~~ suggest the feasibility of generating ~~, that~~ a pan~~PAN~~-LASV vaccine ~~may be possible~~[24,27,28]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a recombinant Vesicular s~~S~~tomatitis v~~V~~irus (rVSV)-based vector expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[29], but also ~~that live attenuated vaccines expressing this same GPC can protect NHP~~protects against heterologous challenge with ~~viruses from:~~ Lineage II[30], ~~Lineage~~ III (Cross, Geisbert unpublished), ~~Lineage~~ V[31], and ~~Lineage~~ VI viruses (Cross and Geisbert Unpublished).

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In **Phase I of RP1**, the ability ~~for~~of an optimized ~~intramuscular (IM)~~ LASV GPe/NP subunit vaccine delivered intramuscularly (IM) ~~to protect against disease after challenge multiple LASV lineages~~ will be assessed in guinea pig and NHP models across multiple viral lineages. We will also assess ~~This~~a ~~formulation will also be assessed using a~~ microneedle skin patch (MNP) formulation optimized in **Core C** to determine if a needle-free vaccination route affords more potent vaccine~~-~~-mediated responses and protection. Similarly, we have ~~already~~ generated MACV GPe immunogens and will design a MACV NP-NTD subunit antigen to test ~~following~~ the same approach ~~approach followed with LASV against both homologous lineage challenge and heterologous challenge of~~ across multiple MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing both ~~emerging~~ OWAV (LUJV) and NWAV (CHAPV) complexes, for which there are no vaccines available. A unique ~~feature of this proposal~~advantage of the PABVAX Center is its access to biosafety level (BSL)-4 containment, allowing us to work with authentic versus surrogate arenaviruses. The BSL4 facilities described in **Core E** are capable of evaluating countermeasures in small animal models and NHPs. ~~It is widely considered that T~~the cynomolgus macaque is the current "gold standard" animal model for LASV, JUNV, and MACV research as ~~as there are a multitude of immunological reagents available and the~~ disease ~~pathogenesis appears to mirror~~recapitulates ~~that of~~ human infection and there are a multitude of immunological reagents available[32-34]. The evaluation of ~~successful countermeasures for the~~ prototype OWAV ~~AND~~and NWAV vaccines and therapies in the NHP model ~~will likely be~~is likely pivotal for IND applications and for future licensure of ~~select~~medical countermeasures for human use.

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Nairoviridae: Unique ~~from~~to most other bunyaviruses, orthonairoviruses are transmitted primarily by ticks[2] ~~in nature (REF)~~. While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[35], Issyk-Kul virus[36], Songling virus[37]), ~~Crimean-Congo Hemorrhagic fever virus (~~CCHFV~~)~~ is by far the most recognized public health threat. Outbreaks of CCHFV have occurred ~~to humans as it responsible for annual outbreaks~~ across Europe, Africa, the Middle East, and Asia. There ~~are~~is growing concern~~s~~ for expansion of the CCHFV endemic r~~ange~~egion ~~expansion~~ due to ~~ongoing range expansion~~increased range of its tick reservoir host~~s~~ (*Hyalomma* spp.) tied to bird migration changes, livestock trade, and ~~even~~ global warming. For example, CCHFV ~~are contributing to increasing numbers of infections in humans in areas otherwise not known to be~~cases have been reported in ~~endemic including central~~ Spain[38], a previously non-endemic region, and ~~*most recently the reservoir*~~*Hyalomma* ~~Hyalomma~~ ticks have been found as north as Sweden[39]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to a ~~cause a~~ severe hemorrhagic fever ~~which may~~ progressing to death in 30% of cases in some regions[40]. ~~CCHFV is listed on the WHO priority pathogen list[13] and is also a NIH-NIAID category A pathogen[41].~~ There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[41] ~~(REF)~~. ~~Ribivirin~~Ribavirin has been used to treat infections with limited success[42].

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CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and ~~a~~ GPC [40]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[40]. Conversely, the CCHFV GPC is considerably less conserved~~, where less than~~ (< 75% amino acid ~~conservation is present~~identity)[40]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location. Thus, creating ~~which creates a challenge in terms of creating~~ a pan-CCHFV vaccine or therapeutic based solely on one clade poses a challenge. N~~one~~evertheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a rVSV vectored vaccine expressing the full GPC of CCHFV[43].

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In phase 1, **RP1 and Core D** will generate CCHFV GP and NP ~~subunits~~immunogens with the highest level of homology across clades. We will ~~and~~ assess their capacity to protect against ~~disease after~~lethal challenge with isolates from multiple CCHFV clades in established mouse[43,44] and NHP[45] models with **Core E**. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best ~~performing~~ formulation of CCHFV GP/NP MNP vaccine ~~approach~~ against the emerging test case ~~mammarenaviruses representing emerging~~nairo ~~bat~~ virus, Kasokero virus, for which there are no vaccines available.

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Paramyxoviridae: ~~*The henipavirus paramyxoviruses.*~~ The ~~recognized, highly pathogenic~~ HNVs ~~,~~ NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality ~~(~~[4,46]~~; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia)~~. HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality[47] ~~(Murray, PMID: 7701348)~~. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs, which served as amplifying hosts ~~(~~[48]~~)~~. NiV and HeV can infect at least 18 animal species across 6 orders of mammals, and among these they ~~will~~ cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans ~~(~~[16,49]~~)~~. The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission ~~(~~[5]~~)~~. Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and ~~South East~~Southeast Asia and Australia. Their broad host tropism and ability ~~of these viruses~~ to infect livestock offers an amplification opportunity ~~and~~ for increased risk of ~~subsequent~~ human ~~infections~~spillover. The ~~henipaviruses~~ HNVs are single-stranded, negative sense, enveloped RNA viruses and their biology ~~also~~ lends themselves to be synthetically produced ~~and~~ as agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS). ~~and~~ The Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents ~~[~~[50]~~]~~, because ~~they~~ the virus could be engineered for mass dissemination and it possesses the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the ~~WHO~~ R&D Blueprint list of priority pathogens with epidemic potential ~~(Sweileh, W. M.~~[51]~~)~~.

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While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb exist. ~~-~~ These p~~p~~ostexposure treatments ~~have been demonstrated to be effective therapies~~conferred protecion against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP) ~~(~~[52-55]~~, 53, 54, 55)~~. The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia ~~(~~[56]~~)~~. In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs, and several vaccine platforms have clear potential for use in people[16] ~~(PMC8782152)~~. The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies[16] ~~(PMC8782152)~~. Such preventive vaccines would have utility for lab workers, first responders or individuals at ~~high risk~~high-risk exposure, and in mitigating the spread of an outbreak ~~; whereas~~ . I~~i~~n the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete

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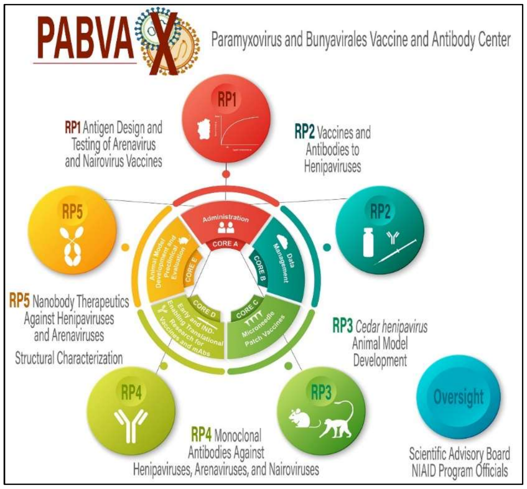
protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent ~~(Middleton, 2014~~[57]~~)~~. It is widely considered that the African green monkey (AGM) is the current "gold standard" animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection[49,58] ~~(Geisbert, 2012;Geisbert, 2010)~~. The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished) ~~(~~[59]~~)~~. The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons~~,~~: 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; and 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. A~~lso, a~~nother divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including ~~the rescue of~~rescuing recombinant LayV (rLayV), ~~explore~~ exploring animal model development, constructing additional LayV and AngV reporter virus tools, and then ~~translate~~ translating and testing the MNP vaccine 'plug-and-play' approach. Finally, we will~~, and also~~ evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME.

A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center's RPs and Core leaders currently have active and productive collaborative research programs in related areas, ~~some~~ most spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.



~~**Figure 1**: Organizational chart of the Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX).~~ ~~**(connecting arrows are needed between elements and the SAB)**~~

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The pandemic preparedness ~~organizational structure~~research gaps addressed by ~~of the~~ PABVAX ~~is~~ are diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **arenaviruses** and **nairoviruses**~~, **Arenaviridae** and **Nairoviridae** viruses~~, and **RP2** will target the prototype **HNVs**~~, **henipaviruses** (HNVs)~~. Both RP1 and RP2 will ~~also be~~ develop~~ing~~ new prototype virus tools (recombinant~~ly generated~~ prototype viruses) along with existing tools, to ~~be used in the analysis of~~analyze vaccine responses ~~as well as in the evaluation of~~and evaluate new mAbs and nabs from the other RPs. A third RP will ~~be engaged in the development of~~develop a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant *Cedar henipavirus* (rCedV) in vivo and a bioluminescence ~~methodology~~ detection method that can longitudinally trace the dynamics and anatomical progression of infection with a luciferase~~-~~ encoding virus ~~infection~~ in individual animals (**RP3**). There will also be two RPs that ~~will target~~focus on the development of broadly reactive human mAbs and nanobodies to the PABVAX selected viral prototypes. **RP4** will isolate human mAbs derived from NiV~~-~~infected survivors with a specific focus on ~~the~~ F ~~glycoprotein~~ glycoprotein-targeting~~ed~~ mAbs. A~~, and a~~dditional human mAbs will be generated from humanized mice by viral prototype antigen ~~-~~immunization. Finally, **RP5** will develop nanobody-

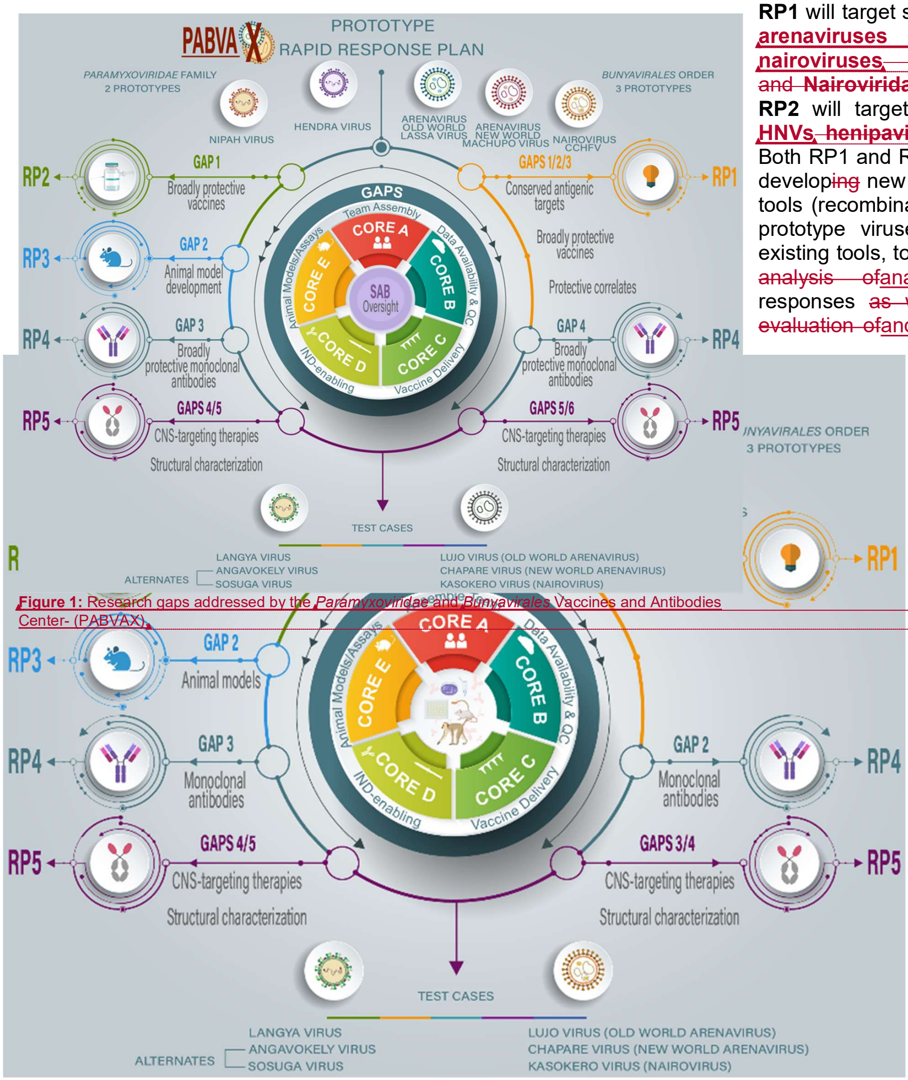

Figure 1: Research gaps addressed by the *Paramyxoviridae* and *Bunyavirales* Vaccines and Antibodies Center- (PABVAX).

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based therapeutics targeting henipaviruses and arenaviruses ~~along ~~with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will e~~be e~~ngage~~d~~ ~~in~~in the manufacture~~ and ~~, quality control, and ~~& ~~testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**). Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core (**Core A**) will provide organizational and programmatic structure to promote research productivity and scientific interactions along with compliance with regulations concerning select agent, vertebrate animal, and human subject use. **Core A** will coordinate all activities with the Scientific Advisory Board (**SAB**) and make data available through the Data Management Core (**Core B**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform.
The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**). This project ~~that has ~~tested a dissolvable MNP-based vaccine technology ~~that is ~~ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine~~ approach (~~[60]~~)~~. ~~This ~~The MNP vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable ~~MNPs ~~microneedles that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants~~ (~~[61]~~)~~ to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as to facilitate ~~the ~~improved vaccine storage and distribution due to their improved thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, ~~a ~~Scientific **Core C**~~:~~ (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ)-adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (*Paramyxoviridae*). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro*, along with ~~and ~~analysis of polyfunctional T-cell responses by ~~in vitro stimulation of isolated cells with ~~antigenic peptide stimulation~~s~~, followed by intracellular cytokine staining (ICS) and flow cytometry~~) responses~~.

Discover effective antibody-based prophylactics and therapies. M
~~PREP can be critical in the absence of available vaccines or cases in which vaccination is not possible.~~
~~HELP~~

~~M~~onoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah[62], Hendra~~-~~[63], Ebola~~-~~[64,65], Marburg~~-~~[66] and Lassa~~-~~[67]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc.*), a feature to be

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exploited in **Core D** by Dr. Gunn. In addition to therapeutic applications, mAbs with Fc mutations ~~to~~ dramatically extend serum half-life~~.~~[68-70] and offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g.* rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization, and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

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Develop new tools and animal models to facilitate the translation of effective countermeasures.

Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. ~~-~~The investigators within the A~~A~~nimal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation S~~s~~cientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including ~~……..~~ animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective ~~therapeutic~~ efficacy of antivirals and vaccines, as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation,~~;~~ including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they are generally less laborious and can be adapted to ~~greater~~ higher ~~-~~throughput platforms~~, are often less laborious, and they can make use of other~~ and/or integrated with other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput fluorescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic NiV~~pah~~ and ~~Hendra~~ HeV ~~virus~~ vaccine~~-~~ induced immune sera, mAbs (**RP4**) ~~and~~, nbs (**RP5**), and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either ~~the~~ HeV or NiV-B envelope GPs~~glycoproteins~~) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals~~, ideally suited~~ to explore nab based therapeutics to CNS resident ~~henipavirus~~HNVs~~(~~[71]~~)~~.

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The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. ~~Data generated to support of possible future regulatory submissions to the Food and Drug Administration (FDA) must be collected in accordance with quality systems, like good laboratory practice (GLP) regulations, to be useful and reliable, and to facilitate FDA review to ensure safety and efficacy.~~ The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the ~~henipaviruses~~ HNVs (*Paramyxoviridae*) and the *Bunyavirales* (*Arenaviridae* and *Nairoviridae*) that will bolster vaccine endurance to storage and transportation; ~~and~~ we also anticipate improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

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The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology, and mAb and vaccine translational drug development along with ~~and includes~~ Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

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THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

***~~Henipaviruses~~Paramyxoviruses*:** ~~Nipah virus (~~NiV ~~)~~ and ~~Hendra virus (~~HeV~~)~~ are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis[4,46] ~~(Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia)~~. HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals. T~~, and~~ hese viruses are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of ~~Cedar virus (~~CedV~~)~~, Gamak virus, Daeryong virus, and ~~Langya virus~~ (LayV)[72] a ~~[xxx] a~~nd five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[73,74] ~~[xxx]~~. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV, are old world pteropid fruit bats[73,75]. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province)[17]. The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**.

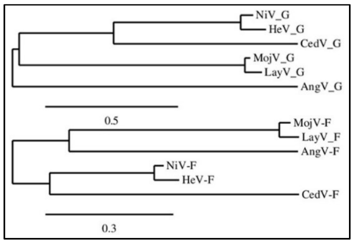

Figure 2: Phylogenetic analysis of select HNV G and F glycoproteins. Trees were constructed for using the PhyML program's maximum likelihood method and aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

~~**Figure 2. Phylogenetic analysis of select HNV G and F glycoproteins**. Phylogenetic trees were constructed for the HNV F and G glycoproteins using the PhyML program's maximum likelihood method. The trees were generated by aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.~~

Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal ~~henipaviral~~ HNV disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters[49] ~~(Geisbert 2012)~~, and ~~African green monkeys~~AGMs (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P)~~, and T~~. The P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists[76-80] ~~(xx).~~ . The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections[81,82]. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature[83]. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2[84] ~~(XXX)~~ and CedV is now recognized as a BSL-2 restricted agent[85] ~~[CDC manual]~~. Because the HNV, LayV, is known

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Lee, S.H.; Kim, K.; Viruses 2021, 13, doi:10.3390/v13102020.

Zhang, X.A.; Li, H.; Jiang, N Engl J Med 2022, 387, 470-472, doi:10.1056/NEJMc2202705.

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Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

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Laing, E.D.; Navaratnarajah, C.K.; Proc Natl Acad Sci U S A 2019, doi:10.1073/pnas.1911773116.

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to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D,** and **E**).

Arenaviruses (old world)~~Old Word Mammarenaviruses (OWAV)~~: For **phase 1**, **RP1** will utilize **LASV as the prototype ~~OWAV~~virus**, due ~~to the~~its significant public health burden ~~it creates~~ throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[86]. LASV ~~has~~ is the most imported viral hemorrhagic fever (VHF) next to D~~d~~engue virus and has been ~~imported to~~documented in several countries outside of Africa over 35 ~~documented~~ times[87]. –C~~ase fatality rates (C~~FR~~s)~~ approaching 70% in hospitalized cases and marked long term sequelae are the result of ~~no~~the lack of approved vaccines or therapeutics, although several live attenuated virus candidates are ~~approaching clinical evaluation~~currently being evaluated with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI)[88]. –Of the vaccines supported by CEPI, 4/5 of these vaccines utilize ~~the~~ only ~~the~~ LASV GP as the target immunogen ~~despite~~.

Investigators in **RP1**, **Core D**, and **Core E** have already demonstrated protective efficacy ~~of~~ against ~~disease after~~ challenge with LASV in outbred guinea pigs ~~in animals~~ vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. –These ~~antigens~~immunogens have been engineered to ~~harness~~express ~~the~~ homologous antigenic regions ~~of both antigens~~ to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach~~,~~ suggests a similar strategy ~~of using a subunit vaccine might also be effective against~~could be implemented against other arenaviruses. T~~While LASV is the most common pathogenic arenavirus in Africa in terms of human infections, t~~he **OWAV test pathogen, ~~Lujo virus~~LUJV,** was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia ~~but~~, which quickly ~~causing~~spread nosocomially~~ infections in care givers after the index case's medical evacuation to South Africa~~[89]. The ~~case fatality rate~~CFR for this ~~incredibly~~ infectious virus was 80% in thi~~s~~e small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from ~~LASV~~ suggest current LASV countermeasures ~~with antigenic targets~~ may be of limited use~~, implying~~. LUJV will ~~be~~serve as an ideal "test case" ~~OWAV~~ to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. -The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

~~New World Mamarenaviruses (NWAV~~Arenaviruses (new world)~~)~~: The~~A~~ live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina[90], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine[91,92]. Furthermore, there ~~are safety concerns related to~~ is the potential for pathogenic reversion ~~to pathogenic versions~~. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest exploring other ~~approaches~~ JUNV countermeasures are warranted. Building on the success of the LASV GPe/NP-NTD~~,~~ -~~i~~Investigators in **RP1 and Cores D and E** will engineer broadly protective MACV GPe and NP-NTD antigens and ~~test for~~perform efficacy testing in established guinea pig and NHP models ~~using MACV from multiple lineages to asses how broadly protective the approach is across MACV lineages~~. We will ~~then~~ extend this plug-and-play approach to ~~other~~ the **NWAV test case, CHAPV**. First reported in 2003 in Bolivia, ~~Chapare virus (~~CHAPV~~)~~ is a rodent-~~-~~borne mammarenavirus associated with the development of severe hemorrhagic fever with case fatality rates up to 60%[93,94]. Recent studies have demonstrated clear human-to-human transmission ~~risk~~ during the acute phase evidenced by several nosocomial infections~~ but also ~~; ~~detection~~isolation of viable virus from semen at 86 days after symptom onset ~~demonstrating~~demonstrates a clear need to implement specialized management and treatment[94]. ~~It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus.~~ The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

N~~Orthon~~airoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as it represents ~~is responsible for~~ the largest global public health burden within the orthonairovirus family ~~responsible for often large~~ with outbreaks ~~of human disease~~ annually across Europe, Africa, and Asia. There are no FDA-~~-~~approved vaccines for CCHFV; however, a suckling mouse brain-~~-~~derived, ~~CHCl2/heat-inactivated aluminum hydroxide adsorbed~~adjuvanted whole virus vaccine for CCHFV has been approved for use ~~only~~ in Bulgaria. This vaccine has ~~since 1974 but has~~ numerous safety concerns and has limited demonstrated efficacy. ~~Since then,~~S~~s~~everal

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experimental vaccine approaches have been e~~xamined~~valuated ranging from viral vectored approaches to DNA-based vaccines with varying efficacy, advantages, and disadvantages[95]; however, only a Modified Vaccinia Ankara virus vaccine (~~n~~ MVA-CCHFV ~~vaccine~~) has begun to advance towards clinical trials[96]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test these for protective efficacy from ~~disease after~~ challenge with 6 different ~~lineages of~~ CCHFV ~~clades~~lineages using ~~in~~ established mouse and NHP models. Next, we will deploy the same approach to ~~a known human pathogen~~the **"test case"** ~~ortho~~nairovirus, **Kasokero virus (KASV)**, for which no vaccine exists.

In 1977, ~~Kasokero virus (KASV), a bat borne Orthonairovirus,~~KASV was first isolated from Ugandan bats and ~~responsible for~~ caus~~ing~~ed several cases of acute febrile illness of laboratory workers from the same lab that processed the infected bats[97]. ~~Sero~~S~~s~~urveys of 109 laboratory workers from the same facility ~~the virus was isolated and worked with~~ demonstrated ~~a~~ 13% seropositivity and ~~where medical surveys revealed~~ clinical symptoms in individuals 4 to 15 months after the virus was isolated. These findings~~,~~ suggest~~ting~~ either continued laboratory-associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the tick reservoir, *Ornithodoros faini*, ~~ticks~~ to participate in enzootic maintenance of KASV[98]. While most chiropteran ticks exhibit host specificity, humans entering caves ~~where~~ these bats inhabit ~~are~~ have reported bites from *O.faini* ticks ~~as well~~. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics ~~make~~ suggest investment in countermeasure development against this virus is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs~~;~~ such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**~~), (~~; ~~a~~arenavirus and nairovirus~~renaviridae and nairoviridae~~ projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine ~~the~~ active and passive immunization approaches to further improve~~d~~ the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

An additional and outstanding advantage of the PABVAX Center is the **Animal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL~~.~~ (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies.

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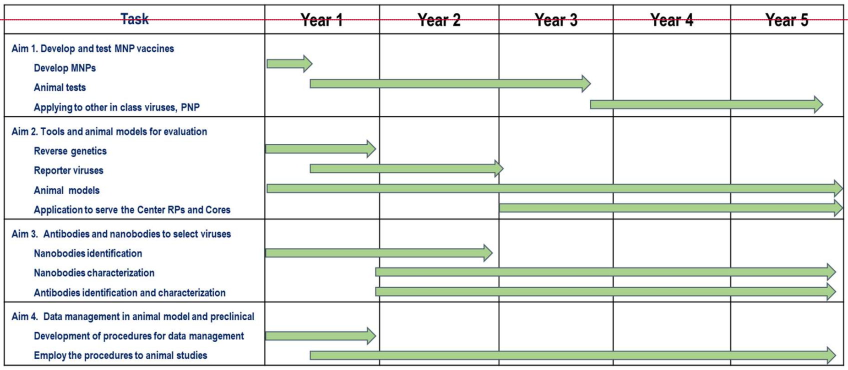
All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for ~~all~~ the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for members of the *Bunyavirales* and *Paramyxoviridae*.**
2. **Develop new tools and animal models for the conduct of cell-based and *in vivo* therapeutic~~s~~ evaluation.**
3. **Develop antibodies and nanobodies to the selected prototype and test viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

~~The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.~~



~~**Figure 3**: Projected PABVAX timeline to achieve the 4 overarching tasks.~~

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.

Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. ~~This is in addition to each PR's individual Milestone plan.~~ The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (d~~D~~etails are specified within the Administration **Core A** Research Strategy). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommending new directions as appropriate, and providing~~e~~ Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

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Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

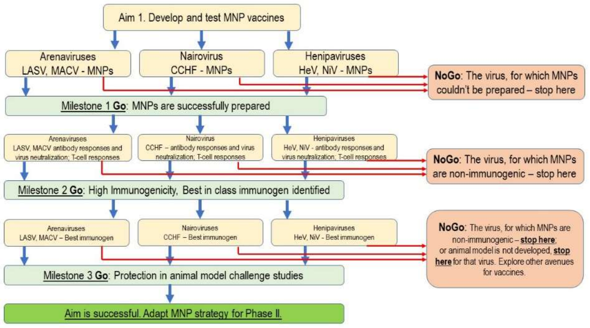

Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

~~**Figure 4.** Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.~~

The first milestone will ~~be~~ address whether ~~proper~~ formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be ~~made based~~established ~~on~~ using immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen~~-~~ specific antibody induction ~~and~~ , virus ~~-~~neutralization, and polyfunctional T-cell responses by *in vitro* stimulation of isolated cells). Go/no go decisions will be made based on ~~the~~ outcomes of these ~~first tier~~first-tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as a success. Comparisons will also be made~~,~~ such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of the best-in-class immunogens, or ~~best~~ immunogen combination~~n immunogen~~s, ~~which~~ will also be explored~~, will be performed in order~~ to down-select vaccine~~-s~~choice. The third milestone will ~~be the determination~~determine ~~of~~ whether MNP vaccination affords protection in animal model challenge experiments. In the case of ~~the~~ NiV and HeV prototypes, the animal models chosen (ferrets and ~~the African green monkey (AGM)~~AGMs) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales*, there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASV~~A~~ and ~~there are~~ guinea pig models exist for some but not all of ~~those~~ these lineages. For MACV, there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys ~~are~~ now ~~each greater than~~exceed costs of $35K each. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of ~~the~~ AGM models for ~~the~~ arenaviruses and possibly ~~also for~~ CCHFV, as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.~~-~~

~~**Figure 5.** Milestones and criteria for progress for **Aim 2** of the PABVAX Center overall objectives.~~

The ~~PABVAX~~ second overall objective of PABVAX is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by

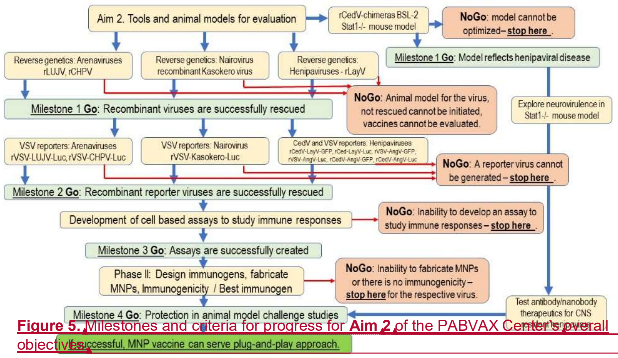

Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center's overall objectives

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reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, ~~a~~ a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing of the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**). T~~t~~he milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new *in vivo* bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease *in vivo* safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**), it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and **RP3** will collaborate with **RP5** in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

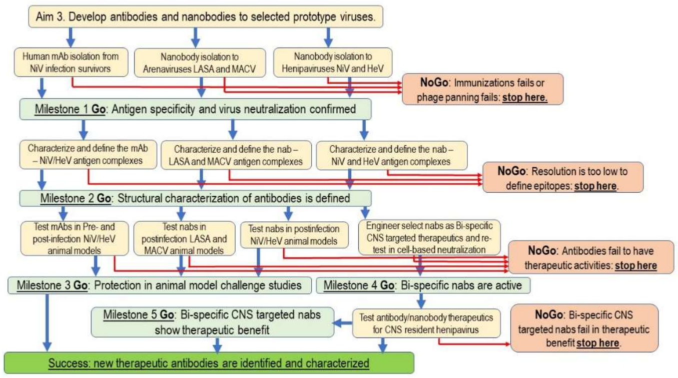

Figure 5. Milestones and criteria for progress for **Aim 3** of the PABVAX Center's overall objectives.

~~**Figure 6.** Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.~~

New human mAbs and ~~nanobodies~~ ~~(~~nbs~~)~~ as tools and potential therapeutics will be developed by **RP4** and **RP5** in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); ~~and~~ transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on ~~the~~ mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs, and in collaboration with **RP2**, neutralization potencies will be rapidly assessed (as well as evaluating synergistic cell-based mAb neutralization) that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, ~~-~~and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

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~~Nanobodies~~Nbs, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, ~~nanobodies~~ nbs can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of ~~nanobodies~~ nbs targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), ~~and also for~~ therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus), and select animal models of LASV and MACV by **Core E**. Success will be measured by the 1) identification of potent virus-neutralizing nbs, 2) ~~potential~~ discovery of more broadly reactive nbs~~,~~ particularly to the multiple LASV~~A~~ clades, 3) ~~the~~ evidence of therapeutic benefit in animal models, and 4) the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.~~-~~

I~~NDUSTRY~~ndustry ~~PARTNERSHIPS~~Partnerships

~~Industry Partnership~~

. Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner ~~which~~ that will provide access to vaccine

expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience in developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX.

~~SPECIAL FEATURES OF THE PABVAX CENTER ENVIRONMENT AND OTHER RESOURCES~~ ??

Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

Galveston National Laboratory (GNL).

The GNL~~,~~ is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics, and therapeutics ~~against them~~. The 7-story GNL offers over

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12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370~~187~~ separate NHP studies employing a total of 2,772~~1,318~~ animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses ~~Ebola and Marburg viruses and also Nipah and Hendra~~ have been successfully performed at the GNL. ~~-~~These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder, and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP~~nonhuman primate~~ facility and a staff of full-time veterinarians. ~~-~~In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of the WRCEVA virus collection within the GNL.

Core E (Animal model development and preclinical evaluation).

Core E operations will be conducted at the GNL, and in conjunction with **Core A**~~-~~, will also include~~s~~ the UTMB Institutional Office of Regulated Nonclinical Studies (~~ONcRS~~ORNcS). The ORNcS~~ONcRS~~'s primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, ~~to meet regulatory requirements by assuring regulatory compliance,~~ quality assurance, and data management. ~~-~~NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. ~~Quality system and regulatory science processes for NHP studies performed at UTMB will be implemented and executed in conjunction with the ORNcS.~~ Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL, and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses, arenaviruses, and ~~the~~ henipaviruses~~ and~~. H~~h~~e has published over 250~~XXX~~ peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI), was the first to develop a NHP model of NiV/HeV infection and disease, and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs).

Mapp Biopharmaceutical has deep experience with antigens and ~~monoclonal antibodies (~~mAbs~~)~~ against these antigens. ~~'s product development team has grown significantly in the last two years in response to the development efforts on its anti-Ebola therapeutic, ZMapp™ which is being advanced to licensure as part of Project Bioshield. OTHER MAbs~~ Their early development and research group has expertise in immunology, infectious diseases, and the production of antigens, antibodies, and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [99] [100]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1~~-~~[101]. ~~-~~Dr. Gunn and Genovac will join the team to analyze antibody Fc effector functions and to provide antigen/antibody discovery resources, respectively. ~~developed multiple products, including a licensed biodefense biologic. As with Profectus, Mapp Bio's expertise and experience will be a very important and valuable asset to the Center.~~

~~Bonnie – Genovac~~

Core A (Administrative Core)

~~***Research Project teams?? Maybe not depending on space***~~

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The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews, and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills, and his experience in translational research activities, qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe's (**RP4**) research focuses on the molecular basis for the development of effective B cell responses to viruses in humans~~ and~~. H~~h~~e has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently ~~henipaviruses~~ HNVs NiV and HeV (owing to the collaboration between the Crowe, Broder and Geisbert laboratories). Perhaps of greatest import, the SARS-CoV-2 mAbs generated by Dr. Crowe's laboratory were used in ~~xxx,000~~thousands of people during the COVID-19 pandemic. RP4 will work closely with Cores D and E in advancing mAb prototypes. ~~The Core B of the Center will work together with RP2 and RP3 along with the Core C to develop the first cocktail human mAb immunotherapeutics, and facilitate the transition products suitable for IND filing and human testing including design, construction, assessment, and optimization of these antibody-based therapies.~~

As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

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1. Lamb RA, Parks GD. Paramyxoviridae: the viruses and their replication. In Fields BN, Knipe DM, Howley PM, editors, Fields Virology: Sixth Edition. Vol. 1. Lippincott, Williams, and Wilkins. 2013. p. 957-995.
2. Boshra H. An Overview of the Infectious Cycle of Bunyaviruses. *Viruses*. Sep 28 2022;14(10)doi:10.3390/v14102139
3. Horne KM, Vanlandingham DL. Bunyavirus-vector interactions. *Viruses*. Nov 13 2014;6(11):4373-97. doi:10.3390/v6114373
4. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia), p 1070-1085.
5. Luby SP. The pandemic potential of Nipah virus. *Antiviral research*. Oct 2013;100(1):38-43. doi:10.1016/j.antiviral.2013.07.011
6. Skowron K, Bauza-Kaszewska J, Grudlewska-Buda K, et al. Nipah Virus-Another Threat From the World of Zoonotic Viruses. *Front Microbiol*. 2021;12:811157. doi:10.3389/fmicb.2021.811157
7. Kummer S, Kranz DC. Henipaviruses-A constant threat to livestock and humans. *PLoS Negl Trop Dis*. Feb 2022;16(2):e0010157. doi:10.1371/journal.pntd.0010157
8. Gouglas D, Christodoulou M, Plotkin SA, Hatchett R. CEPI: Driving Progress Toward Epidemic Preparedness and Response. *Epidemiol Rev*. Jan 31 2019;41(1):28-33. doi:10.1093/epirev/mxz012
9. Maes P, Adkins S, Alkhovsky SV, et al. Taxonomy of the order Bunyavirales: second update 2018. *Arch Virol*. Mar 2019;164(3):927-941. doi:10.1007/s00705-018-04127-3
10. Silva-Ramos CR, Montoya-Ruíz C, Faccini-Martínez Á A, Rodas JD. An updated review and current challenges of Guanarito virus infection, Venezuelan hemorrhagic fever. *Arch Virol*. Sep 2022;167(9):1727-1738. doi:10.1007/s00705-022-05453-3
11. Garry RF. Lassa fever - the road ahead. *Nat Rev Microbiol*. Feb 2023;21(2):87-96. doi:10.1038/s41579-022-00789-8
12. Mehand MS, Al-Shorbaji F, Millett P, Murgue B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research*. Nov 2018;159:63-67. doi:10.1016/j.antiviral.2018.09.009
13. WHO. List of Blueprint Priority Diseases. Accessed November 14, 2018. http://www.who.int/blueprint/priority-diseases/en/
14. NIH-NIAID. NIAID Emerging Infectious Diseases/ Pathogens. Accessed 04 Jun 2023, https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens
15. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol*. Jan 2006;4(1):23-35. doi:10.1038/nrmicro1323
16. Amaya M, Broder CC. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol*. Sep 29 2020;7(1):447-473. doi:10.1146/annurev-virology-021920-113833
17. Zhang XA, Li H, Jiang FC, et al. A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med*. Aug 4 2022;387(5):470-472. doi:10.1056/NEJMc2202705
18. Radoshitzky SR, Buchmeier MJ, Charrel RN, et al. ICTV Virus Taxonomy Profile: Arenaviridae. *J Gen Virol*. Aug 2019;100(8):1200-1201. doi:10.1099/jgv.0.001280
19. Hallam SJ, Koma T, Maruyama J, Paessler S. Review of Mammarenavirus Biology and Replication. *Front Microbiol*. 2018;9:1751. doi:10.3389/fmicb.2018.01751
20. Forni D, Pontremoli C, Pozzoli U, Clerici M, Cagliani R, Sironi M. Ancient Evolution of Mammarenaviruses: Adaptation via Changes in the L Protein and No Evidence for Host-Virus Codivergence. *Genome Biol Evol*. 2018/03// 2018;10(3):863-874. doi:10.1093/gbe/evy050
21. Shaffer JG, Grant DS, Schieffelin JS, et al. Lassa Fever in Post-Conflict Sierra Leone. *PLOS Neglected Tropical Diseases*. 2014;8(3):e2748. doi:10.1371/journal.pntd.0002748
22. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. Review. *Antiviral research*. Apr 2008;78(1):132-9. doi:10.1016/j.antiviral.2007.10.010
23. Kilgore PE, Peters CJ, Mills JN, et al. Prospects for the control of Bolivian hemorrhagic fever. *Emerg Infect Dis*. Jul-Sep 1995;1(3):97-100. doi:10.3201/eid0103.950308

24. Heinrich ML, Boisen ML, Nelson DKS, et al. Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages. *Scientific Reports*. 2020/09/29 2020;10(1):16030. doi:10.1038/s41598-020-72539-w
25. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nature Medicine*. 2017/10/01 2017;23(10):1146-1149. doi:10.1038/nm.4396
26. Hastie KM, Zandonatti MA, Kleinfelter LM, et al. Structural basis for antibody-mediated neutralization of Lassa virus. *Science*. Jun 2 2017;356(6341):923-928. doi:10.1126/science.aam7260
27. Sullivan BM, Sakabe S, Hartnett JN, et al. High crossreactivity of human T cell responses between Lassa virus lineages. *PLoS Pathog*. Mar 2020;16(3):e1008352. doi:10.1371/journal.ppat.1008352
28. Ugwu C, Olumade T, Nwakpakpa E, et al. Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria. *Scientific Reports*. 2022/12/25 2022;12(1):22330. doi:10.1038/s41598-022-26045-w
29. Geisbert TW, Jones S, Fritz EA, et al. Development of a New Vaccine for the Prevention of Lassa Fever. *PLOS Medicine*. 2005;2(6):e183. doi:10.1371/journal.pmed.0020183
30. Cross RW, Woolsey C, Prasad AN, et al. A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever. *Cell Rep*. Jul 19 2022;40(3):111094. doi:10.1016/j.celrep.2022.111094
31. Safronetz D, Mire C, Rosenke K, et al. A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses. *PLOS Neglected Tropical Diseases*. 2015;9(4):e0003736. doi:10.1371/journal.pntd.0003736
32. Hensley LE, Smith MA, Geisbert JB, et al. Pathogenesis of Lassa fever in cynomolgus macaques. *Virol J*. May 6 2011;8:205. doi:10.1186/1743-422x-8-205
33. Zeitlin L, Cross RW, Geisbert JB, et al. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. *Proceedings of the National Academy of Sciences*. 2021;118(11):e2023332118. doi:doi:10.1073/pnas.2023332118
34. Bell TM, Shaia CI, Bunton TE, et al. Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure. *Veterinary Pathology*. 2015;52(1):26-37. doi:10.1177/0300985814540544
35. Burt FJ, Spencer DC, Leman PA, Patterson B, Swanepoel R. Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia. *Epidemiol Infect*. Jun 1996;116(3):353-61. doi:10.1017/s0950268800052687
36. Brinkmann A, Kohl C, Radonić A, et al. First detection of bat-borne Issyk-Kul virus in Europe. *Sci Rep*. Dec 24 2020;10(1):22384. doi:10.1038/s41598-020-79468-8
37. Ma J, Lv XL, Zhang X, et al. Identification of a new orthonairovirus associated with human febrile illness in China. *Nat Med*. Mar 2021;27(3):434-439. doi:10.1038/s41591-020-01228-y
38. Lorenzo Juanes HM, Carbonell C, Sendra BF, et al. Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021. *Emerg Infect Dis*. Feb 2023;29(2):252-259. doi:10.3201/eid2902.220677
39. Grandi G, Chitimia-Dobler L, Choklikitumnuey P, et al. First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden. *Ticks Tick Borne Dis*. May 2020;11(3):101403. doi:10.1016/j.ttbdis.2020.101403
40. Hawman DW, Feldmann H. Crimean–Congo haemorrhagic fever virus. *Nature Reviews Microbiology*. 2023/03/14 2023;doi:10.1038/s41579-023-00871-9
41. Keshtkar-Jahromi M, Kuhn JH, Christova I, Bradfute SB, Jahrling PB, Bavari S. Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies. *Antiviral research*. May 2011;90(2):85-92. doi:10.1016/j.antiviral.2011.02.010
42. Soares-Weiser K, Thomas S, G GT, Garner P. Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis. *BMC Infectious Diseases*. 2010/07/13 2010;10(1):207. doi:10.1186/1471-2334-10-207
43. Rodriguez SE, Cross RW, Fenton KA, Bente DA, Mire CE, Geisbert TW. Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever. *Sci Rep*. May 23 2019;9(1):7755. doi:10.1038/s41598-019-44210-6
44. Bente DA, Alimonti JB, Shieh WJ, et al. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol*. Nov 2010;84(21):11089-100. doi:10.1128/jvi.01383-10
45. Cross RW, Prasad AN, Borisevich V, et al. Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys. *PLoS Negl Trop Dis*. Aug 2020;14(8):e0008637. doi:10.1371/journal.pntd.0008637

46. Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York, p 519-536.

47. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science*. Apr 7 1995;268(5207):94-7. doi:10.1126/science.7701348

48. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science (New York, NY)*. 2000/05// 2000;288(5470):1432-1435. doi:10.1126/science.288.5470.1432

49. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol*. 2012;359:153-77. doi:10.1007/82_2012_208

50. *Department of Health and Human Services. Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087*.

51. Sweileh WM. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health*. Feb 8 2017;13(1):9. doi:10.1186/s12992-017-0233-9

52. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Science translational medicine*. 2011/10// 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901

53. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine*. 2014/06// 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929

54. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep*. Aug 3 2016;6:30916. doi:10.1038/srep30916

55. Mire CE, Chan YP, Borisevich V, et al. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. *J Infect Dis*. May 11 2020;221(Suppl 4):S471-s479. doi:10.1093/infdis/jiz515

56. Playford EG, Munro T, Mahler SM, et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*. Apr 2020;20(4):445-454. doi:10.1016/s1473-3099(19)30634-6

57. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerging infectious diseases*. 2014/03// 2014;20(3):372-379. doi:10.3201/eid2003.131159

58. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One*. May 18 2010;5(5):e10690. doi:10.1371/journal.pone.0010690

59. Cheliout Da Silva S, Yan L, Dang HV, et al. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses*. Mar 22 2021;13(3)doi:10.3390/v13030517

60. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo LD, Jr. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. *Expert Opin Drug Deliv*. Feb 2021;18(2):151-167. doi:10.1080/17425247.2021.1823964

61. Andrianov AK, DeCollibus DP, Gillis HA, et al. Poly[di(carboxylatophenoxy)phosphazene] is a potent adjuvant for intradermal immunization. *Proc Natl Acad Sci U S A*. Nov 10 2009;106(45):18936-41. doi:10.1073/pnas.0908842106

62. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med*. Jun 25 2014;6(242):242ra82. doi:10.1126/scitranslmed.3008929

63. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med*. Oct 19 2011;3(105):105ra103. doi:10.1126/scitranslmed.3002901

64. Pettitt J, Zeitlin L, Kim do H, et al. Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail. *Sci Transl Med*. Aug 21 2013;5(199):199ra113. doi:10.1126/scitranslmed.3006608

65. Qiu X, Wong G, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature*. Oct 2 2014;514(7520):47-53. doi:10.1038/nature13777

66. Mire CE, Geisbert JB, Borisevich V, et al. Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci Transl Med*. Apr 5 2017;9(384)doi:10.1126/scitranslmed.aai8711

67. Mire CE, Cross RW, Geisbert JB, et al. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. *Nat Med*. Oct 2017;23(10):1146-1149. doi:10.1038/nm.4396

68. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. *mAbs*. Sep-Oct 2011;3(5):422-30. doi:10.4161/mabs.3.5.16983

69. Robbie GJ, Criste R, Dall'acqua WF, et al. A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study. *Antimicrobial agents and chemotherapy*. Sep 30 2013;doi:10.1128/aac.01285-13

70. Gaudinski MR, Coates EE, Houser KV, et al. Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults. *PLoS Med*. Jan 2018;15(1):e1002493. doi:10.1371/journal.pmed.1002493

71. Mastraccio KE, Huaman C, Laing ED, Broder CC, Schaefer BC. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. *Methods Mol Biol*. 2022;2524:369-394. doi:10.1007/978-1-0716-2453-1_30

72. Rima B, Balkema-Buschmann A, Dundon WG, et al. ICTV Virus Taxonomy Profile: Paramyxoviridae. *J Gen Virol*. Dec 2019;100(12):1593-1594. doi:10.1099/jgv.0.001328

73. Madera S, Kistler A, Ranaivoson HC, et al. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol*. Sep 28 2022;96(18):e0092122. doi:10.1128/jvi.00921-22

74. Vanmechelen B, Meurs S, Horemans M, et al. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol*. 2022;8(2):veac061. doi:10.1093/ve/veac061

75. Voigt K, Hoffmann M, Drexler JF, et al. Fusogenicity of the Ghana Virus (Henipavirus: Ghanaian bat henipavirus) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. *Viruses*. Aug 29 2019;11(9)doi:10.3390/v11090800

76. Childs K, Randall R, Goodbourn S. Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction. *J Virol*. Apr 2012;86(7):3411-21. doi:10.1128/jvi.06405-11

77. Davis ME, Wang MK, Rennick LJ, et al. Antagonism of the phosphatase PP1 by the measles virus V protein is required for innate immune escape of MDA5. *Cell Host Microbe*. Jul 9 2014;16(1):19-30. doi:10.1016/j.chom.2014.06.007

78. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol*. Nov 2002;76(22):11476-83. doi:10.1128/jvi.76.22.11476-11483.2002

79. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol*. May 2005;79(10):6078-88. doi:10.1128/jvi.79.10.6078-6088.2005

80. Basler CF. Nipah and hendra virus interactions with the innate immune system. *Curr Top Microbiol Immunol*. 2012;359:123-52. doi:10.1007/82_2012_209

81. Yoneda M, Guillaume V, Sato H, et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One*. Sep 15 2010;5(9):e12709. doi:10.1371/journal.pone.0012709

82. Satterfield BA, Cross RW, Fenton KA, et al. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J Virol*. Jul 15 2016;90(14):6326-6343. doi:10.1128/jvi.00215-16

83. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog*. 2012;8(8):e1002836. doi:10.1371/journal.ppat.1002836

84. Amaya M, Cheng H, Borisevich V, et al. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research*. Sep 2021;193:105084. doi:10.1016/j.antiviral.2021.105084

85. CDC. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. . Updated June 2020. https://www.cdc.gov/labs/pdf/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

86. Organization WH. Lassa fever fact sheet. *World Health Organization*. 2017;

87. Wolf T, Ellwanger R, Goetsch U, Wetzstein N, Gottschalk R. Fifty years of imported Lassa fever: a systematic review of primary and secondary cases. *J Travel Med*. Jul 14 2020;27(4)doi:10.1093/jtm/taaa035

88. Purushotham J, Lambe T, Gilbert SC. Vaccine platforms for the prevention of Lassa fever. *Immunol Lett*. Nov 2019;215:1-11. doi:10.1016/j.imlet.2019.03.008

89. Sewlall NH, Richards G, Duse A, et al. Clinical features and patient management of Lujo hemorrhagic fever. *PLoS Negl Trop Dis*. 2014;8(11):e3233. doi:10.1371/journal.pntd.0003233

90. Saito T, Reyna RA, Taniguchi S, Littlefield K, Paessler S, Maruyama J. Vaccine Candidates against Arenavirus Infections. *Vaccines*. 2023;11(3):635.

91. Koma T, Patterson M, Huang C, et al. Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic. *J Virol*. Feb 1 2016;90(3):1290-7. doi:10.1128/jvi.02615-15

92. Jahrling P TR, Barrero O. . Crossprotection against Machupo virus with Candid 1 Junin virus vaccine. 1988:53.
93. Delgado S, Erickson BR, Agudo R, et al. Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia. *PLOS Pathogens*. 2008;4(4):e1000047. doi:10.1371/journal.ppat.1000047
94. Loayza Mafayle R, Morales-Betoulle ME, Romero C, et al. Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019. *New England Journal of Medicine*. 2022;386(24):2283-2294. doi:10.1056/NEJMoa2110339
95. Tipih T, Burt FJ. Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development. *Biores Open Access*. 2020;9(1):137-150. doi:10.1089/biores.2019.0057
96. UHS-NHS. Healthy volunteers needed for research investigating world-first vaccine for viral disease. 2023. Accessed 03/06, 2023. https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease
97. Kalunda M, Mukwaya LG, Mukuye A, et al. Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda. *The American Journal of Tropical Medicine and Hygiene*. 01 Mar. 1986 1986;35(2):387-392. doi:10.4269/ajtmh.1986.35.387
98. Schuh AJ, Amman BR, Patel K, Sealy TK, Swanepoel R, Towner JS. Human-Pathogenic Kasokero Virus in Field-Collected Ticks. *Emerg Infect Dis*. Dec 2020;26(12):2944-2950. doi:10.3201/eid2612.202411
99. Mulangu S, Dodd LE, Davey RT, Jr., et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. *N Engl J Med*. Dec 12 2019;381(24):2293-2303. doi:10.1056/NEJMoa1910993
100. Group PIW, Multi-National PIIST, Davey RT, Jr., et al. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. *N Engl J Med*. Oct 13 2016;375(15):1448-1456. doi:10.1056/NEJMoa1604330
101. Politch JA, Cu-Uvin S, Moench TR, et al. Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial. *PLoS Med*. Feb 2021;18(2):e1003495. doi:10.1371/journal.pmed.1003495

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Page 5: [29] Commented [CRW3] **Cross, Robert W.** **6/3/2023 11:55:00 PM**

Tom asked me to insert this to see how it would look so we can see what changes are needed to ensure legibility since the figure needed to get shrunk to fit. Some of the graphics dead center might be a little too small, not sure anything can be done there…

Maybe be good to have the whole title per RP listed instead of abbreviated form?

May need to identify what is meant by Gaps as its not identified in this document, I think maybe it is spelled out in Core E document?. Assuming maybe these are Gaps identified in the main RFA?

Just an idea…. having RP4 and RP5 bubbles duplicated to demonstrate different GAPs addressed is a little confusing to me, maybe if we do end up enumerating the gaps somewhere in the text, it might simplify things by identify what gaps are addressed by each RP so that each RP only has one bubble? EG:

- RP1=gap 1
- RP2=gap 1
- RP3=gap 2
- RP4=gap 2,3
- RP5= gap 3,4,5

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Page 5: [31] Commented [LZ4R3] **Larry Zeitlin** **6/4/2023 7:05:00 AM**

It would be nice if the emergent/moderna logos can be squeezed in somewhere…

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Page 7: [64] Commented [CB5] **CHRISTOPHER BRODER** **5/31/2023 8:30:00 AM**

Larry/Dafna you are needed for this.

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Page 7: [66] Commented [LZ6R5] **Larry Zeitlin** **6/1/2023 8:56:00 AM**

Here is something though we probably need a few sentences on nanobodies…

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CENTER OVERVIEW: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction

Commented [CB1]: I think as you review this doc, perhaps specifically name the PIs and PLs in certain areas?

Emerging and periodically reemerging pathogenic enveloped RNA viruses are ~~a~~ continuous ~~infectious disease and potentially~~ pandemic threats to global public health. Among the many groups of these types of pathogens are viral zoonoses within the family **Paramyxoviridae** and order ***Bunyavirales*** where humans can acquire infections directly from animal reservoir hosts such as rodents or bats~~,~~ through an intermediate amplifying host such as livestock, or from arthropod vectors such as mosquitos and ticks (Fields virology). More often than not, humans represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur, and should RNA viral pathogens mutate and adapt to more efficient replication and shedding in the human host, the epidemic potential of these viruses could manifest (PMID: 23911335 -Nipah / others?). The viral order *Bunyavirales* is composed of several viral families known to contain multiple high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic fever diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and are also identified as NIAID Category A pathogens due to their ease of dissemination and/or abilities to transmit person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special actions for public health preparedness. Also, within the WHO Priority Pathogens list is Nipah virus (NiV) and henipaviral disease which includes Hendra virus (HeV) which are zoonotic henipaviruses (HNVs) in the family **Paramyxoviridae**. NiV and HeV have long been considered the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease (PMID: 16357858). The genus now includes nine additional reported HNVs but only HeV and NiV are known to be associated with severe and often fatal henipaviral disease in humans and a number of animal species (PMID: 32991264). Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illnesses in humans [PMID: 35921459].

Taken together, these viral threats to global public health are heightened due to the absence of approved vaccines or therapeutics. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for vaccine and monoclonal antibody (mAb) countermeasures in developing prototype candidates (**Table 1**) against a selection of these viruses. In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Table 1. PABVAX selected prototype and test case viral agents.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old world), Machupo (New world)	Lujo (Old world), Chapare (New world)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Bunyavirales:

Arenaviridae: The family Arenaviridae is composed of three genera: Mammarenaviridae, Reptarenavirus, and Hartmanivirus. The Mammarenaviridae are composed of a large number rodent borne viruses, some of which are demonstrated human pathogens responsible for causing severe hemorrhagic fever, neurological damage, and if not fatal can result permanent neurological sequelae including hearing loss, vision impairment, and balance [1]derangement. While these genera exhibit a global distribution, two geographically distinct sub-genera have formed due to the separation across continents by the Atlantic and Pacific Ocean. Old World Mammarenaviruses (Lassa Virus (LASV), Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered Lujo Virus (LUJV)) and the New World Arenaviruses (Junin Virus (JUNV), Machupo Virus (MACV), Sabia Virus (SABV), and the newly emerging Chapare Virus (CHAPV)). Many of these viruses are identified as NIAID Category A pathogens requiring biosafety level 4 (BSL4) containment [2]. This classification is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness. While several

promising medical countermeasures are in development, there are currently no US FDA approved vaccines or therapeutics for any mammarenaviruses, though the attenuated JUNV “Candid-1” vaccine is approved for use in Argentina.

Infection in humans is often the result of exposure to rodent excreta from infected animals where disease severity and case fatality rates (CFR) can vary across the genera and even within genetic lineages as can be the case for infections with LASV where genetic diversity can be high, even within clades. While this genetic variability creates significant challenges for LASV molecular diagnostics, antigen-based diagnostics have seen high levels of cross reactivity across the seven known lineages of LASV suggesting cross protective antigens may be possible[3]. Lassa virus, the causative agent of Lassa fever (LF), likely causes the largest number of annual cases per year of LF, where its understood up to 500,000 cases per year can occur where the CFR can approach 70% in hospitalized cases [4]. Ribivirin has been used to treat infections with uncertain success [5]. Before the introduction of the JUNV Candid-1 vaccine, its believed that upwards of 60,000 cases of JUNV infection, also known as Argentine Hemorrhagic Fever (AHF) per year occurred where CFRs ranged from 15-30 %. Convalescent plasma from JUNV survivors is still the primary means to treat acute JUNV infections in Argentina [6]. The initial outbreak of Machupo virus had over 1000 cases and CFRs have ranged from 5-30%; ~~with a CFR~~, ~~where~~ outbreak frequency is thought to have been reduced through enhanced rodent abatement practices and potentially cross-protective immunity in populations who have taken the JUNV Candid-1 vaccine [7].

All mammarenaviruses share a common genomic organization consisting of two ambi-sense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP) [1]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [8]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [9]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. To test these notions, investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccine approaches to test this idea and have demonstrated protection against lethal LASV-Josiah challenge using a stabilized prefusion, trimeric GPe, a highly conserved NP NTD epitope, or a combination of the two antigens using a lethal outbred guinea pig model.

Despite the considerable molecular diversity in LASV strains, there is understood that there is some conservation in key antigenic sites within both the GPe and the NP, suggesting that a PAN-LASV vaccine may be possible[3, 10, 11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

In **Phase I of RP1**, the ability for an optimized intramuscular (IM) LASV GPe/NP subunit vaccine to protect against disease after challenge multiple LASV lineages will be assessed in guinea pig and NHP models. This formulation will also be assessed using a microneedle skin patch (MNP) formulation optimized in Core C to determine if a needle-free vaccination route affords more potent vaccine mediated responses and protection. Similarly, we have already generated MACV GPe and will design a MACV NP-NTD subunit antigen to test following the same approach followed with LASV against both homologous lineage challenge and heterologous challenge of MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing

emerging OWAV (LUJV) and NWAV (CHAPV) for which there are no vaccines available. A unique feature of this proposal is its access to BSL4 facilities described in **Core E** capable of evaluating countermeasures in NHP. It is widely considered that the cynomolgus macaque is the current "gold standard" animal model for LASV, JUNV, and MACV research as there are a multitude of immunological reagents available and the disease pathogenesis appears to mirror that of human infection[15-17]. The evaluation of successful countermeasures for the prototype OWAV AND NWAV in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

Unique from most other bunyaviruses, orthonairoviruses are transmitted primarily by ticks in nature (REF). While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[18], Issyk-Kul virus[19], Songling virus[20]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[21] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[22]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[23]. CCHFV is listed on the WHO priority pathogen list[24] and is also a NIH-NIAID category A pathogen[2]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[25] (REF). Ribivirin has been used to treat infections with limited success[26].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [23]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[23]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[23]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[27].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP subunits with the highest level of homology across clades and assess their capacity to protect against disease after challenge with multiple CCHFV clades in established mouse[27, 28] and NHP[29] models with Core E. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best performing formulation of CCHFV GP/NP MNP vaccine approach against emerging test case mammarenaviruses representing emerging bat virus, Kasokero virus for which there are no vaccines available.

~~The *Bunyavirales*.~~

~~Fill out~~

The henipavirus paramyxoviruses. The recognized, highly pathogenic HNVs, NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality (Murray, PMID: 7701348). NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts (Chua, PMID: 10827955). NiV and HeV

can infect at least 18 animal species across 6 orders of mammals, and among these they will cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans (PMID: 32991264; PMID: 22476556). The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission (PMID: 23911335). Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and South East Asia and Australia. Their broad host tropism and ability of these viruses to infect livestock offers an amplification opportunity and increased risk of subsequent human infections. The henipaviruses are single-stranded, negative sense, enveloped RNA viruses and their biology also lends themselves to be synthetically produced and agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents [Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; Department of Health and Human Services (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087]. because they could be engineered for mass dissemination and possess the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the WHO R&D Blueprint list of priority pathogens with epidemic potential (Sweileh, W. M. PMC5299748).

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb, postexposure treatments have been demonstrated to be effective therapies against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP) (PMID: 22013123, PMID: 24964990, PMID: 27484128, PMID: 31686101). The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia (PMID: 32027842). In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs and several vaccine platforms have clear potential for use in people (PMC8782152). The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies (PMC8782152). Such preventive vaccines would have utility for lab workers, first responders or individuals at high risk exposure and in mitigating the spread of an outbreak; whereas in the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent (Middleton, 2014 PMID: 24572697). It is widely considered that the African green monkey (AGM) is the current "gold standard" animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection (Geisbert, 2012;Geisbert, 2010). The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished) (PMID: 33809833). The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons; 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Also, another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including the rescue of recombinant LayV (rLayV), explore animal model development, construct additional LayV and AngV reporter virus tools, and then translate and test the MNP

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vaccine ‘plug-and-play’ approach, and also evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME

A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center’s RPs and Core leaders currently have active and productive collaborative research programs in related areas, some spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

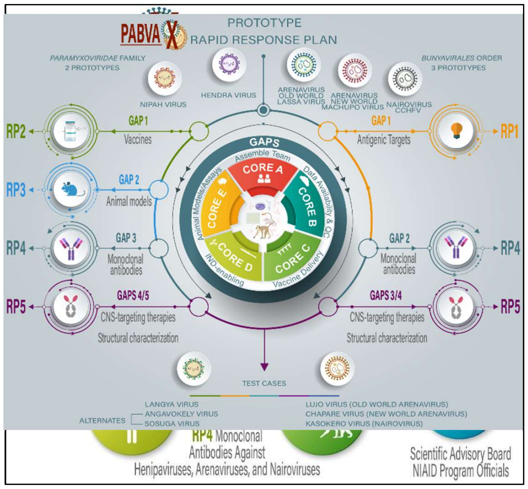

Figure 1: Organizational chart of the Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center-(PABVAX). **(connecting arrows are needed between elements and the SAB)**

The organizational structure of the PABVAX is diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **Arenaviridae** and **Nairoviridae** viruses, and **RP2** will target the prototype **henipaviruses** (HNVs). Both RP1 and RP2 will also be developing new prototype virus tools (recombinantly generated prototype viruses) along with existing tools, to be used in the analysis of vaccine responses as well as in the evaluation of new mAbs and nabs from the other RPs. A third RP will be engaged in the development of a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant Cedar henipavirus (rCedV) in vivo and a bioluminescence methodology that can longitudinally trace the dynamics and anatomical progression of luciferase encoding virus infection in individual animals (**RP3**). There will also be two RPs that will target the development of human mAbs and nanobodies to the PABVAX select viral prototypes. **RP4** will isolate human mAbs derived from NiV infected survivors with a specific focus on the F glycoprotein targeted mAbs, and additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses along with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will be engaged in manufacture and quality control & testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform.
The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from

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Maybe be good to have the whole title per RP listed instead of abbreviated form?

May need to identify what is meant by Gaps as its not identified in this document, I think maybe it is spelled out in Core E document?. Assuming maybe these are Gaps identified in the main RFA?

Just an idea…. having RP4 and RP5 bubbles duplicated to demonstrate different GAPs addressed is a little confusing to me, maybe if we do end up enumerating the gaps somewhere in the text, it might simplify things by identify what gaps are addressed by each RP so that each RP only has one bubble? EG:

- RP1=gap 1
- RP2=gap 1
- RP3=gap 2
- RP4=gap 2,3
- RP5= gap 3,4,5

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the UPitt and IBBR/UMD (**Core C**) that has tested a dissolvable MNP-based vaccine technology that is ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine approach (PMID: 32924651). This vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable MNPs that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants (PMID: 19864632) to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as facilitate the improved vaccine storage and distribution due to their thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, a Scientific **Core C**: (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ) adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (Paramyxoviridae). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity in vitro and polyfunctional T-cell responses by in vitro stimulation of isolated cells with antigenic peptides, intracellular cytokine staining (ICS) and flow cytometry) responses.

Discover effective antibody-based ~~prophylactics and~~ therapies.

~~PREP can be critical in the absence of available vaccines or cases in which vaccination is not possible.~~

HELP

Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah [30], Hendra [31], Ebola [32, 33], Marburg [34] and Lassa [35]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc.*), a feature to be exploited in **Core D**. In addition to therapeutic applications, mAbs with Fc mutations to dramatically extend serum half-life [36-38] offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g.* rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures.

Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the A~~A~~nimal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation S~~s~~cientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including ~~……..~~ animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective ~~therapeutic~~ efficacy of antivirals and vaccines as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation; including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera

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or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they can be adapted to greater high-throughput platforms, are often less laborious, and they can make use of other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput florescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic Nipah and Hendra virus vaccine induced immune sera, mAbs (**RP4**) and nbs (**RP5**) and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either the HeV or NiV-B envelope glycoproteins) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals, ideally suited to explore nab based therapeutics to CNS resident henipavirus (PMID: 35821488).

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. ~~Data generated to support of possible future regulatory submissions to the Food and Drug Administration (FDA) must be collected in accordance with quality systems, like good laboratory practice (GLP) regulations, to be useful and reliable, and to facilitate FDA review to ensure safety and efficacy.~~ The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the henipaviruses (Paramyxoviridae) and the Bunyavirales (Arenaviridae and Nairoviridae) that will bolster vaccine endurance to storage and transportation; and also improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology and mAb and vaccine translational drug development and includes Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Henipaviruses: Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals and are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of Cedar virus (CedV), Gamak virus, Daeryong virus, and Langya virus (LayV) [xxx] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV) [xxx]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV,

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Lee, S.H.; Kim, K.; Viruses 2021, 13, doi:10.3390/v13102020.

Zhang, X.A.; Li, H.; Jiang, N Engl J Med 2022, 387, 470-472, doi:10.1056/NEJMc2202705.

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

are old world pteropid fruit bats. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province). The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**.

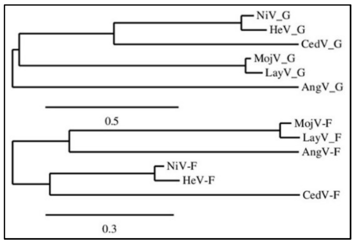

Figure 2. Phylogenetic analysis of select HNV G and F glycoproteins. Phylogenetic trees were constructed for the HNV F and G glycoproteins using the PhyML program's maximum likelihood method. The trees were generated by aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal henipaviral disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters (Geisbert 2012), and African green monkeys (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P), and the P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists (xx). The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2 (XXX) and CedV is now recognized as a BSL-2 restricted agent [CDC manual]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D, and E**).

Old Word Mammarenaviruses (OWAV): For **phase 1**, **RP1** will utilize **LASV as the prototype OWAV** due to the significant public health burden it creates throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[39]. LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to several countries outside of Africa over 35 documented times [40]. Case fatality rates (CFR) approaching 70% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) [41]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize the only the LASV GP as the target immunogen despite

Investigators in RP1, Core D, and Core E have already demonstrated protective efficacy of against disease after challenge with LASV in outbred guinea pigs in animals vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These antigens have been engineered to harness the homologous antigenic regions of both antigens to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach, suggest a similar strategy of using a subunit vaccine might also be effective against other arenaviruses. While LASV is the most common pathogenic arenavirus in Africa in terms of human infections, Lujo virus was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [42]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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Laing, E.D.; Navaratnarajah, C.K.; Proc Natl Acad Sci U S A 2019, doi:10.1073/pnas.1911773116.

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New World Mamarenaviruses (NWAV): A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [43], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [44, 45]. Further, there are safety concerns related to potential for reversion to pathogenic versions. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted. Building on the success of the LASV GPe/NP-NTD. Investigators in **RP1 and Cores D and E** will engineer MACV GPe and NP-NTD antigens and test for efficacy in established guinea pig and NHP models using MACV from multiple lineages to asses how broadly protective the approach is across MACV lineages. We will then extend this plug-and-play approach to other NWAV. First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [46, 47]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [47]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Orthonairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family responsible for often large outbreaks of human disease annually across Europe, Africa, and Asia. There are no FDA approved vaccines for CCHFV; however, a suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974 but has numerous safety concerns and limited demonstrated efficacy. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[48]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[49]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test for protective efficacy from disease after challenge with 6 different lineages of CCHFV clades using in established mouse and NHP models. Next we will deploy the same approach to a known human pathogen "test case" orthonairovirus for which no vaccine exists.

In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [50]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV [51]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as

for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs; such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**), (aarenavirus and nairovirus~~renaviridae and nairoviridae~~ projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine the active and passive immunization approaches to further improved the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

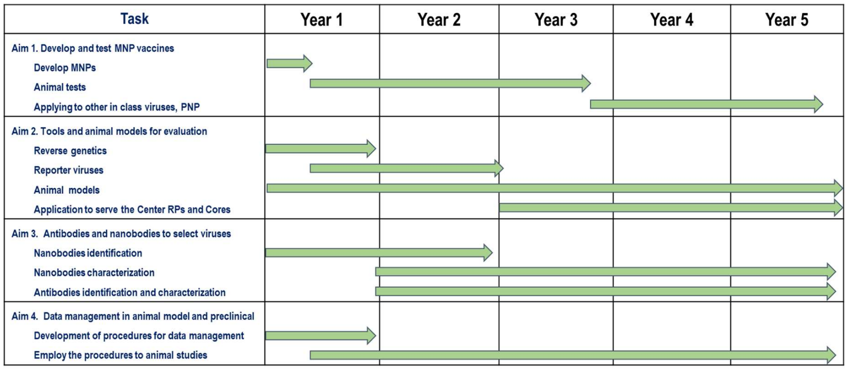
An additional and outstanding advantage of the PABVAX Center is the **Animal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL. (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for all the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.**
2. **Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.**
3. **Develop antibodies and nanobodies to selected prototype viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.



Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. This is in addition to each PR's individual Milestone plan. The oversight and evaluation of progress will be in concert with quarterly reports to the

PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (Details are within the Administration **Core A**). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommend new directions as appropriate and provide Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

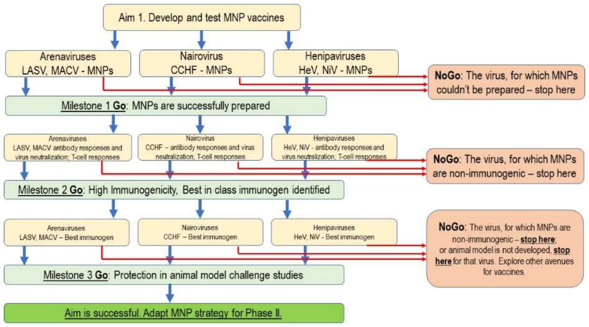


Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will be whether proper formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be made based on immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction and virus-neutralization and polyfunctional T-cell responses by in vitro stimulation of isolated cells). Go/no go decisions will be made based on the outcomes of these first tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as success. Comparisons will also be made, such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of best-in-class immunogens, or best combination immunogens which will also be explored, will be performed in order to down-select vaccine choice. The third milestone will be the determination of whether MNP vaccination affords protection in animal model challenge experiments. In the case of the NiV and HeV prototypes the animal models chosen (ferret and the African green monkey (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales* there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASA and there are guinea pig models for some but not all of those lineages. For MACV there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys are now each greater than 35K. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of the AGM for the arenaviruses and possibly also for CCHF as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

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Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.

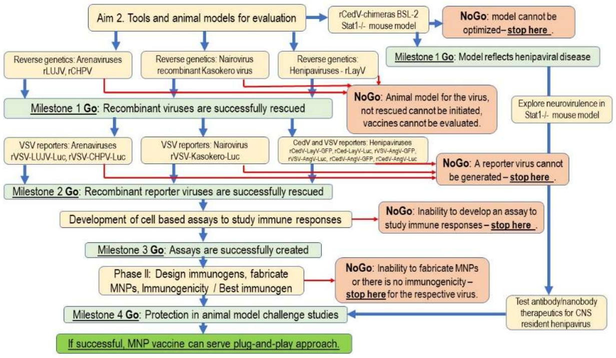

Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center overall objectives.

The PABVAX second overall objective is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined in vivo (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, a a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**) the milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new in vivo bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease in vivo safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**) it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and RP3 will collaborate with RP5 in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

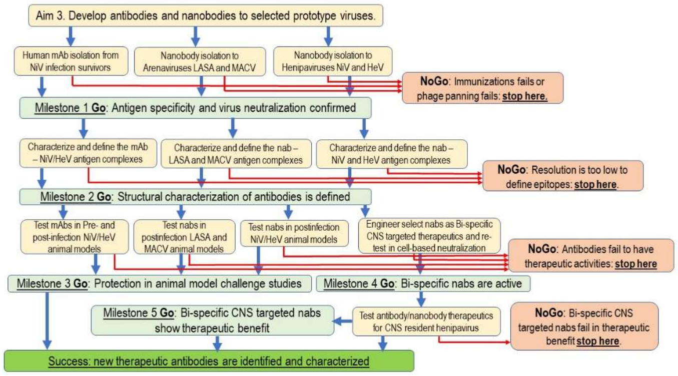

Figure 6. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

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New human mAbs and nanobodies (nbs) as tools and potential therapeutics will be developed by RP4 and RP5 in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); and transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on the mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs and in collaboration with **RP2** neutralization potencies will be rapidly assessed as well as evaluating synergistic cell-based mAb neutralization that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

Nanobodies, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nanobodies can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nanobodies targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), and also for therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus) and select animal models of LASV and MACV by **Core E**. Success will be measured by the identification of virus-neutralizing nbs, potential discovery of more broadly reactive nbs, particularly to the multiple LASA clades, the evidence of therapeutic benefit in animal models, and the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

INDUSTRY PARTNERSHIPS

Industry Partnership

Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine

expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX.

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SPECIAL FEATURES OF THE PABVAX CENTER ENVIRONMENT AND OTHER RESOURCES ??

Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

Galveston National Laboratory (GNL)

The GNL, is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics and therapeutics against them. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370~~187~~ separate NHP studies employing a total of 2,772~~1,318~~ animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses ~~Ebola and Marburg viruses and also Nipah and Hendra~~ have been successfully performed at the GNL. These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP~~nonhuman primate~~ facility and a staff of full-time veterinarians. In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of WRCEVA virus collection within the GNL.

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Core E (Animal model development and preclinical evaluation)

Core E operations will be conducted at the GNL, and in conjunction with **Core A** also includes the UTMB Institutional Office of Regulated Nonclinical Studies (ONcRS). The ONcRS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, ~~to meet regulatory requirements by assuring regulatory compliance,~~ quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. ~~Quality system and regulatory science processes for NHP studies performed at UTMB will be implemented and executed in conjunction with the ORNcS.~~ Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses and the henipaviruses and he has published over 250~~XXX~~ peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI) was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

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Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs)

Mapp Biopharmaceutical has deep experience with antigens and monoclonal antibodies (mAbs) against these antigens. ~~'s product development team has grown significantly in the last two years in response to the development efforts on its anti-Ebola therapeutic, ZMapp™ which is being advanced to licensure as part of Project Bioshield. OTHER MAbs~~ Their early development and research group has expertise in immunology, infectious diseases, and production of antigens, antibodies and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [52] [53]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1 [54]. ~~developed multiple products, including a licensed biodefense biologic. As with Profectus, Mapp Bio's expertise and experience will be a very important and valuable asset to the Center.~~

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Core A (Administrative Core)

Research Project teams?? Maybe not depending on space

The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills and his experience in translational research activities qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe's (**RP4**) research focuses on the molecular basis for the development of effective B cell responses to viruses in humans and he has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently henipaviruses NiV and HeV owing to the collaboration between the Crowe, Broder and Geisbert laboratories. Perhaps of greatest import, the SARS-CoV-2 mAbs generated by Dr. Crowe's laboratory were used in xxx,000 of people during the COVID-19 pandemic. RP4 will work closely with Cores D and E in advancing mAb prototypes. ~~The Core B of the Center will work together with RP2 and RP3 along with the Core C to develop the first cocktail human mAb immunotherapeutics, and facilitate the transition products suitable for IND filing and human testing including design, construction, assessment, and optimization of these antibody-based therapies.~~

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As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

1. Hallam, S.J., et al., *Review of Mammarenavirus Biology and Replication.* Front Microbiol, 2018. **9**: p. 1751.

2. NIH-NIAID. *NIAID Emerging Infectious Diseases/ Pathogens*. 2023 [cited 2023 03/06]; Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
3. Heinrich, M.L., et al., *Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages.* Scientific Reports, 2020. **10**(1): p. 16030.
4. Shaffer, J.G., et al., *Lassa Fever in Post-Conflict Sierra Leone.* PLOS Neglected Tropical Diseases, 2014. **8**(3): p. e2748.
5. Salam, A.P., et al., *Time to reconsider the role of ribavirin in Lassa fever.* PLOS Neglected Tropical Diseases, 2021. **15**(7): p. e0009522.
6. Enria, D.A., A.M. Briggiler, and Z. Sanchez, *Treatment of Argentine hemorrhagic fever.* Antiviral Res, 2008. **78**(1): p. 132-9.
7. Kilgore, P.E., et al., *Prospects for the control of Bolivian hemorrhagic fever.* Emerg Infect Dis, 1995. **1**(3): p. 97-100.
8. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nature Medicine, 2017. **23**(10): p. 1146-1149.
9. Hastie, K.M., et al., *Structural basis for antibody-mediated neutralization of Lassa virus.* Science, 2017. **356**(6341): p. 923-928.
10. Sullivan, B.M., et al., *High crossreactivity of human T cell responses between Lassa virus lineages.* PLoS Pathog, 2020. **16**(3): p. e1008352.
11. Ugwu, C., et al., *Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria.* Scientific Reports, 2022. **12**(1): p. 22330.
12. Geisbert, T.W., et al., *Development of a New Vaccine for the Prevention of Lassa Fever.* PLOS Medicine, 2005. **2**(6): p. e183.
13. Cross, R.W., et al., *A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever.* Cell Rep, 2022. **40**(3): p. 111094.
14. Safronetz, D., et al., *A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses.* PLOS Neglected Tropical Diseases, 2015. **9**(4): p. e0003736.
15. Hensley, L.E., et al., *Pathogenesis of Lassa fever in cynomolgus macaques.* Virol J, 2011. **8**: p. 205.
16. Zeitlin, L., et al., *Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody.* Proceedings of the National Academy of Sciences, 2021. **118**(11): p. e2023332118.
17. Bell, T.M., et al., *Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure.* Veterinary Pathology, 2015. **52**(1): p. 26-37.
18. Burt, F.J., et al., *Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia.* Epidemiol Infect, 1996. **116**(3): p. 353-61.
19. Brinkmann, A., et al., *First detection of bat-borne Issyk-Kul virus in Europe.* Sci Rep, 2020. **10**(1): p. 22384.
20. Ma, J., et al., *Identification of a new orthonairovirus associated with human febrile illness in China.* Nat Med, 2021. **27**(3): p. 434-439.
21. Lorenzo Juanes, H.M., et al., *Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021.* Emerg Infect Dis, 2023. **29**(2): p. 252-259.
22. Grandi, G., et al., *First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden.* Ticks Tick Borne Dis, 2020. **11**(3): p. 101403.
23. Hawman, D.W. and H. Feldmann, *Crimean–Congo haemorrhagic fever virus.* Nature Reviews Microbiology, 2023.
24. World.Health.Organisation. *List of Blueprint Priority Diseases*. 2018 [cited 2018 November 14]; Available from: http://www.who.int/blueprint/priority-diseases/en/.
25. Keshtkar-Jahromi, M., et al., *Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies.* Antiviral Res, 2011. **90**(2): p. 85-92.
26. Soares-Weiser, K., et al., *Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis.* BMC Infectious Diseases, 2010. **10**(1): p. 207.
27. Rodriguez, S.E., et al., *Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever.* Sci Rep, 2019. **9**(1): p. 7755.
28. Bente, D.A., et al., *Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model.* J Virol, 2010. **84**(21): p. 11089-100.

29. Cross, R.W., et al., *Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys.* PLoS Negl Trop Dis, 2020. **14**(8): p. e0008637.
30. Geisbert, T.W., et al., *Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody.* Sci Transl Med, 2014. **6**(242): p. 242ra82.
31. Bossart, K.N., et al., *A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge.* Sci Transl Med, 2011. **3**(105): p. 105ra103.
32. Pettitt, J., et al., *Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail.* Sci Transl Med, 2013. **5**(199): p. 199ra113.
33. Qiu, X., et al., *Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp.* Nature, 2014. **514**(7520): p. 47-53.
34. Mire, C.E., et al., *Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody.* Sci Transl Med, 2017. **9**(384).
35. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nat Med, 2017. **23**(10): p. 1146-1149.
36. Kuo, T.T. and V.G. Aveson, *Neonatal Fc receptor and IgG-based therapeutics.* MAbs, 2011. **3**(5): p. 422-30.
37. Robbie, G.J., et al., *A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study.* Antimicrob Agents Chemother, 2013.
38. Gaudinski, M.R., et al., *Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults.* PLoS Med, 2018. **15**(1): p. e1002493.
39. Organization, W.H., *Lassa fever fact sheet.* World Health Organization, 2017.
40. Wolf, T., et al., *Fifty years of imported Lassa fever: a systematic review of primary and secondary cases.* J Travel Med, 2020. **27**(4).
41. Purushotham, J., T. Lambe, and S.C. Gilbert, *Vaccine platforms for the prevention of Lassa fever.* Immunol Lett, 2019. **215**: p. 1-11.
42. Sewlall, N.H., et al., *Clinical features and patient management of Lujo hemorrhagic fever.* PLoS Negl Trop Dis, 2014. **8**(11): p. e3233.
43. Saito, T., et al., *Vaccine Candidates against Arenavirus Infections.* Vaccines, 2023. **11**(3): p. 635.
44. Koma, T., et al., *Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic.* J Virol, 2016. **90**(3): p. 1290-7.
45. Jahrling P, T.R., Barrero O. . *Crossprotection against Machupo virus with Candid 1 Junin virus vaccine*. in *2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region*. 1988. Mar del Plata, Argentina.
46. Delgado, S., et al., *Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia.* PLOS Pathogens, 2008. **4**(4): p. e1000047.
47. Loayza Mafayle, R., et al., *Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019.* New England Journal of Medicine, 2022. **386**(24): p. 2283-2294.
48. Tipih, T. and F.J. Burt, *Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development.* Biores Open Access, 2020. **9**(1): p. 137-150.
49. UHS-NHS. *Healthy volunteers needed for research investigating world-first vaccine for viral disease*. 2023 [cited 2023 03/06]; Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.
50. Kalunda, M., et al., *Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda.* The American Journal of Tropical Medicine and Hygiene, 1986. **35**(2): p. 387-392.
51. Schuh, A.J., et al., *Human-Pathogenic Kasokero Virus in Field-Collected Ticks.* Emerg Infect Dis, 2020. **26**(12): p. 2944-2950.
52. Mulangu, S., et al., *A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics.* N Engl J Med, 2019. **381**(24): p. 2293-2303.
53. Group, P.I.W., et al., *A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection.* N Engl J Med, 2016. **375**(15): p. 1448-1456.
54. Politch, J.A., et al., *Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial.* PLoS Med, 2021. **18**(2): p. e1003495.

CENTER OVERVIEW: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction

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Emerging and periodically reemerging pathogenic enveloped RNA viruses are ~~a~~ continuous ~~infectious disease and potentially~~ pandemic threats to global public health. Among the many groups of these types of pathogens are viral zoonoses within the family **Paramyxoviridae** and order ***Bunyavirales*** where humans can acquire infections directly from animal reservoir hosts such as rodents or bats~~,~~ through an intermediate amplifying host such as livestock, or from arthropod vectors such as mosquitos and ticks (Fields virology). More often than not, humans represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur, and should RNA viral pathogens mutate and adapt to more efficient replication and shedding in the human host, the epidemic potential of these viruses could manifest (PMID: 23911335 -Nipah / others?). The viral order *Bunyavirales* is composed of several viral families known to contain multiple high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic fever diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and are also identified as NIAID Category A pathogens due to their ease of dissemination and/or abilities to transmit person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special actions for public health preparedness. Also, within the WHO Priority Pathogens list is Nipah virus (NiV) and henipaviral disease which includes Hendra virus (HeV) which are zoonotic henipaviruses (HNVs) in the family **Paramyxoviridae**. NiV and HeV have long been considered the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease (PMID: 16357858). The genus now includes nine additional reported HNVs but only HeV and NiV are known to be associated with severe and often fatal henipaviral disease in humans and a number of animal species (PMID: 32991264). Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illnesses in humans [PMID: 35921459].

Taken together, these viral threats to global public health are heightened due to the absence of approved vaccines or therapeutics. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for vaccine and monoclonal antibody (mAb) countermeasures in developing prototype candidates (**Table 1**) against a selection of these viruses. In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Table 1. PABVAX selected prototype and test case viral agents.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old world), Machupo (New world)	Lujo (Old world), Chapare (New world)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Bunyavirales:

Arenaviridae: The family Arenaviridae is composed of three genera: Mammarenaviridae, Reptarenavirus, and Hartmanivirus. The Mammarenaviridae are composed of a large number rodent borne viruses, some of which are demonstrated human pathogens responsible for causing severe hemorrhagic fever, neurological damage, and if not fatal can result permanent neurological sequelae including hearing loss, vision impairment, and balance [1]derangement. While these genera exhibit a global distribution, two geographically distinct sub-genera have formed due to the separation across continents by the Atlantic and Pacific Ocean. Old World Mammarenaviruses (Lassa Virus (LASV), Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered Lujo Virus (LUJV)) and the New World Arenaviruses (Junin Virus (JUNV), Machupo Virus (MACV), Sabia Virus (SABV), and the newly emerging Chapare Virus (CHAPV)). Many of these viruses are identified as NIAID Category A pathogens requiring biosafety level 4 (BSL4) containment [2]. This classification is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness. While several

promising medical countermeasures are in development, there are currently no US FDA approved vaccines or therapeutics for any mammarenaviruses, though the attenuated JUNV “Candid-1” vaccine is approved for use in Argentina.

Infection in humans is often the result of exposure to rodent excreta from infected animals where disease severity and case fatality rates (CFR) can vary across the genera and even within genetic lineages as can be the case for infections with LASV where genetic diversity can be high, even within clades. While this genetic variability creates significant challenges for LASV molecular diagnostics, antigen-based diagnostics have seen high levels of cross reactivity across the seven known lineages of LASV suggesting cross protective antigens may be possible[3]. Lassa virus, the causative agent of Lassa fever (LF), likely causes the largest number of annual cases per year of LF, where its understood up to 500,000 cases per year can occur where the CFR can approach 70% in hospitalized cases [4]. Ribivirin has been used to treat infections with uncertain success [5]. Before the introduction of the JUNV Candid-1 vaccine, its believed that upwards of 60,000 cases of JUNV infection, also known as Argentine Hemorrhagic Fever (AHF) per year occurred where CFRs ranged from 15-30 %. Convalescent plasma from JUNV survivors is still the primary means to treat acute JUNV infections in Argentina [6]. The initial outbreak of Machupo virus had over 1000 cases and CFRs have ranged from 5-30%;~~ with a CFR~~, ~~where ~~outbreak frequency is thought to have been reduced through enhanced rodent abatement practices and potentially cross-protective immunity in populations who have taken the JUNV Candid-1 vaccine [7].

All mammarenaviruses share a common genomic organization consisting of two ambi-sense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP) [1]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [8]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [9]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. To test these notions, investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccine approaches to test this idea and have demonstrated protection against lethal LASV-Josiah challenge using a stabilized prefusion, trimeric GPe, a highly conserved NP NTD epitope, or a combination of the two antigens using a lethal outbred guinea pig model.

Despite the considerable molecular diversity in LASV strains, there is understood that there is some conservation in key antigenic sites within both the GPe and the NP, suggesting that a PAN-LASV vaccine may be possible[3, 10, 11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

In **Phase I of RP1**, the ability for an optimized intramuscular (IM) LASV GPe/NP subunit vaccine to protect against disease after challenge multiple LASV lineages will be assessed in guinea pig and NHP models. This formulation will also be assessed using a microneedle skin patch (MNP) formulation optimized in Core C to determine if a needle-free vaccination route affords more potent vaccine mediated responses and protection. Similarly, we have already generated MACV GPe and will design a MACV NP-NTD subunit antigen to test following the same approach followed with LASV against both homologous lineage challenge and heterologous challenge of MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing

emerging OWAV (LUJV) and NWAV (CHAPV) for which there are no vaccines available. A unique feature of this proposal is its access to BSL4 facilities described in **Core E** capable of evaluating countermeasures in NHP. It is widely considered that the cynomolgus macaque is the current "gold standard" animal model for LASV, JUNV, and MACV research as there are a multitude of immunological reagents available and the disease pathogenesis appears to mirror that of human infection[15-17]. The evaluation of successful countermeasures for the prototype OWAV AND NWAV in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

Unique from most other bunyaviruses, orthonairoviruses are transmitted primarily by ticks in nature (REF). While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[18], Issyk-Kul virus[19], Songling virus[20]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[21] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[22]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[23]. CCHFV is listed on the WHO priority pathogen list[24] and is also a NIH-NIAID category A pathogen[2]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[25] (REF). Ribivirin has been used to treat infections with limited success[26].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [23]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[23]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[23]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[27].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP subunits with the highest level of homology across clades and assess their capacity to protect against disease after challenge with multiple CCHFV clades in established mouse[27, 28] and NHP[29] models with Core E. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best performing formulation of CCHFV GP/NP MNP vaccine approach against emerging test case mammarenaviruses representing emerging bat virus, Kasokero virus for which there are no vaccines available.

~~The *Bunyavirales*.~~

~~Fill out~~

The henipavirus paramyxoviruses. The recognized, highly pathogenic HNVs, NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality (Murray, PMID: 7701348). NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts (Chua, PMID: 10827955). NiV and HeV

can infect at least 18 animal species across 6 orders of mammals, and among these they will cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans (PMID: 32991264; PMID: 22476556). The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission (PMID: 23911335). Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and South East Asia and Australia. Their broad host tropism and ability of these viruses to infect livestock offers an amplification opportunity and increased risk of subsequent human infections. The henipaviruses are single-stranded, negative sense, enveloped RNA viruses and their biology also lends themselves to be synthetically produced and agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents [Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; Department of Health and Human Services (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087]. because they could be engineered for mass dissemination and possess the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the WHO R&D Blueprint list of priority pathogens with epidemic potential (Sweileh, W. M. PMC5299748).

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb, postexposure treatments have been demonstrated to be effective therapies against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP) (PMID: 22013123, PMID: 24964990, PMID: 27484128, PMID: 31686101). The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia (PMID: 32027842). In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs and several vaccine platforms have clear potential for use in people (PMC8782152). The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies (PMC8782152). Such preventive vaccines would have utility for lab workers, first responders or individuals at high risk exposure and in mitigating the spread of an outbreak; whereas in the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent (Middleton, 2014 PMID: 24572697). It is widely considered that the African green monkey (AGM) is the current "gold standard" animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection (Geisbert, 2012;Geisbert, 2010). The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished) (PMID: 33809833). The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons; 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Also, another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including the rescue of recombinant LayV (rLayV), explore animal model development, construct additional LayV and AngV reporter virus tools, and then translate and test the MNP

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vaccine 'plug-and-play' approach, and also evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME

A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center's RPs and Core leaders currently have active and productive collaborative research programs in related areas, some spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

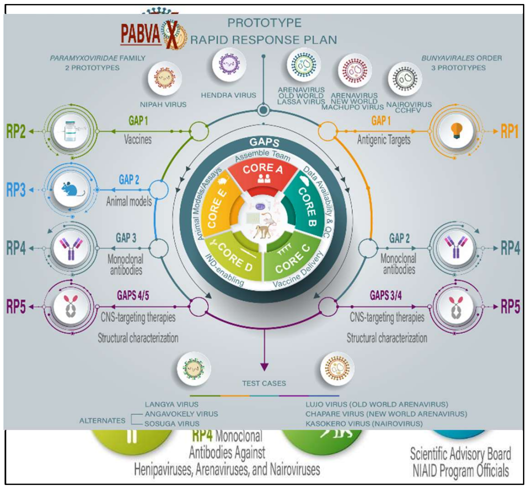

Figure 1: Organizational chart of the Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center-(PABVAX). **(connecting arrows are needed between elements and the SAB)**

The organizational structure of the PABVAX is diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **Arenaviridae** and **Nairoviridae** viruses, and **RP2** will target the prototype **henipaviruses** (HNVs). Both RP1 and RP2 will also be developing new prototype virus tools (recombinantly generated prototype viruses) along with existing tools, to be used in the analysis of vaccine responses as well as in the evaluation of new mAbs and nabs from the other RPs. A third RP will be engaged in the development of a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant Cedar henipavirus (rCedV) in vivo and a bioluminescence methodology that can longitudinally trace the dynamics and anatomical progression of luciferase encoding virus infection in individual animals (**RP3**). There will also be two RPs that will target the development of human mAbs and nanobodies to the PABVAX select viral prototypes. **RP4** will isolate human mAbs derived from NiV infected survivors with a specific focus on the F glycoprotein targeted mAbs, and additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses along with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will be engaged in manufacture and quality control & testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based prophylactics and therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform.
The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from

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Maybe be good to have the whole title per RP listed instead of abbreviated form?

May need to identify what is meant by Gaps as its not identified in this document, I think maybe it is spelled out in Core E document?. Assuming maybe these are Gaps identified in the main RFA?

Just an idea…. having RP4 and RP5 bubbles duplicated to demonstrate different GAPs addressed is a little confusing to me, maybe if we do end up enumerating the gaps somewhere in the text, it might simplify things by identify what gaps are addressed by each RP so that each RP only has one bubble? EG:

- RP1=gap 1
- RP2=gap 1
- RP3=gap 2
- RP4=gap 2,3
- RP5= gap 3,4,5

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the UPitt and IBBR/UMD (**Core C**) that has tested a dissolvable MNP-based vaccine technology that is ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine approach (PMID: 32924651). This vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable MNPs that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants (PMID: 19864632) to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as facilitate the improved vaccine storage and distribution due to their thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, a Scientific **Core C**: (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ) adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (Paramyxoviridae). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity in vitro and polyfunctional T-cell responses by in vitro stimulation of isolated cells with antigenic peptides, intracellular cytokine staining (ICS) and flow cytometry) responses.

Discover effective antibody-based prophylactics and therapies.
~~PREP can be critical in the absence of available vaccines or cases in which vaccination is not possible.~~
HELP

Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah [30], Hendra [31], Ebola [32, 33], Marburg [34] and Lassa [35]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc*.), a feature to be exploited in **Core D**. In addition to therapeutic applications, mAbs with Fc mutations to dramatically extend serum half-life [36-38] offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g.* rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures.
Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the A~~A~~nimal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation S~~s~~cientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective ~~therapeutic~~ efficacy of antivirals and vaccines as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation; including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera

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or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they can be adapted to greater high-throughput platforms, are often less laborious, and they can make use of other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput florescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic Nipah and Hendra virus vaccine induced immune sera, mAbs (**RP4**) and nbs (**RP5**) and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either the HeV or NiV-B envelope glycoproteins) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals, ideally suited to explore nab based therapeutics to CNS resident henipavirus (PMID: 35821488).

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. ~~Data generated to support of possible future regulatory submissions to the Food and Drug Administration (FDA) must be collected in accordance with quality systems, like good laboratory practice (GLP) regulations, to be useful and reliable, and to facilitate FDA review to ensure safety and efficacy.~~ The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the henipaviruses (Paramyxoviridae) and the Bunyavirales (Arenaviridae and Nairoviridae) that will bolster vaccine endurance to storage and transportation; and also improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology and mAb and vaccine translational drug development and includes Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Henipaviruses: Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals and are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of Cedar virus (CedV), Gamak virus, Daeryong virus, and Langya virus (LayV) [xxx] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV) [xxx]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV,

Commented [CB7]: Marsh, G.A.; PLoS pathogens 2012, 8, e1002836, doi:10.1371/journal.ppat.1002836.

Lee, S.H.; Kim, K.; Viruses 2021, 13, doi:10.3390/v13102020.

Zhang, X.A.; Li, H.; Jiang, N Engl J Med 2022, 387, 470-472, doi:10.1056/NEJMc2202705.

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

are old world pteropid fruit bats. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province). The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**.

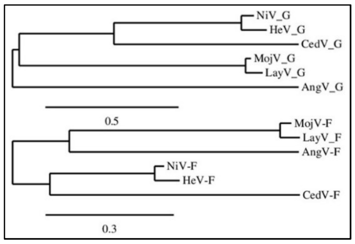

Figure 2. Phylogenetic analysis of select HNV G and F glycoproteins. Phylogenetic trees were constructed for the HNV F and G glycoproteins using the PhyML program's maximum likelihood method. The trees were generated by aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal henipaviral disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters (Geisbert 2012), and African green monkeys (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P), and the P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists (xx). The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2 (XXX) and CedV is now recognized as a BSL-2 restricted agent [CDC manual]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D, and E**).

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Laing, E.D.; Navaratnarajah, C.K.; Proc Natl Acad Sci U S A 2019, doi:10.1073/pnas.1911773116.

Commented [CB11]: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. HHS Publication No. (CDC) 300859. US Government Printing Office: Washington, 2020; pp. 248-250.

Old Word Mammarenaviruses (OWAV): For **phase 1**, **RP1** will utilize **LASV as the prototype OWAV** due to the significant public health burden it creates throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[39]. LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to several countries outside of Africa over 35 documented times [40]. Case fatality rates (CFR) approaching 70% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) [41]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize the only the LASV GP as the target immunogen despite

Investigators in RP1, Core D, and Core E have already demonstrated protective efficacy of against disease after challenge with LASV in outbred guinea pigs in animals vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These antigens have been engineered to harness the homologous antigenic regions of both antigens to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach, suggest a similar strategy of using a subunit vaccine might also be effective against other arenaviruses. While LASV is the most common pathogenic arenavirus in Africa in terms of human infections, Lujo virus was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [42]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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New World Mamarenaviruses (NWAV): A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [43], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [44, 45]. Further, there are safety concerns related to potential for reversion to pathogenic versions. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted. Building on the success of the LASV GPe/NP-NTD. Investigators in **RP1 and Cores D and E** will engineer MACV GPe and NP-NTD antigens and test for efficacy in established guinea pig and NHP models using MACV from multiple lineages to asses how broadly protective the approach is across MACV lineages. We will then extend this plug-and-play approach to other NWAV. First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [46, 47]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [47]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Orthonairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family responsible for often large outbreaks of human disease annually across Europe, Africa, and Asia. There are no FDA approved vaccines for CCHFV; however, a suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974 but has numerous safety concerns and limited demonstrated efficacy. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[48]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[49]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test for protective efficacy from disease after challenge with 6 different lineages of CCHFV clades using in established mouse and NHP models. Next we will deploy the same approach to a known human pathogen "test case" orthonairovirus for which no vaccine exists.

In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [50]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV [51]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as

for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs; such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**), (aarenavirus and nairovirus~~renaviridae and nairoviridae~~ projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine the active and passive immunization approaches to further improved the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

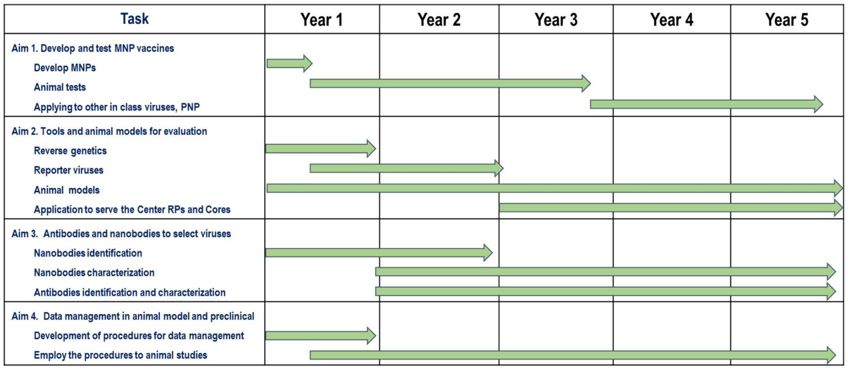
An additional and outstanding advantage of the PABVAX Center is the **Animal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL. (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for all the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.**
2. **Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.**
3. **Develop antibodies and nanobodies to selected prototype viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.



Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. This is in addition to each PR's individual Milestone plan. The oversight and evaluation of progress will be in concert with quarterly reports to the

PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (Details are within the Administration **Core A**). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommend new directions as appropriate and provide Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

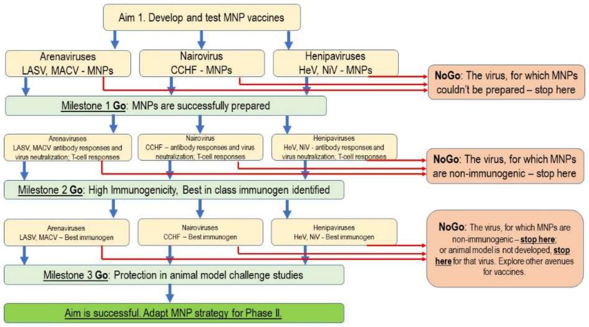


Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will be whether proper formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be made based on immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction and virus-neutralization and polyfunctional T-cell responses by in vitro stimulation of isolated cells). Go/no go decisions will be made based on the outcomes of these first tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as success. Comparisons will also be made, such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of best-in-class immunogens, or best combination immunogens which will also be explored, will be performed in order to down-select vaccine choice. The third milestone will be the determination of whether MNP vaccination affords protection in animal model challenge experiments. In the case of the NiV and HeV prototypes the animal models chosen (ferret and the African green monkey (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales* there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASA and there are guinea pig models for some but not all of those lineages. For MACV there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys are now each greater than 35K. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of the AGM for the arenaviruses and possibly also for CCHF as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

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Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.

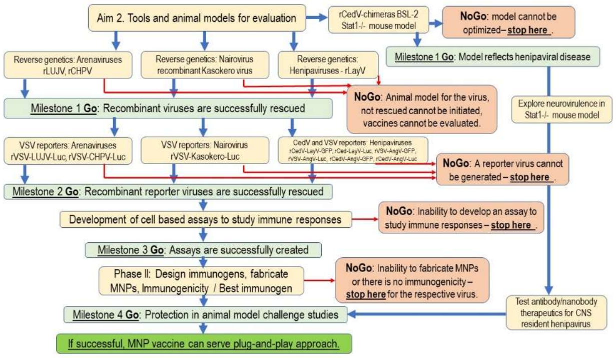

Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center overall objectives.

The PABVAX second overall objective is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined in vivo (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, a a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**) the milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new in vivo bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease in vivo safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**) it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and RP3 will collaborate with RP5 in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

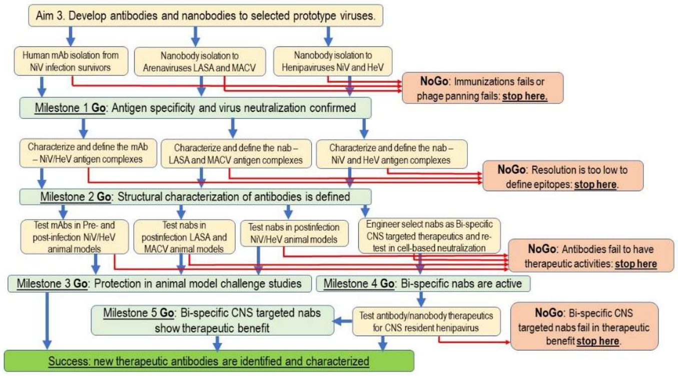

Figure 6. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

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New human mAbs and nanobodies (nbs) as tools and potential therapeutics will be developed by RP4 and RP5 in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); and transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on the mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs and in collaboration with **RP2** neutralization potencies will be rapidly assessed as well as evaluating synergistic cell-based mAb neutralization that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

Nanobodies, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nanobodies can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nanobodies targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), and also for therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus) and select animal models of LASV and MACV by **Core E**. Success will be measured by the identification of virus-neutralizing nbs, potential discovery of more broadly reactive nbs, particularly to the multiple LASA clades, the evidence of therapeutic benefit in animal models, and the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

INDUSTRY PARTNERSHIPS

Industry Partnership

Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine

expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX.

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SPECIAL FEATURES OF THE PABVAX CENTER ENVIRONMENT AND OTHER RESOURCES ??

Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

Galveston National Laboratory (GNL)

The GNL, is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics and therapeutics against them. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370~~187~~ separate NHP studies employing a total of 2,772~~1,318~~ animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses ~~Ebola and Marburg viruses and also Nipah and Hendra~~ have been successfully performed at the GNL. These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP~~nonhuman primate~~ facility and a staff of full-time veterinarians. In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of WRCEVA virus collection within the GNL.

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Core E (Animal model development and preclinical evaluation)

Core E operations will be conducted at the GNL, and in conjunction with **Core A** also includes the UTMB Institutional Office of Regulated Nonclinical Studies (ONcRS). The ONcRS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, ~~to meet regulatory requirements by assuring regulatory compliance,~~ quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. ~~Quality system and regulatory science processes for NHP studies performed at UTMB will be implemented and executed in conjunction with the ORNcS.~~ Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses and the henipaviruses and he has published over 250~~XXX~~ peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI) was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

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Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs)

Mapp Biopharmaceutical has deep experience with antigens and monoclonal antibodies (mAbs) against these antigens. ~~'s product development team has grown significantly in the last two years in response to the development efforts on its anti-Ebola therapeutic, ZMapp^{TM} which is being advanced to licensure as part of Project Bioshield. OTHER MAbs~~ Their early development and research group has expertise in immunology, infectious diseases, and production of antigens, antibodies and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies [52] [53]; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1 [54]. ~~developed multiple products, including a licensed biodefense biologic. As with Profectus, Mapp Bio's expertise and experience will be a very important and valuable asset to the Center.~~

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Core A (Administrative Core)

Research Project teams?? Maybe not depending on space

The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills and his experience in translational research activities qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe's (**RP4**) research focuses on the molecular basis for the development of effective B cell responses to viruses in humans and he has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently henipaviruses NiV and HeV owing to the collaboration between the Crowe, Broder and Geisbert laboratories. Perhaps of greatest import, the SARS-CoV-2 mAbs generated by Dr. Crowe's laboratory were used in xxx,000 of people during the COVID-19 pandemic. RP4 will work closely with Cores D and E in advancing mAb prototypes. ~~The Core B of the Center will work together with RP2 and RP3 along with the Core C to develop the first cocktail human mAb immunotherapeutics, and facilitate the transition products suitable for IND filing and human testing including design, construction, assessment, and optimization of these antibody-based therapies.~~

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As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

1. Hallam, S.J., et al., *Review of Mammarenavirus Biology and Replication.* Front Microbiol, 2018. **9**: p. 1751.

2. NIH-NIAID. *NIAID Emerging Infectious Diseases/ Pathogens*. 2023 [cited 2023 03/06]; Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
3. Heinrich, M.L., et al., *Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages.* Scientific Reports, 2020. **10**(1): p. 16030.
4. Shaffer, J.G., et al., *Lassa Fever in Post-Conflict Sierra Leone.* PLOS Neglected Tropical Diseases, 2014. **8**(3): p. e2748.
5. Salam, A.P., et al., *Time to reconsider the role of ribavirin in Lassa fever.* PLOS Neglected Tropical Diseases, 2021. **15**(7): p. e0009522.
6. Enria, D.A., A.M. Briggiler, and Z. Sanchez, *Treatment of Argentine hemorrhagic fever.* Antiviral Res, 2008. **78**(1): p. 132-9.
7. Kilgore, P.E., et al., *Prospects for the control of Bolivian hemorrhagic fever.* Emerg Infect Dis, 1995. **1**(3): p. 97-100.
8. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nature Medicine, 2017. **23**(10): p. 1146-1149.
9. Hastie, K.M., et al., *Structural basis for antibody-mediated neutralization of Lassa virus.* Science, 2017. **356**(6341): p. 923-928.
10. Sullivan, B.M., et al., *High crossreactivity of human T cell responses between Lassa virus lineages.* PLoS Pathog, 2020. **16**(3): p. e1008352.
11. Ugwu, C., et al., *Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria.* Scientific Reports, 2022. **12**(1): p. 22330.
12. Geisbert, T.W., et al., *Development of a New Vaccine for the Prevention of Lassa Fever.* PLOS Medicine, 2005. **2**(6): p. e183.
13. Cross, R.W., et al., *A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever.* Cell Rep, 2022. **40**(3): p. 111094.
14. Safronetz, D., et al., *A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses.* PLOS Neglected Tropical Diseases, 2015. **9**(4): p. e0003736.
15. Hensley, L.E., et al., *Pathogenesis of Lassa fever in cynomolgus macaques.* Virol J, 2011. **8**: p. 205.
16. Zeitlin, L., et al., *Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody.* Proceedings of the National Academy of Sciences, 2021. **118**(11): p. e2023332118.
17. Bell, T.M., et al., *Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure.* Veterinary Pathology, 2015. **52**(1): p. 26-37.
18. Burt, F.J., et al., *Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia.* Epidemiol Infect, 1996. **116**(3): p. 353-61.
19. Brinkmann, A., et al., *First detection of bat-borne Issyk-Kul virus in Europe.* Sci Rep, 2020. **10**(1): p. 22384.
20. Ma, J., et al., *Identification of a new orthonairovirus associated with human febrile illness in China.* Nat Med, 2021. **27**(3): p. 434-439.
21. Lorenzo Juanes, H.M., et al., *Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021.* Emerg Infect Dis, 2023. **29**(2): p. 252-259.
22. Grandi, G., et al., *First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden.* Ticks Tick Borne Dis, 2020. **11**(3): p. 101403.
23. Hawman, D.W. and H. Feldmann, *Crimean–Congo haemorrhagic fever virus.* Nature Reviews Microbiology, 2023.
24. World.Health.Organisation. *List of Blueprint Priority Diseases*. 2018 [cited 2018 November 14]; Available from: http://www.who.int/blueprint/priority-diseases/en/.
25. Keshtkar-Jahromi, M., et al., *Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies.* Antiviral Res, 2011. **90**(2): p. 85-92.
26. Soares-Weiser, K., et al., *Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis.* BMC Infectious Diseases, 2010. **10**(1): p. 207.
27. Rodriguez, S.E., et al., *Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever.* Sci Rep, 2019. **9**(1): p. 7755.
28. Bente, D.A., et al., *Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model.* J Virol, 2010. **84**(21): p. 11089-100.

29. Cross, R.W., et al., *Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys.* PLoS Negl Trop Dis, 2020. **14**(8): p. e0008637.
30. Geisbert, T.W., et al., *Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody.* Sci Transl Med, 2014. **6**(242): p. 242ra82.
31. Bossart, K.N., et al., *A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge.* Sci Transl Med, 2011. **3**(105): p. 105ra103.
32. Pettitt, J., et al., *Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail.* Sci Transl Med, 2013. **5**(199): p. 199ra113.
33. Qiu, X., et al., *Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp.* Nature, 2014. **514**(7520): p. 47-53.
34. Mire, C.E., et al., *Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody.* Sci Transl Med, 2017. **9**(384).
35. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nat Med, 2017. **23**(10): p. 1146-1149.
36. Kuo, T.T. and V.G. Aveson, *Neonatal Fc receptor and IgG-based therapeutics.* MAbs, 2011. **3**(5): p. 422-30.
37. Robbie, G.J., et al., *A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study.* Antimicrob Agents Chemother, 2013.
38. Gaudinski, M.R., et al., *Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults.* PLoS Med, 2018. **15**(1): p. e1002493.
39. Organization, W.H., *Lassa fever fact sheet.* World Health Organization, 2017.
40. Wolf, T., et al., *Fifty years of imported Lassa fever: a systematic review of primary and secondary cases.* J Travel Med, 2020. **27**(4).
41. Purushotham, J., T. Lambe, and S.C. Gilbert, *Vaccine platforms for the prevention of Lassa fever.* Immunol Lett, 2019. **215**: p. 1-11.
42. Sewlall, N.H., et al., *Clinical features and patient management of Lujo hemorrhagic fever.* PLoS Negl Trop Dis, 2014. **8**(11): p. e3233.
43. Saito, T., et al., *Vaccine Candidates against Arenavirus Infections.* Vaccines, 2023. **11**(3): p. 635.
44. Koma, T., et al., *Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic.* J Virol, 2016. **90**(3): p. 1290-7.
45. Jahrling P, T.R., Barrero O. . *Crossprotection against Machupo virus with Candid 1 Junin virus vaccine*. in *2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region*. 1988. Mar del Plata, Argentina.
46. Delgado, S., et al., *Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia.* PLOS Pathogens, 2008. **4**(4): p. e1000047.
47. Loayza Mafayle, R., et al., *Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019.* New England Journal of Medicine, 2022. **386**(24): p. 2283-2294.
48. Tipih, T. and F.J. Burt, *Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development.* Biores Open Access, 2020. **9**(1): p. 137-150.
49. UHS-NHS. *Healthy volunteers needed for research investigating world-first vaccine for viral disease*. 2023 [cited 2023 03/06]; Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.
50. Kalunda, M., et al., *Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda.* The American Journal of Tropical Medicine and Hygiene, 1986. **35**(2): p. 387-392.
51. Schuh, A.J., et al., *Human-Pathogenic Kasokero Virus in Field-Collected Ticks.* Emerg Infect Dis, 2020. **26**(12): p. 2944-2950.
52. Mulangu, S., et al., *A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics.* N Engl J Med, 2019. **381**(24): p. 2293-2303.
53. Group, P.I.W., et al., *A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection.* N Engl J Med, 2016. **375**(15): p. 1448-1456.
54. Politch, J.A., et al., *Safety, acceptability, and pharmacokinetics of a monoclonal antibody-based vaginal multipurpose prevention film (MB66): A Phase I randomized trial.* PLoS Med, 2021. **18**(2): p. e1003495.

CENTER OVERVIEW: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction

Emerging and periodically reemerging pathogenic enveloped RNA viruses are ~~a~~ continuous ~~infectious disease and potentially~~ pandemic threats to global public health. Among the many groups of these types of pathogens are viral zoonoses within the family **Paramyxoviridae** and order ***Bunyavirales*** where humans can acquire infections directly from animal reservoir hosts such as rodents or bats~~,~~ through an intermediate amplifying host such as livestock, or from arthropod vectors such as mosquitos and ticks (Fields virology). More often than not, humans represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur, and should RNA viral pathogens mutate and adapt to more efficient replication and shedding in the human host, the epidemic potential of these viruses could manifest (PMID: 23911335 -Nipah / others?). The viral order *Bunyavirales* is composed of several viral families known to contain multiple high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic fever diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and are also identified as NIAID Category A pathogens due to their ease of dissemination and/or abilities to transmit person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special actions for public health preparedness. Also, within the WHO Priority Pathogens list is Nipah virus (NiV) and henipaviral disease which includes Hendra virus (HeV) which are zoonotic henipaviruses (HNVs) in the family **Paramyxoviridae**. NiV and HeV have long been considered the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease (PMID: 16357858). The genus now includes nine additional reported HNVs but only HeV and NiV are known to be associated with severe and often fatal henipaviral disease in humans and a number of animal species (PMID: 32991264). Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illnesses in humans [PMID: 35921459].

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Taken together, these viral threats to global public health are heightened due to the absence of approved vaccines or therapeutics. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for vaccine and monoclonal antibody (mAb) countermeasures in developing prototype candidates (**Table 1**) against a selection of these viruses. In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Table 1. PABVAX selected prototype and test case viral agents.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old world), Machupo (New world)	Lujo (Old world), Chapare (New world)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

Bunyavirales:

Arenaviridae: The family Arenaviridae is composed of three genera: Mammarenaviridae, Reptarenavirus, and Hartmanivirus. The Mammarenaviridae are composed of a large number rodent borne viruses, some of which are demonstrated human pathogens responsible for causing severe hemorrhagic fever, neurological damage, and if not fatal can result permanent neurological sequelae including hearing loss, vision impairment, and balance [1]derangement. While these genera exhibit a global distribution, two geographically distinct sub-genera have formed due to the separation across continents by the Atlantic and Pacific Ocean. Old World Mammarenaviruses (Lassa Virus (LASV), Lymphocytic Choriomeningitis Virus (LCMV), and the newly discovered Lujo Virus (LUJV)) and the New World Arenaviruses (Junin Virus (JUNV), Machupo Virus (MACV), Sabia Virus (SABV), and the newly emerging Chapare Virus (CHAPV)). Many of these viruses are identified as NIAID Category A pathogens requiring biosafety level 4 (BSL4) containment [2]. This classification is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness. While several

promising medical countermeasures are in development, there are currently no US FDA approved vaccines or therapeutics for any mammarenaviruses, though the attenuated JUNV “Candid-1” vaccine is approved for use in Argentina.

Infection in humans is often the result of exposure to rodent excreta from infected animals where disease severity and case fatality rates (CFR) can vary across the genera and even within genetic lineages as can be the case for infections with LASV where genetic diversity can be high, even within clades. While this genetic variability creates significant challenges for LASV molecular diagnostics, antigen-based diagnostics have seen high levels of cross reactivity across the seven known lineages of LASV suggesting cross protective antigens may be possible[3]. Lassa virus, the causative agent of Lassa fever (LF), likely causes the largest number of annual cases per year of LF, where its understood up to 500,000 cases per year can occur where the CFR can approach 70% in hospitalized cases [4]. Ribivirin has been used to treat infections with uncertain success [5]. Before the introduction of the JUNV Candid-1 vaccine, its believed that upwards of 60,000 cases of JUNV infection, also known as Argentine Hemorrhagic Fever (AHF) per year occurred where CFRs ranged from 15-30 %. Convalescent plasma from JUNV survivors is still the primary means to treat acute JUNV infections in Argentina [6]. The initial outbreak of Machupo virus had over 1000 cases with a CFR, where outbreak frequency is thought to have been reduced through enhanced rodent abatement practices and potentially cross-protective immunity in populations who have taken the JUNV Candid-1 vaccine [7].

All mammarenaviruses share a common genomic organization consisting of two ambi-sense (bi-directional reading frame) RNA segments. Each segment contains two open reading frames encoding two proteins per strand, these include the Z protein (matrix), the L protein (polymerase), glycoprotein precursor (GPC), and the nucleoprotein (NP) [1]. Despite general similarity of their genomic architecture, these groups exhibit marked differences in their replication life cycles. This includes differences in attachment, entry, and immune evasion; nonetheless, the dominant antigenic proteins driving immune responses for both OWAV and NWAV are the glycoprotein (GP) and NP.

Monoclonal antibodies derived from human LASV survivors have been demonstrated to protect non-human primates (NHP), against lethal challenge [8]. Recent crystal structure studies have identified binding interactions with these antibodies using stabilized, pre-fusion trimeric LASV glycoproteins (GPe) [9]. These findings suggest a stabilized form soluble LASV glycoprotein could serve as a viable vaccine antigen. Humoral responses to LASV NP in survivors from Sierra Leone (LASV Lineage IV endemic region) and Nigeria (LASV Lineages II, III, and VI endemic region) are directed toward the highly conserved N-terminal domain (NTD) of NP, suggesting using this domain as a vaccine antigen may also be possible. To test these notions, investigators in the Center **RP1**, **Core E**, and **Core D** have developed adjuvanted vaccine approaches to test this idea and have demonstrated protection against lethal LASV-Josiah challenge using a stabilized prefusion, trimeric GPe, a highly conserved NP NTD epitope, or a combination of the two antigens using a lethal outbred guinea pig model.

Despite the considerable molecular diversity in LASV strains, there is understood that there is some conservation in key antigenic sites within both the GPe and the NP, suggesting that a PAN-LASV vaccine may be possible[3, 10, 11]. Indeed, **RP1** and **Core E** investigators have demonstrated that using a Vesicular Stomatitis Virus expressing the LASV-Josiah GPC is not only protective against homologous challenge with LASV Josiah (Lineage IV-Sierra Leone) in a lethal NHP model[12], but also that live attenuated vaccines expressing this same GPC can protect NHP against challenge with viruses from: Lineage II[13] , Lineage III (Cross, Geisbert unpublished), Lineage V[14] and Lineage VI (Cross and Geisbert Unpublished).

In **Phase I of RP1**, the ability for an optimized intramuscular (IM) LASV GPe/NP subunit vaccine to protect against disease after challenge multiple LASV lineages will be assessed in guinea pig and NHP models. This formulation will also be assessed using a microneedle skin patch (MNP) formulation optimized in Core C to determine if a needle-free vaccination route affords more potent vaccine mediated responses and protection. Similarly, we have already generated MACV GPe and will design a MACV NP-NTD subunit antigen to test following the same approach followed with LASV against both homologous lineage challenge and heterologous challenge of MACV genetic lineages. In **Phase II of RP1**, we will assess the **plug-and-play** potential of our stabilized GPe/NP-NTD MNP vaccine approach against emerging test case mammarenaviruses representing

emerging OWAV (LUJV) and NWAV (CHAPV) for which there are no vaccines available. A unique feature of this proposal is its access to BSL4 facilities described in **Core E** capable of evaluating countermeasures in NHP. It is widely considered that the cynomolgus macaque is the current “gold standard” animal model for LASV, JUNV, and MACV research as there are a multitude of immunological reagents available and the disease pathogenesis appears to mirror that of human infection[15-17]. The evaluation of successful countermeasures for the prototype OWAV AND NWAV in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

Unique from most other bunyaviruses, orthonairoviruses are transmitted primarily by ticks in nature (REF). While there are a number of human pathogenic orthonairoviruses (e.g. Dugbe virus[18], Issyk-Kul virus[19], Songling virus[20]), Crimean-Congo Hemorrhagic fever virus (CCHFV) is by far the most recognized public health threat to humans as it responsible for annual outbreaks across Europe, Africa, the Middle East, and Asia. There are growing concerns for endemic range expansion due to ongoing range expansion of tick hosts tied to bird migration changes, livestock trade, and even global warming are contributing to increasing numbers of infections in humans in areas otherwise not known to be endemic including central Spain[21] and most recently the reservoir Hyalomma ticks have been found as north as Sweden[22]. Infection in humans begins as a nonspecific febrile illness but can rapidly progress to cause a severe hemorrhagic fever which may progress to death in 30% of cases in some regions[23]. CCHFV is listed on the WHO priority pathogen list[24] and is also a NIH-NIAID category A pathogen[2]. There are no FDA approved vaccines or therapeutics for CCHF; however, a mouse-brain derived inactivated whole virus vaccine has been in use in Bulgaria with limited efficacy[25] (REF). Ribivirin has been used to treat infections with limited success[26].

CCHFV has a trisegmented genome encoding 4 proteins, NP, small non-structural protein (NSs), L polymerase protein, and a GPC [23]. Similar to mammarenaviruses, the NP and GP are the primary antigenic targets of the host immune response. The NP of CCFHV is highly conserved across the 6 genomic clades with up to 95% amino acid homology[23]. Conversely, he CCHFV GPC is considerably less conserved, where less than 75% amino acid conservation is present[23]. The genetic diversity of CCHFV correlates strongly with geography, and clades of CCHFV segregate based on geographical location which creates a challenge in terms of creating a pan-CCHFV vaccine or therapeutic based solely on one clade. Nonetheless, we have demonstrated that heterologous vaccine mediated protection can be afforded using a VSV vectored vaccine expressing the full GPC of CCHF[27].

In phase 1, **RP1 and Core D** will generate CCHFV GP and NP subunits with the highest level of homology across clades and assess their capacity to protect against disease after challenge with multiple CCHFV clades in established mouse[27, 28] and NHP[29] models with Core E. CCHFV GP and NP subunit vaccines will also be coupled to MNP (**Core C**) for comparison with IM vaccination. In **Phase II of RP1**, we will assess the **plug-and-play** potential of the best performing formulation of CCHFV GP/NP MNP vaccine approach against emerging test case mammarenaviruses representing emerging bat virus, Kasokero virus for which there are no vaccines available.

~~The *Bunyavirales*.~~

~~Fill out~~

The henipavirus paramyxoviruses. The recognized, highly pathogenic HNVs, NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality (Murray, PMID: 7701348). NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts (Chua, PMID: 10827955). NiV and HeV

can infect at least 18 animal species across 6 orders of mammals, and among these they will cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans (PMID: 32991264; PMID: 22476556). The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission (PMID: 23911335). Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and South East Asia and Australia. Their broad host tropism and ability of these viruses to infect livestock offers an amplification opportunity and increased risk of subsequent human infections. The henipaviruses are single-stranded, negative sense, enveloped RNA viruses and their biology also lends themselves to be synthetically produced and agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents [Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; Department of Health and Human Services (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087]. because they could be engineered for mass dissemination and possess the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the WHO R&D Blueprint list of priority pathogens with epidemic potential (Sweileh, W. M. PMC5299748).

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb, postexposure treatments have been demonstrated to be effective therapies against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP) (PMID: 22013123, PMID: 24964990, PMID: 27484128, PMID: 31686101). The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia (PMID: 32027842). In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs and several vaccine platforms have clear potential for use in people (PMC8782152). The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies (PMC8782152). Such preventive vaccines would have utility for lab workers, first responders or individuals at high risk exposure and in mitigating the spread of an outbreak; whereas in the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent (Middleton, 2014 PMID: 24572697). It is widely considered that the African green monkey (AGM) is the current "gold standard" animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection (Geisbert, 2012;Geisbert, 2010). The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished) (PMID: 33809833). The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons; 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Also, another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including the rescue of recombinant LayV (rLayV), explore animal model development, construct additional LayV and AngV reporter virus tools, and then translate and test the MNP

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vaccine ‘plug-and-play’ approach, and also evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME

A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center’s RPs and Core leaders currently have active and productive collaborative research programs in related areas, some spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

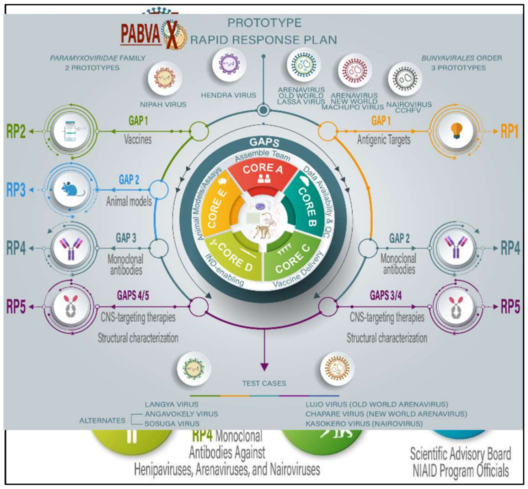

Figure 1: Organizational chart of the Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center-(PABVAX). **(connecting arrows are needed between elements and the SAB)**

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Maybe be good to have the whole title per RP listed instead of abbreviated form?

May need to identify what is meant by Gaps as its not identified in this document, I think maybe it is spelled out in Core E document?. Assuming maybe these are Gaps identified in the main RFA?

Just an idea…. having RP4 and RP5 bubbles duplicated to demonstrate different GAPs addressed is a little confusing to me, maybe if we do end up enumerating the gaps somewhere in the text, it might simplify things by identify what gaps are addressed by each RP so that each RP only has one bubble? EG:

- RP1=gap 1
- RP2=gap 1
- RP3=gap 2
- RP4=gap 2,3
- RP5= gap 3,4,5

The organizational structure of the PABVAX is diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **Arenaviridae** and **Nairoviridae** viruses, and **RP2** will target the prototype **henipaviruses** (HNVs). Both RP1 and RP2 will also be developing new prototype virus tools (recombinantly generated prototype viruses) along with existing tools, to be used in the analysis of vaccine responses as well as in the evaluation of new mAbs and nabs from the other RPs. A third RP will be engaged in the development of a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant Cedar henipavirus (rCedV) in vivo and a bioluminescence methodology that can longitudinally trace the dynamics and anatomical progression of luciferase encoding virus infection in individual animals (**RP3**). There will also be two RPs that will target the development of human mAbs and nanobodies to the PABVAX select viral prototypes. **RP4** will isolate human mAbs derived from NiV infected survivors with a specific focus on the F glycoprotein targeted mAbs, and additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses along with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will be engaged in manufacture and quality control & testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform.
The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from

the UPitt and IBBR/UMD (**Core C**) that has tested a dissolvable MNP-based vaccine technology that is ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine approach (PMID: 32924651). This vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable MNPs that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants (PMID: 19864632) to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as facilitate the improved vaccine storage and distribution due to their thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, a Scientific **Core C**: (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ) adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (Paramyxoviridae). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity in vitro and polyfunctional T-cell responses by in vitro stimulation of isolated cells with antigenic peptides, intracellular cytokine staining (ICS) and flow cytometry) responses.

Discover effective antibody-based prophylactics and therapies.
~~PREP can be critical in the absence of available vaccines or cases in which vaccination is not possible.~~
HELP

Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah [30], Hendra [31], Ebola [32, 33], Marburg [34] and Lassa [35]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc.*), a feature to be exploited in **Core D**. In addition to therapeutic applications, mAbs with Fc mutations to dramatically extend serum half-life [36-38] offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g.* rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

Develop new tools and animal models to facilitate the translation of effective countermeasures.
Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the A~~A~~nimal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation S~~s~~cientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including ~~……..~~ animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective ~~therapeutic~~ efficacy of antivirals and vaccines as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation; including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera

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or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they can be adapted to greater high-throughput platforms, are often less laborious, and they can make use of other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput florescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic Nipah and Hendra virus vaccine induced immune sera, mAbs (**RP4**) and nbs (**RP5**) and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either the HeV or NiV-B envelope glycoproteins) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals, ideally suited to explore nab based therapeutics to CNS resident henipavirus (PMID: 35821488).

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. ~~Data generated to support of possible future regulatory submissions to the Food and Drug Administration (FDA) must be collected in accordance with quality systems, like good laboratory practice (GLP) regulations, to be useful and reliable, and to facilitate FDA review to ensure safety and efficacy.~~ The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the henipaviruses (Paramyxoviridae) and the Bunyavirales (Arenaviridae and Nairoviridae) that will bolster vaccine endurance to storage and transportation; and also improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology and mAb and vaccine translational drug development and includes Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Henipaviruses: Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals and are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of Cedar virus (CedV), Gamak virus, Daeryong virus, and Langya virus (LayV) [xxx] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV) [xxx]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV,

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Lee, S.H.; Kim, K.; Viruses 2021, 13, doi:10.3390/v13102020.

Zhang, X.A.; Li, H.; Jiang, N Engl J Med 2022, 387, 470-472, doi:10.1056/NEJMc2202705.

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

are old world pteropid fruit bats. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province). The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**.

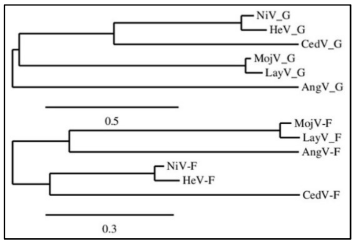

Figure 2. Phylogenetic analysis of select HNV G and F glycoproteins. Phylogenetic trees were constructed for the HNV F and G glycoproteins using the PhyML program's maximum likelihood method. The trees were generated by aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal henipaviral disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters (Geisbert 2012), and African green monkeys (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P), and the P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists (xx). The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2 (XXX) and CedV is now recognized as a BSL-2 restricted agent [CDC manual]. Because the HNV, LayV, is known to be a human pathogen and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D, and E**).

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Laing, E.D.; Navaratnarajah, C.K.; Proc Natl Acad Sci U S A 2019, doi:10.1073/pnas.1911773116.

Commented [CB10]: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. HHS Publication No. (CDC) 300859. US Government Printing Office: Washington, 2020; pp. 248-250.

Old Word Mammarenaviruses (OWAV): For **phase 1**, **RP1** will utilize **LASV as the prototype OWAV** due to the significant public health burden it creates throughout West Africa with hundreds of thousands of estimated cases and approximately 5000 deaths annually[39]. LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to several countries outside of Africa over 35 documented times [40]. Case fatality rates (CFR) approaching 70% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) [41]. Of the vaccines supported by CEPI, 4/5 of these vaccines utilize the only the LASV GP as the target immunogen despite

Investigators in RP1, Core D, and Core E have already demonstrated protective efficacy of against disease after challenge with LASV in outbred guinea pigs in animals vaccinated IM with engineered stabilized, prefusion LASV GPe, NP-NTD, or a combination of both antigens. These antigens have been engineered to harness the homologous antigenic regions of both antigens to increase potential for pan-LASV lineage protection from disease after vaccination. Success with this approach, suggest a similar strategy of using a subunit vaccine might also be effective against other arenaviruses. While LASV is the most common pathogenic arenavirus in Africa in terms of human infections, Lujo virus was isolated over 10 years ago from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [42]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, implying LUJV will be an ideal "test case" OWAV to assess the plug-and-play approach of stabilized, prefusion LUJV GPe/ NP-NTD vaccination. The PABVAX Center will explore LUJV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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New World Mamarenaviruses (NWAV): A live attenuated vaccine for JUNV (Candid-1) is currently in use in Argentina [43], but there is conflicting evidence of whether cross protection with other NWAV is possible with this vaccine [44, 45]. Further, there are safety concerns related to potential for reversion to pathogenic versions. For **Phase 1, RP1** will use **MACV and JUNV** as the **prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted. Building on the success of the LASV GPe/NP-NTD. Investigators in **RP1 and Cores D and E** will engineer MACV GPe and NP-NTD antigens and test for efficacy in established guinea pig and NHP models using MACV from multiple lineages to asses how broadly protective the approach is across MACV lineages. We will then extend this plug-and-play approach to other NWAV. First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [46, 47]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [47]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective against CHAPV. Otherwise, there are no known vaccines demonstrated effective for this virus. The PABVAX Center will explore CHAPV as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

Orthonairoviruses: Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family responsible for often large outbreaks of human disease annually across Europe, Africa, and Asia. There are no FDA approved vaccines for CCHFV; however, a suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974 but has numerous safety concerns and limited demonstrated efficacy. Since then, several vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages[48]; however only an MVA-CCHFV vaccine has begun to advance towards clinical trials[49]. Investigators in **RP1 and Cores D and E** will engineer CCHFV GP and NP subunit antigens and test for protective efficacy from disease after challenge with 6 different lineages of CCHFV clades using in established mouse and NHP models. Next we will deploy the same approach to a known human pathogen "test case" orthonairovirus for which no vaccine exists.

In 1977, **Kasokero virus (KASV),** a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [50]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for the reservoir, *Ornithodoros faini,* ticks to participate in enzootic maintenance of KASV [51]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised. The PABVAX Center will explore **KASV** as part of the Center's **Phase II** research plan (**RP1** with **Cores C, D, and E**).

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EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as

for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs; such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**), (aarenavirus and nairovirus~~renaviridae and nairoviridae~~ projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine the active and passive immunization approaches to further improved the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

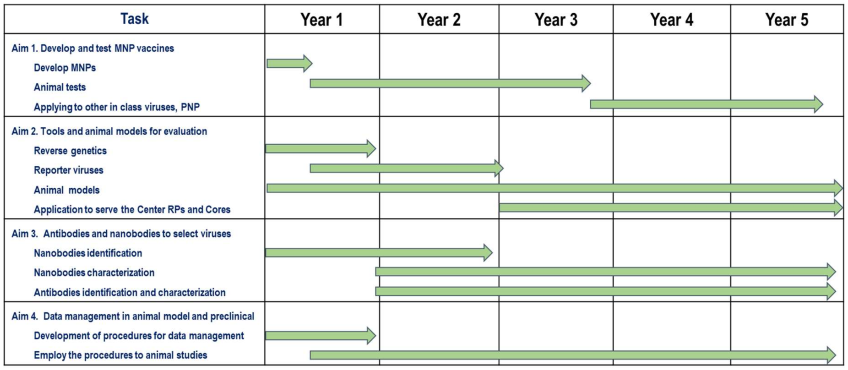
An additional and outstanding advantage of the PABVAX Center is the **Animal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL. (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for all the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.**
2. **Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.**
3. **Develop antibodies and nanobodies to selected prototype viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.



Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. This is in addition to each PR's individual Milestone plan. The oversight and evaluation of progress will be in concert with quarterly reports to the

PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (Details are within the Administration **Core A**). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommend new directions as appropriate and provide Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

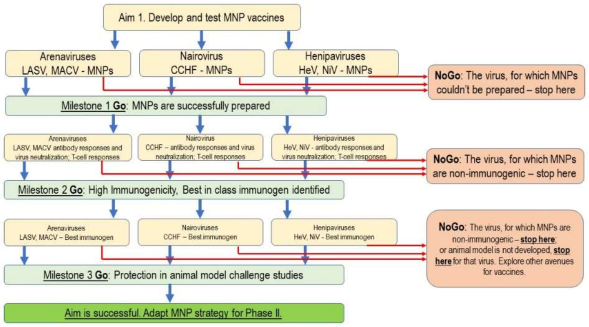


Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will be whether proper formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be made based on immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction and virus-neutralization and polyfunctional T-cell responses by in vitro stimulation of isolated cells). Go/no go decisions will be made based on the outcomes of these first tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as success. Comparisons will also be made, such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of best-in-class immunogens, or best combination immunogens which will also be explored, will be performed in order to down-select vaccine choice. The third milestone will be the determination of whether MNP vaccination affords protection in animal model challenge experiments. In the case of the NiV and HeV prototypes the animal models chosen (ferret and the African green monkey (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales* there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASA and there are guinea pig models for some but not all of those lineages. For MACV there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys are now each greater than 35K. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of the AGM for the arenaviruses and possibly also for CCHF as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.

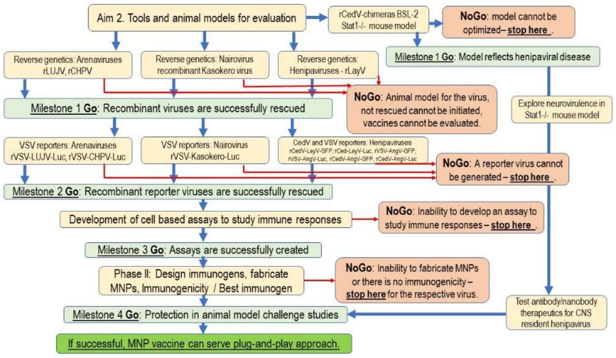


Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center overall objectives.

The PABVAX second overall objective is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined in vivo (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, a a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**) the milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new in vivo bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease in vivo safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**) it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and RP3 will collaborate with RP5 in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

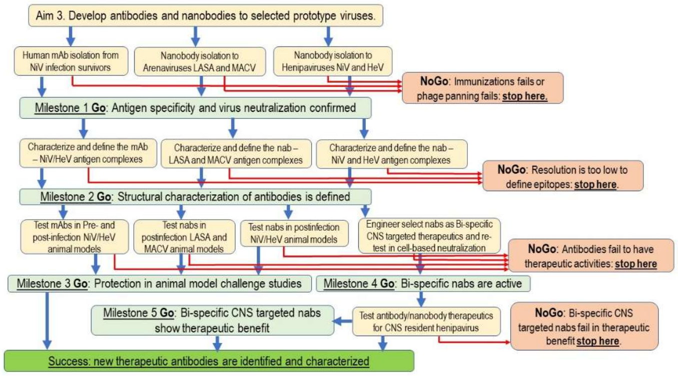


Figure 6. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

New human mAbs and nanobodies (nbs) as tools and potential therapeutics will be developed by RP4 and RP5 in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); and transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on the mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs and in collaboration with **RP2** neutralization potencies will be rapidly assessed as well as evaluating synergistic cell-based mAb neutralization that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

Nanobodies, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nanobodies can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nanobodies targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), and also for therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus) and select animal models of LASV and MACV by **Core E**. Success will be measured by the identification of virus-neutralizing nbs, potential discovery of more broadly reactive nbs, particularly to the multiple LASA clades, the evidence of therapeutic benefit in animal models, and the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

INDUSTRY PARTNERSHIPS

Industry Partnership

Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine

expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX.

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SPECIAL FEATURES OF THE PABVAX CENTER ENVIRONMENT AND OTHER RESOURCES ??

Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

Galveston National Laboratory (GNL)

The GNL, is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics and therapeutics against them. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370~~187~~ separate NHP studies employing a total of 2,772~~1,318~~ animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses ~~Ebola and Marburg viruses and also Nipah and Hendra~~ have been successfully performed at the GNL. These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP~~nonhuman primate~~ facility and a staff of full-time veterinarians. In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of WRCEVA virus collection within the GNL.

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Core E (Animal model development and preclinical evaluation)

Core E operations will be conducted at the GNL, and in conjunction with **Core A** also includes the UTMB Institutional Office of Regulated Nonclinical Studies (ONcRS). The ONcRS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, ~~to meet regulatory requirements by assuring regulatory compliance,~~ quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. ~~Quality system and regulatory science processes for NHP studies performed at UTMB will be implemented and executed in conjunction with the ORNcS.~~ Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses and the henipaviruses and he has published over 250~~XXX~~ peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI) was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

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Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs)

Mapp Biopharmaceutical has deep experience with antigens and monoclonal antibodies (mAbs) against these antigens. ~~'s product development team has grown significantly in the last two years in response to the development efforts on its anti-Ebola therapeutic, ZMapp^{TM} which is being advanced to licensure as part of Project Bioshield. OTHER MAbs~~ Their early development and research group has expertise in immunology, infectious diseases, and production of antigens, antibodies and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies {Mulangu, 2019 #818} {Group, 2016 #649}; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1 {Politch, 2021 #897}. ~~developed multiple products, including a licensed biodefense biologic. As with Profectus, Mapp Bio's expertise and experience will be a very important and valuable asset to the Center.~~

Bonnie Genovac

Core A (Administrative Core)

Research Project teams?? Maybe not depending on space

The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills and his experience in translational research activities qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe's (**RP4**) research focuses on the molecular basis for the development of effective B cell responses to viruses in humans and he has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently henipaviruses NiV and HeV owing to the collaboration between the Crowe, Broder and Geisbert laboratories. Perhaps of greatest import, the SARS-CoV-2 mAbs generated by Dr. Crowe's laboratory were used in xxx,000 of people during the COVID-19 pandemic. RP4 will work closely with Cores D and E in advancing mAb prototypes. ~~The Core B of the Center will work together with RP2 and RP3 along with the Core C to develop the first cocktail human mAb immunotherapeutics, and facilitate the transition products suitable for IND filing and human testing including design, construction, assessment, and optimization of these antibody-based therapies.~~

As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

BIBLIOGRAPHY AND REFERENCES CITED

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1. Hallam, S.J., et al., *Review of Mammarenavirus Biology and Replication.* Front Microbiol, 2018. **9**: p. 1751.
2. NIH-NIAID. *NIAID Emerging Infectious Diseases/ Pathogens*. 2023 [cited 2023 03/06]; Available from: https://www.niaid.nih.gov/research/emerging-infectious-diseases-pathogens.
3. Heinrich, M.L., et al., *Antibodies from Sierra Leonean and Nigerian Lassa fever survivors cross-react with recombinant proteins representing Lassa viruses of divergent lineages.* Scientific Reports, 2020. **10**(1): p. 16030.
4. Shaffer, J.G., et al., *Lassa Fever in Post-Conflict Sierra Leone.* PLOS Neglected Tropical Diseases, 2014. **8**(3): p. e2748.
5. Salam, A.P., et al., *Time to reconsider the role of ribavirin in Lassa fever.* PLOS Neglected Tropical Diseases, 2021. **15**(7): p. e0009522.
6. Enria, D.A., A.M. Briggiler, and Z. Sánchez, *Treatment of Argentine hemorrhagic fever.* Antiviral Res, 2008. **78**(1): p. 132-9.
7. Kilgore, P.E., et al., *Prospects for the control of Bolivian hemorrhagic fever.* Emerg Infect Dis, 1995. **1**(3): p. 97-100.
8. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nature Medicine, 2017. **23**(10): p. 1146-1149.
9. Hastie, K.M., et al., *Structural basis for antibody-mediated neutralization of Lassa virus.* Science, 2017. **356**(6341): p. 923-928.
10. Sullivan, B.M., et al., *High crossreactivity of human T cell responses between Lassa virus lineages.* PLoS Pathog, 2020. **16**(3): p. e1008352.
11. Ugwu, C., et al., *Humoral and cellular immune responses to Lassa fever virus in Lassa fever survivors and their exposed contacts in Southern Nigeria.* Scientific Reports, 2022. **12**(1): p. 22330.
12. Geisbert, T.W., et al., *Development of a New Vaccine for the Prevention of Lassa Fever.* PLOS Medicine, 2005. **2**(6): p. e183.
13. Cross, R.W., et al., *A recombinant VSV-vectored vaccine rapidly protects nonhuman primates against heterologous lethal Lassa fever.* Cell Rep, 2022. **40**(3): p. 111094.
14. Safronetz, D., et al., *A Recombinant Vesicular Stomatitis Virus-Based Lassa Fever Vaccine Protects Guinea Pigs and Macaques against Challenge with Geographically and Genetically Distinct Lassa Viruses.* PLOS Neglected Tropical Diseases, 2015. **9**(4): p. e0003736.
15. Hensley, L.E., et al., *Pathogenesis of Lassa fever in cynomolgus macaques.* Virol J, 2011. **8**: p. 205.
16. Zeitlin, L., et al., *Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody.* Proceedings of the National Academy of Sciences, 2021. **118**(11): p. e2023332118.
17. Bell, T.M., et al., *Pathology of Experimental Machupo Virus Infection, Chicava Strain, in Cynomolgus Macaques (Macaca fascicularis) by Intramuscular and Aerosol Exposure.* Veterinary Pathology, 2015. **52**(1): p. 26-37.
18. Burt, F.J., et al., *Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in a patient with prolonged thrombocytopenia.* Epidemiol Infect, 1996. **116**(3): p. 353-61.
19. Brinkmann, A., et al., *First detection of bat-borne Issyk-Kul virus in Europe.* Sci Rep, 2020. **10**(1): p. 22384.
20. Ma, J., et al., *Identification of a new orthonairovirus associated with human febrile illness in China.* Nat Med, 2021. **27**(3): p. 434-439.
21. Lorenzo Juanes, H.M., et al., *Crimean-Congo Hemorrhagic Fever, Spain, 2013-2021.* Emerg Infect Dis, 2023. **29**(2): p. 252-259.
22. Grandi, G., et al., *First records of adult Hyalomma marginatum and H. rufipes ticks (Acari: Ixodidae) in Sweden.* Ticks Tick Borne Dis, 2020. **11**(3): p. 101403.
23. Hawman, D.W. and H. Feldmann, *Crimean–Congo haemorrhagic fever virus.* Nature Reviews Microbiology, 2023.
24. World.Health.Organisation. *List of Blueprint Priority Diseases*. 2018 [cited 2018 November 14]; Available from: http://www.who.int/blueprint/priority-diseases/en/.
25. Keshtkar-Jahromi, M., et al., *Crimean-Congo hemorrhagic fever: current and future prospects of vaccines and therapies.* Antiviral Res, 2011. **90**(2): p. 85-92.
26. Soares-Weiser, K., et al., *Ribavirin for Crimean-Congo hemorrhagic fever: systematic review and meta-analysis.* BMC Infectious Diseases, 2010. **10**(1): p. 207.
27. Rodriguez, S.E., et al., *Vesicular Stomatitis Virus-Based Vaccine Protects Mice against Crimean-Congo Hemorrhagic Fever.* Sci Rep, 2019. **9**(1): p. 7755.
28. Bente, D.A., et al., *Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model.* J Virol, 2010. **84**(21): p. 11089-100.

29. Cross, R.W., et al., *Crimean-Congo hemorrhagic fever virus strains Hoti and Afghanistan cause viremia and mild clinical disease in cynomolgus monkeys.* PLoS Negl Trop Dis, 2020. **14**(8): p. e0008637.
30. Geisbert, T.W., et al., *Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody.* Sci Transl Med, 2014. **6**(242): p. 242ra82.
31. Bossart, K.N., et al., *A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge.* Sci Transl Med, 2011. **3**(105): p. 105ra103.
32. Pettitt, J., et al., *Therapeutic intervention of Ebola virus infection in rhesus macaques with the MB-003 monoclonal antibody cocktail.* Sci Transl Med, 2013. **5**(199): p. 199ra113.
33. Qiu, X., et al., *Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp.* Nature, 2014. **514**(7520): p. 47-53.
34. Mire, C.E., et al., *Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody.* Sci Transl Med, 2017. **9**(384).
35. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nat Med, 2017. **23**(10): p. 1146-1149.
36. Kuo, T.T. and V.G. Aveson, *Neonatal Fc receptor and IgG-based therapeutics.* MAbs, 2011. **3**(5): p. 422-30.
37. Robbie, G.J., et al., *A Novel Investigational Fc Modified Humanized Monoclonal Antibody, Motavizumab-YTE, Has an Extended Half-life in Healthy Adults: A Randomized Study.* Antimicrob Agents Chemother, 2013.
38. Gaudinski, M.R., et al., *Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: A Phase 1 open-label clinical trial in healthy adults.* PLoS Med, 2018. **15**(1): p. e1002493.
39. Organization, W.H., *Lassa fever fact sheet.* World Health Organization, 2017.
40. Wolf, T., et al., *Fifty years of imported Lassa fever: a systematic review of primary and secondary cases.* J Travel Med, 2020. **27**(4).
41. Purushotham, J., T. Lambe, and S.C. Gilbert, *Vaccine platforms for the prevention of Lassa fever.* Immunol Lett, 2019. **215**: p. 1-11.
42. Sewlall, N.H., et al., *Clinical features and patient management of Lujo hemorrhagic fever.* PLoS Negl Trop Dis, 2014. **8**(11): p. e3233.
43. Saito, T., et al., *Vaccine Candidates against Arenavirus Infections.* Vaccines, 2023. **11**(3): p. 635.
44. Koma, T., et al., *Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic.* J Virol, 2016. **90**(3): p. 1290-7.
45. Jahrling P, T.R., Barrero O. . *Crossprotection against Machupo virus with Candid 1 Junin virus vaccine*. in *2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region*. 1988. Mar del Plata, Argentina.
46. Delgado, S., et al., *Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia.* PLOS Pathogens, 2008. **4**(4): p. e1000047.
47. Loayza Mafayle, R., et al., *Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019.* New England Journal of Medicine, 2022. **386**(24): p. 2283-2294.
48. Tipih, T. and F.J. Burt, *Crimean-Congo Hemorrhagic Fever Virus: Advances in Vaccine Development.* Biores Open Access, 2020. **9**(1): p. 137-150.
49. UHS-NHS. *Healthy volunteers needed for research investigating world-first vaccine for viral disease*. 2023 [cited 2023 03/06]; Available from: https://clinicalresearch.uhs.nhs.uk/news/healthy-volunteers-needed-for-research-investigating-world-first-vaccine-for-viral-disease.
50. Kalunda, M., et al., *Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda.* The American Journal of Tropical Medicine and Hygiene, 1986. **35**(2): p. 387-392.
51. Schuh, A.J., et al., *Human-Pathogenic Kasokero Virus in Field-Collected Ticks.* Emerg Infect Dis, 2020. **26**(12): p. 2944-2950.

From: Woolsey, Courtney B. (Micro) on behalf of Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
To: Geisbert, Thomas W.
Cc: Larry Zeitlin; CHRISTOPHER BRODER; Dafna Abelson; Antony Dimitrov; Haller, Sherry; Stutz, Sonja J.; Cross, Robert W.; Moushimi Amaya
Subject: Re: overall 12 page res plan
Date: Monday, June 5, 2023 10:04:08 AM
Attachments: Overall_Research-Strategy_PABVAX-12pages-060523v2.docx

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Removed three non-critical references and Endnote updated to NIH style to include PMCID numbers.

On Jun 5, 2023, at 7:56 AM, Geisbert, Thomas W. <twgeisbe@utmb.edu> wrote:

OK here are edits from Courtney and then me. This looks really good. Sherry, I have two questions. Are References limited to 100? If so we will need to lose three? Second, do we have to put PMCID numbers into references?

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Monday, June 5, 2023 6:42 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
Cc: Dafna Abelson <dafna.abelson@mappbio.com>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Haller, Sherry <shhaller@UTMB.EDU>; Stutz, Sonja J. <sjstutz@utmb.edu>; Cross, Robert W. <rwcross@UTMB.EDU>; Moushimi Amaya <moushimi.amaya@usuhs.edu>
Subject: Re: overall 12 page res plan

Here it is – resolved my two comments and deleted some extraneous text left over from RFA instructions…

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Date: Monday, June 5, 2023 at 7:32 AM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
Cc: Dafna Abelson <dafna.abelson@mappbio.com>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>, Haller, Sherry <shhaller@UTMB.EDU>, Stutz, Sonja J. <sjstutz@utmb.edu>, Cross, Robert W. <rwcross@UTMB.EDU>, Moushimi Amaya <moushimi.amaya@usuhs.edu>
Subject: Re: overall 12 page res plan

I think there is something that Chris mentioned on a comment from you. Can you go through it and then I will go through it after you? Many thanks!

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Monday, June 5, 2023 6:31 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
Cc: Dafna Abelson <dafna.abelson@mappbio.com>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Haller, Sherry <shhaller@UTMB.EDU>; Stutz, Sonja J. <sjstutz@utmb.edu>; Cross, Robert W. <rwcross@UTMB.EDU>; Moushimi Amaya <moushimi.amaya@usuhs.edu>
Subject: Re: overall 12 page res plan

I haven’t touched this. Happy to though if you want me to do a review.

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>

Date: Monday, June 5, 2023 at 7:29 AM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
Cc: Larry Zeitlin <larry.zeitlin@mappbio.com>, Dafna Abelson <dafna.abelson@mappbio.com>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>, Haller, Sherry <shhaller@UTMB.EDU>, Stutz, Sonja J. <sjstutz@utmb.edu>, Cross, Robert W. <rwcross@UTMB.EDU>, Moushimi Amaya <moushimi.amaya@usuhs.edu>
Subject: Re: overall 12 page res plan

Larry,

I don't want to duplicate effort. Are you working on this now?

Many thanks!

Tom

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Sunday, June 4, 2023 9:30 PM
To: Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
Cc: Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Haller, Sherry <shhaller@UTMB.EDU>; Stutz, Sonja J. <sjstutz@utmb.edu>; Cross, Robert W. <rwcross@UTMB.EDU>; Moushimi Amaya <moushimi.amaya@usuhs.edu>
Subject: Re: overall 12 page res plan

here is 12 pages
there is ~10% left space on page 12

i did not mess with formatting, so if you want an UNformatted
version , then use this to unformat

i edited throughout to tighten up, did not remove any reffs

Only larry's comment (2) remain to finalize

On Sun, Jun 4, 2023 at 8:46 PM Woolsey, Courtney B. (Micro) <cbwillia@utmb.edu> wrote:

> Okay, here is where I'm at on the overall research strategy (tracked changes and clean versions). We still need to get rid of a little over half of a page.
>
> > On Jun 4, 2023, at 9:56 AM, Cross, Robert W. <rwcross@UTMB.EDU> wrote:
> >
> > Got it, yes I will add connection to RP5, I initially missed this, but caught it when trying to make a small directional figure to add to RP1 to demonstrate connections to other cores.

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Sunday, June 4, 2023 9:35 AM
To: Cross, Robert W. <rwcross@UTMB.EDU>
Cc: Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>; Haller, Sherry <shhaller@UTMB.EDU>; Stutz, Sonja J. <sjstutz@utmb.edu>
Subject: Re: overall 12 page res plan

problem is jims aims draft did even mention
alloy mice and LagV or bunya

you can definitely say you wll test nanobodies in SNTs to arenas
and then retest nanobody bi-specifics and then end with testing candidates
in vivo
we could use both big figures one in Admin core the other in the overalll

On Sun, Jun 4, 2023 at 9:45 AM Cross, Robert W. <rwcross@utmb.edu> wrote:

> I am crashing on RP1 today in order to get the final to the group for another round of review by tomorrow morning.
>
> Might be good if someone else has some time today to go through and accept the edits and fill in the refs. I can send the associated endnote library from my stuff if needed. Also, I'm good if we need to condense any of my section to be more concise, I figured I'd put more than needed and we could reduce and refine to make it all fit.
>
> I'm in the dark on a lot of the other projects beyond the specific aims as I've not even seen working drafts for really anything other than cores C, D, and E. So I'm trying my best to ensure cohesion with at least these cores, but might be good to ensure I'm on the right track in terms of ensuring formatting is consistent and I'm not missing any sections. I can't say how my RP will interact much with the other RPs as I don't fully know what they are doing and/or if it even makes sense to interact. BTW, did we ever get a specific aims page from Jim? I'm not sure I fully understand the direction he is going in and if I need to demonstrate interaction with his RP.
>
> ---
>
> **From:** Broder, Christopher <christopher.broder@usuhs.edu>
> **Sent:** Sunday, June 4, 2023 7:26 AM
> **To:** Larry Zeitlin <larry.zeitlin@mappbio.com>
> **Cc:** Cross, Robert W. <rwcross@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
> **Subject:** Re: overall 12 page res plan
>
>
>
> hi all
>
> i am working on a final version of RP2 now, Tony and Moushimi got
> stuff to me last night. i am putting in the last section on the milestones/timelines/industry interfacing ec,,,
> i see Larry your comment about alloy mice and RP4 but it was never clear to me what his plan was
>
> the new fig in the overall is really cool,
> i see Bob's point about the RP titles. ect,,, but the fig does have a lot more info
> that can be referred to,   in order to see how it could be edited and using it larger

someone has to take this version, and go through an accept edits and get the remaining refs in remove comments unless there is a need for one more round to discuss

then we can see where it stands?

cb

On Sun, Jun 4, 2023 at 7:12 AM Larry Zeitlin <larry.zeitlin@mappbio.com> wrote:

> Thanks Bob. I had a few light edits and a couple of comments.
>
> ---
>
> **From:** Cross, Robert W. <rwcross@UTMB.EDU>
> **Date:** Sunday, June 4, 2023 at 1:03 AM
> **To:** CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Larry Zeitlin <larry.zeitlin@mappbio.com>, Dafna Abelson <dafna.abelson@mappbio.com>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>, Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
> **Subject:** RE: overall 12 page res plan
>
> Now attached
>
> ---
>
> **From:** Cross, Robert W.
> **Sent:** Sunday, June 4, 2023 12:03 AM
> **To:** Broder, Christopher <christopher.broder@usuhs.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Dafna Abelson <dafna.abelson@mappbio.com>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>; Woolsey, Courtney B. (Micro) <cbwillia@UTMB.EDU>
> **Subject:** RE: overall 12 page res plan
>
> Added sections on Bunyas for overall, my sections had endnote added. Also sending a separate version also with endnote citations added in case anything gets fudged up. Can also export and send the ENL if needed.
>
> Tom asked me to add the first draft of Courtney's overall figure to this version to see how it fit in place of the first version. Looks good, maybe a few small refinements to streamline. CC'd Court here so she can get up to speed on the document as well and ensure she gets timely feedback.
>
> Working on RP1 tomorrow and will send full version tomorrow as soon as I can, likely late afternoon or evening.
>
> ---
>
> **From:** Cross, Robert W.
> **Sent:** Saturday, June 3, 2023 4:38 PM
> **To:** Broder, Christopher <christopher.broder@usuhs.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Dafna Abelson <dafna.abelson@mappbio.com>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
> **Subject:** RE: overall 12 page res plan
>
> Filling in the bunya sections today, will have to you all by tonight.
>
> ---
>
> **From:** Broder, Christopher <christopher.broder@usuhs.edu>
> **Sent:** Wednesday, May 31, 2023 5:59 PM
> **To:** Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Dafna Abelson <dafna.abelson@mappbio.com>; Cross, Robert W. <rwcross@UTMB.EDU>;

> > Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
> > **Subject:** overall 12 page res plan
> >
> > **External Email Warning:** Do not click links or open attachments unless you recognize the sender and expect the content. UTMB Email Phishing Awareness
> >
> > ok,
> > based on the RFA i made sections to address the requirements
> > some bits are from the CETR
> >
> > The biggest thing this RFA wanted was the Milestones and go and no go
> > and timelines and diagrams.
> >
> > needed items are green. need your magic guys especially industry and mabs and bunyas
> >
> > there are never to many refs needed, yellow, so best to
> > have Sherry and Sonya do this (add them from your library) like the admin ect,,
> >
> > pending space, the other bits are yellow and optional depending on what you think
> >
> > cb

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PROJECT SUMMARY/ABSTRACT - RP3 (Cedar Henipavirus Animal Model)

Pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. Among these are the two emerged zoonotic paramyxoviruses: the ‘prototype’ henipaviruses (HNVs); Nipah virus (NiV) and Hendra virus (HeV), discovered in the mid to late 1990s. The prototype HNVs have a uniquely broad tropism capable of infecting 18 species across 6 orders of mammals using the conserved ephrin-B2 and -B3 ligands as entry receptors. NiV and HeV infection often results in a systemic and fatal respiratory and/or neurological disease in multiple mammalian species including humans. NiV and henipaviral disease are also included in the WHO List of Priority Pathogens, and they are significant biothreats to humans and livestock in South and Southeast Asia and Australia. HNVs have two membrane glycoproteins: the attachment glycoprotein (G) and the fusion glycoprotein (F) that are the major targets of neutralizing antibodies and the focus of all vaccine and antiviral monoclonal antibody (mAb) strategies. The most extensively studied HNV vaccine is a soluble recombinant form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including nonhuman primates (NHPs). With no licensed vaccines or treatments available for human use, this project will use the prototype HNVs as a model to develop, test, and translate a novel vaccination platform (a thermostable, needle-free, adaptable, and widely deployable polyphosphazene (PPZ) adjuvanted dissolvable microneedle patch (MNP)). Preliminary data demonstrates potent immunogenicity of a PPZ-MNP-HeV-sGvaccine in mice, inducing robust antigen-specific HNV cross-neutralizing antibody and cellular immune responses. The MNP vaccines will be used to evaluate HeV-sG and compare stabilized soluble forms of HNV G and F (sGtet and sFstb) and combinations in challenge studies. A reverse genetics system has been developed to generate recombinant, nonpathogenic Cedar virus (rCedV) and rCedV chimeric virus tools by swapping the G and F glycoproteins with those of NiV/HeV as a rapid, surrogate, BSL-2 high-throughput neutralization platform to be used to rapidly evaluate HNV vaccine responses. Also, divergent and pathogenic zoonotic HNVs have been discovered including the related rodent borne Mòjiāng virus and Langya virus (LayV)), and the divergent bat-borne Angavokely virus (AngV). This project will develop and test the rapid, adaptable and readily deployable MNP vaccine platform together with new virological tools to facilitate the translational nature of this pandemic preparedness approach. Specifically, we will: 1) Develop PPZ-MNP-based sGtet and sFstb prototype HNV vaccines; 2) Construct new reporter virus tools; 3) Evaluate vaccine-induced immune responses and HNV-specific mAbs and nbs; and 4) Validate the PPZ-MNP platform with another paramyxovirus antigen.

FACILITIES AND OTHER RESOURCES - RP3 (Cedar Henipavirus Animal Model)

USUHS

Project Lead: Brian Schaefer, Ph.D.

Overall Scientific Environment: Uniformed Services University (USU) is the medical school at which approximately half of the physicians in the Armed Services receive their graduate training. Research at USU is supported primarily by extramural grants, as in other medical schools. Dr. Schaefer is a tenured Professor in the Department of Microbiology and Immunology, which includes 12 full-time Faculty members. The overall focus of the Department is mechanisms of infectious diseases and the host response/immunology. Faculty interests and active research programs at USU are diverse, with many nationally- and internationally-known investigators, including the Department of Microbiology and Immunology chair, Dr. Christopher Broder, who is an internationally-known virologist (and centrally involved in this proposal).

USU is directly across the street from the main NIH campus and close to Johns Hopkins, the University of Maryland and several other outstanding institutions, facilitating opportunities to establish productive collaborations with world-class investigators. For example, Dr. Schaefer has had a multi-PI U01 grant with Dr. Wolfgang Losert (Dept of Physics, University of Maryland) as the co-PI and Dr. Hari Shroff (NIH/NIBIB) as a collaborator. Dr. Schaefer is also primary PI on a multi-PI R01 from NIAID with Dr. YouWen He at Duke University Medical Center, and Dr. Thomas Conrads (Women's Health Integrated Research Center). Dr. Schaefer and his staff have also collaborated with the Advanced Imaging Center (AIC) at the Howard Hughes Medical Institute (HHMI) for some of their imaging-based research. Overall, the broader scientific environment at USU and the greater Bethesda/Washington D.C. metropolitan region is highly conducive to productive collaborations. Dr. Schaefer has been using these resources to his advantage, and has been very active in establishing collaborative relationships to facilitate his research objectives. Notably, Dr. Schaefer and Dr. Broder have been collaborating for several years on lyssavirus- and henipavirus-related project, and they have a very strong working relationship.

Members of the Schaefer lab participate in a weekly multi-lab Immunology journal club, Immunology data club, virology journal club, and virology data club. Approximately 10 groups regularly participate in the immunology journal/data club and 5 groups regularly participate in the virology journal/data club. Also, members of the Schaefer lab participate in a bi-weekly data presentation/discussion group which meets monthly.

Dr. Schaefer has been well-supported by the Department of Microbiology and Immunology and the USU Office of Research. Although USU is a modest-sized medical school, the technical resources are outstanding, rivaling many very large institutions (see below). The modest size of the school is an advantage, as key pieces of equipment (e.g., The IVIS® Spectrum CT preclinical in vivo imaging system; Cytek Aurora spectral flow cytometer), and 10x Genomics Visium Spatial Transcriptomics instruments are not overbooked and therefore readily available to laboratory personnel. Dr. Schaefer is also Chair of the USU Biomedical Instrumentation Center (BIC), and thus plays a central role in directing equipment purchases, ensuring that needed technologies are available for the research of the PI and his USU colleagues. In addition to University funds, we have successfully used the NIH S10 mechanism to purchase core facility equipment. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional, as is illustrated by recent USU purchases of cutting-edge equipment including a Perkin-Elmer IVIS® Spectrum CT preclinical in vivo imaging system, a Zeiss Elyra PS.1 super-resolution microscope, an Agilant Seahorse XPe96 Analyzer, a BD FACSAria Fusion flow sorter, and many other pieces of advanced research equipment. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional.

USU has several Ph.D. programs, including an Emerging Infectious Diseases (EID) program, a Molecular and Cellular Biology (MCB) program and a Neuroscience (NES) program. Dr. Schaefer holds secondary appointments in all three programs and is currently Interim Director of the Emerging Infectious Diseases Graduate Program. Ds. Schaefer has been very active in training graduate students, having trained >10 Ph.D. students. USU recruits strong graduate students, and Dr. Schaefer's students have been very productive, during and beyond their dissertation studies. The availability of talented graduate students is a further resource, promoting productivity and enhancing the overall vibrancy of the scientific environment at USU. **Laboratory**: Dr. Schaefer's laboratory area consists of three rooms of 450 sq. ft, 300 sq. ft and 225 sq.ft., and a dedicated microscopy suite of 110 sq. ft. The Schaefer lab contains routine equipment for microbiology, molecular biology, and biochemistry including incubators, centrifuges, inverted microscopes, gel electrophoresis equipment for protein and DNA gels, thermal cyclers, balances, pH meters, refrigerators, freezers, platform orbital shakers, sonicator, UV transilluminator, shakers, heating blocks, waterbaths, free-standing biosafety cabinets, and CO_2-tissue culture incubators. Additionally, Dr. Schaefer's lab is equipped

with an epifluorescence microscopy system (a Zeiss AxioObserver inverted fluorescence microscope), a BioRad CFX Connect real-time PCR system, a Coulter Z1-Dual cell counter, a DeNovix CellDrop-FL cell counter and a Lonza Nucleofector 4D Shuttle. Postdocs, students, and technicians have desks and computers in the laboratory.

Animal: Animals are maintained in University facilities under the supervision of a full-time veterinarian. The USUHS maintains a modern AAALC Accredited, Central Animal Facility of about 50,000 square feet. The facility also includes two preclinical behavior cores (one for mice, one for rats). The facility and the University's animal care and use program is managed by the Department of Laboratory Animal Medicine (DLAR), which is directed by a veterinarian who is an ACLAM Diplomate and staffed with three other veterinarians, a graduate animal husbandryman, and about 30 technicians. The University is able to provide appropriate care for a wide variety of laboratory animal species, from invertebrates and lower vertebrates to higher vertebrates including non-human primates and domestic livestock, as well as more commonly used species such as rodents and rabbits. The facility also includes a number of properly equipped ABSL-2 rooms.

Computer: Windows-based computers (Intel i5/i7 or similar processors and ≥8GB of RAM) are available for routine use by postdoctoral fellows, graduate students, and technicians located in the laboratories of both investigators. Dr. Schaefer's laboratory also has a dedicated workstation for use for analysis of microscopy and in vivo imaging data. Image analysis software in the Dr. Schaefer's lab includes Zeiss Zen, with a digital deconvolution module and numerous tools for quantitative image analysis. The University maintains site licenses for other image and data analysis software, including Zeiss Axiovision, VisioPharm, and OriginPro. Dr. Schaefer also maintains a license for FlowJo software, which can be used on any networked computer in the laboratory. The computers are connected by a central server to each other and to the Internet. A variety of USU-supported software programs are available, including EndNote, Microsoft Office, Adobe Creative Cloud, Geneious Prime and Graph Pad Prism 9.

Office: Drs. Schaefer's office is separate from but proximal to each of his laboratories. Trainees and technicians have desks in the laboratory. Four full-time administrative officers and one part-time administrator are available to provide support within the department.

Clinical: N/A

Other: The Biostatistics Consulting Center (BCC), a service of the Department of Preventive Medicine and Biometrics, provides statistical consulting to USUHS scientific investigators. We routinely meet with BCC consultants regarding proper design of experiments for statistical testing and for statistical analysis of the resulting data.

The USU Biomedical Research Imaging Core (BRIC) houses state-of-the art equipment for pre-clinical studies, including a IVIS® Spectrum CT, a Siemens Inveon SPECT/PET/CT Scanner and a Bruker Biospec 70/20 USR Magnetic Resonance Imaging system. A Scientist and several staff provide technical support and manage the facility.

The USU American Genome Center (TAGC) is one of only four academic genome centers in the United States. It is the Department of Defense operations subsidiary of the Collaborative Health Initiative Research Program (CHIRP) established between the National Heart, Lung, and Blood Institute (NHLBI) and USU. Since inception, TAGC has generated more than 40,000 whole Genome Sequences across 107 research studies sourced from NHLBI, other NIH Institutes, DoD and USU investigators. TAGC is also the sequencing center and genome data analysis center for the APOLLO Cancer Moonshot project, and collaborates for this purpose with the Murtha Cancer Center (MCC) at Walter Reed, the National Cancer Institute, and the Veterans Administration. Through a collaborative relationship with 10x Genomics, USU has early access to newly released platforms such as Visium Spatial Transcriptomics (including improvements to that platform).

The USU TAGC includes a large array of next-generation sequencing instruments including Illumina NovaSeq 6000 systems, Illumina HiSeq X systems, and an Illumina NextSeq 500 system. A 10x Genomics Chromium Connect automated single-cell NGS library workstation is available for automated single cell partitioning, barcoding and NGS library preparation. A 10x Genomics Chromium Controller massively parallel sample partitioning platform is available for barcoding single cell RNA for library preparation and for single cell transcriptome profiling, including CITE-seq workflows. 10x Genomics Visium CyAssist transcriptomic probe transfer system is available to facilitate spatial transcriptomics workflows. Electrophoresis systems include Agilent Fragment Analyzers and a Bio-Rad Experion LabChip Platform. Sequencing platforms are connected to a high-speed network switch for data storage on a petabyte-scale Isilon SAN.

The USU Biomedical Instrumentation Center (BIC) consists of five core equipment facilities for use by investigators throughout the University. Instrumentation is available either free or on a fee-for-service basis, depending on which instruments have annual service contracts (which are paid largely through per-hour use fees). Each core is run by a full-time Director with an advanced science background (most hold a PhD) and a high degree of technical expertise. The majority of cores have at least one additional employee to help manage the core and provide guidance and assistance to users.

The BIC Flow Cytometry Core includes three analyzers (Cytek Aurora 5-laser, 64-channel Spectral Analyzer; Becton-Dickinson 5-laser, 30-parameter FACSymphony A5; Becton-Dickinson 4-laser, 15-parameter LSRII) and two sorters (BD FACSAria Fusion 4-laser, 18-parameter flow sorter; BD FACSAria IIIu 4-laser, 17-parameter flow sorter) and an Amnis Image Stream X Mark II imaging flow cytometer. Other equipment includes a Bio-Rad Bio-Plex 200 multi-analyte detection system and an Agilant Seahorse XPe96 Analyzer. The core has several off-line analysis workstations with FlowJo and other data analysis platforms.

The BIC Imaging Core houses a wide array of microscopes, including a Zeiss Elyra PS.1 super-resolution microscope, which is capable of 4-parameter SR-SIM (super-resolution structured illumination) imaging, 3-parameter PALM (Photoactivation localization microscopy) and dSTORM (direct stochastic optical reconstruction microscopy), as well as 3D-PALM/dSTORM. Other microscopes include aZeiss 980 Confocal with Airyscan2, a Zeiss 700 confocal system, a Zeiss 7 MP Multi-photon Confocal Electrophysiology System, a Zeiss Lightsheet Z.1 and a Zeiss Axioscan Z1 Slide Scanner. The facility also includes several additional wide-field fluorescence microscopes, and three offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize. For electron microscopy needs, the core is equipped with a JEOL JEM-1011 transmission electron microscope, an ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment), and a grid stainer (Leica EM AC20).

The BIC Genomics core includes a 3500 XL Applied Biosystems Genetic Analyzer, a Gene Forge 3900 and an ABI 3900 and DNA Synthesizer, a U-3000 Thermo-Scientific HPLC system, an Agilent 1200 HPLC, a QX200 Droplet Digital PCR System, a Roche LightCycler 480 Real-Time PCR System and an Agilent Capillary Electrophoresis Fragment Analyzer.

The BIC Structural Biology and Proteomics Core includes a GE Healthcare Biacore 3000 Surface Plasmon Resonance instrument, an AB Sciex 4000 QTRAP LC/MS/MS System, an Agilent 1200 HPLC, a Wyatt DynaPro NanoStar Dynamic Light Scattering analyzer, an Art Robbins Instruments Phoenix Crystallization Robot, and a JANSi UVEX Fluorescence Microscope (for crystal inspection).

The BIC Histopathology Core includes a Leica Autostainer XL, a Leica CV 5030 Robotic Coverslipper, a Sakura Tissue-Tek VIP tissue Processor, a Leica CM 3050 S Cryostat, and a Microm HM 325 Microtome.

The University also has an equipment repair service, central duplicating service, audiovisual service, and microcomputer support service. The University Learning Resource Center is a high quality medical and scientific library with additional microcomputers and support. A wide variety of scientific journals are available via remote computer access. A central autoclave/glassware washroom serves the Department of Microbiology and Immunology.

Washington State University

Paul G. Allen School of Global Health at Washington State University in Pullman, WA (Bronwyn Gunn)

The Paul G. Allen Center for Global Health at Washington State University, completed in 2012, is a 62,000-square-foot state-of-the-art infectious disease research facility equipped to meet today's standards for investigating pathogens and emerging diseases. The facility includes first floor administrative offices and 5,000 sq. ft. of CDC- and USDA-certified Biosafety Level 3 (BSL3) laboratory space, and two floors with meeting rooms, faculty BSL2 laboratories and office space. Meeting rooms are well-equipped for presentations and connecting with colleagues across the globe, while open spaces on each floor also encourage informal interactions among the students, faculty, and staff. Shared facilities including common equipment rooms and licensed work sites for bioinformatics are also available throughout the building.

Laboratory: The Gunn laboratory is located on the 2nd floor of the Paul G. Allen School of Global Health at Washington State University in Pulllman, WA. The BSL2 laboratory space includes 8 six-ft benches, equipped with standard laboratory equipment (pipettes, serological pipet-aids, vortex, heat blocks, PCR machine, 4C refrigerator, -20C freezer, -80C freezer etc), with a dedicated space for molecular DNA and protein analysis. There are two class II biosafetly cabinets located in a separate room attached to the main lab space that contains a double stack New Brunswick 37C 5% CO_2 incubator and a shaking CO_2 incubator (New Brunswick S41i) for culture of suspension cell lines. A Luminex MagPix and a magneic plate washer (AquaMax4000, Molecular Devices) and an OctetRed 96 biolayer interferometry instrument (ForteBio/Sartorius) are housed within the Gunn lab space for multiplexed analysis of antibody levels and affinity measurements, respectively. The Gunn lab also houses a Cytek Aurora Spectral Flow Cytometer, equipped with 5 lasers (UV, violet, blue, yellow-green, red) and automated 96 well high throughput sampler that is used to analyze all cell-based effector assays. Dr. Gunn also has access to a Biotek Synergy HTX multimode microplate reader equipped with luminescence and fluorescence detectors for highthroughput analysis of pseudotyped virus entry.

Dr. Gunn also has access to additional facilities and resources within the rest of the Paul G. Allen School of Global Health. All investigators' laboratories and the core facilities are fully equipped for research in molecular biology and infectious diseases. The BSL2 laboratories have been approved for pathogen use, recombinant DNA experiments, and radioisotope use. The BSL-3 and ABSL-3 labs are USDA and CDC certified for select agent use. A full range of centrifuges, a scintillation counter, microscopes, spinning disc fluorescence microscope, spectrophotometers, electrophoresis instrumentation, thermal cyclers for PCR, real-time PCR and digital PCR, multi-plate fluorimeters and proteomic instrumentation (isoelectric focuser) as well as other general equipment are available on-site, and gamma counters, HPLC and FPLC are available nearby. Major equipment in the Allen Center also includes instrumentation for microarray fabrication and an Illumina MiSeq system that is used routinely for DNA sequencing and RNAseq experiments. Shared instrumentation includes photographic equipment, an automatic radiograph processor, an upright flourescent microscope, image analysis equipment, large-scale incubators, ultra - and high-speed centrifuges.

BSL-3 laboratory: Dr. Gunn has dedicated BSL-3 space in the Paul G. Allen School of Global Health BSL-3 suite and currently approved to work with SARS-CoV-2. Equipment in the BSL-3 laboratory space include a Class II biosafety cabinet, centrifuge equipped with biocontainment rotors, and 37C 5% CO_2 tissue culture incubator.

Computer: All members of the Gunn laboratory are supplied a laptop 13" MacBook Pro for their research needs, with licenses to Office Software (Word, Excel, PowerPoint, Outlook), Adobe Creative Cloud (Illustrator, Photoshop, Acrobat), GraphPad Prism, and EndNote20. In addition, two lab desktop computers are available that have licenses to Cytek SpectroFlo analysis software and Geneious. Computers are networked and wireless connectivity is available throughout the building. High-performance computing needs on campus are met by an SGI Power Challenge L computer, an NSF-funded 16-processor IBM SP2 parallel computer, and the Amazon Cloud (AWS). Critical data bases including various DNA and protein sequence data bases (GenBank, EMBL, SwissPro and others), the Brookhaven Protein Data Bank, and the Cambridge Crystallographic Structure Data Bank for small molecules are available locally and through network access. Software for sequence analysis, clustering orthologous proteins, detecting SNPs, and exploration of phylogenetic relationships is available, as are numerous programs available via network access.

Office: Office space for graduate students and post-docs in the Gunn lab are located directly outside of the laboratory space, allowing for easy access and transition between spaces. Dr. Gunn's office is located adjacent to the general office space, allowing for easy communication and interactions.

Animal: All animals on the WSU campus are maintained in accordance with USDA and NIH regulations that include twice-yearly inspections of all facilities by the Institutional Animal Care and Use Committee (IACUC). The facilities and procedures for maintenance and care of animals are accredited by the American Association for Accreditation of Laboratory Animal Care. Personnel trained in the care of laboratory animals staff all animal facilities.

Scientific Environment: The research environment is strengthened by an interactive faculty. These include interactions with many faculty members within the College of Veterinary Medicine Departments of Veterinary Microbiology and Pathology (VMP), Integrated Physiology and Neurosciences (IPN) and the School of Molecular Biosciences (SMB) which provide opportunities for scientific exchange and collaborations as well as extended expertise in infectious diseases, cell biology and biochemistry.

Palouse Virology group: There is a bi-weekly Virology Group meeting of laboratories from WSU, USDA, and the University of Idaho that contributes to the interactive intellectual environment. Participating virologists are from the Nicola, Bose, Cunha, Fortunato, Goodman, Gunn, Koehler, Letko, Miura, Ramsay, Rowley, Schwartz and Seifert laboratories, covering a range of viral pathogens including those impacting human and animal species.

Research in Progress (RIP): Weekly meetings where students, staff, and faculty from VMP, Allen School, and USDA present their research in 20- or 45-minute seminars. RIP is well attended both in person and on Zoom.

EQUIPMENT - RP3 (Cedar Henipavirus Animal Model)

USUHS

Project Lead: Brian Schaefer, Ph.D.

Schaefer Laboratory

Epifluorescence microscopy system (Fully-automated Zeiss AxioObserver epifluorescence microscope)
BioRad CFX Connect real-time PCR system
Lonza Nucleofector 4D Shuttle – core unit and X-unit
Coulter Z1-Dual cell counter
DeNovix CellDrop-FL cell counter with fluorescence
centrifuges: Eppendorf 5425 microfuges (3); Beckman Allegra 25R; Thermo-Fisher Jouan GR4i; various rotors NestAdvance Bullet Blender bead mills (2)
Miltenyi QuadroMACS separator (2)
inverted microscopes for tissue culture (2)
gel electrophoresis equipment for protein (3) and DNA (5) gels
semi-dry protein transfer apparatus (2)
Bio-Rad thermal cyclers (2)
balances (4)
pH meter
refrigerators
freezers (-20 C, -80 C)
platform orbital shakers (5)
sonicator with cup horn and microtip
bacterial shaker
heating blocks (6)
waterbaths (8)
biosafety cabinets (2)
CO_2-tissue culture incubators (3)
Thermo-Fisher Locator 4 liquid nitrogen storage tank with electronic monitoring

Departmental

GE Healthcare Life Sciences Amersham Imager 680 (for imaging of protein and DNA gels and blots)
Alpha-Innotech gel documentation system (EtBr-stained gels)
2D gel electrophoresis system (protein)
MilliQ ultra-pure water system
Nanodrop spectrophotometer
Luminometer
Fluorescence plate readers (2)

USU CORE FACILITIES (fee for service or free)

Biomedical Research Imaging Core (BRIC)

IVIS® Spectrum CT preclinical *in vivo* imaging system (2D and 3D bioluminescence and CT imaging)
Siemens Inveon SPECT/PET/CT Scanner
Bruker Biospec 70/20 USR MRI
Image Guided Therapy 7T MR guided Focused Ultrasound System for neurological applications

The American Genome Center (TAGC)

Illumina NovaSeq 6000 systems (7)
Illumina HiSeq X systems (6)
Illumina NextSeq 500 system
10x Genomics Visium CyAssist transcriptomic probe transfer system
10x Genomics Chromium Connect automated single-cell NGS library workstation
10x Genomics Chromium Controller massively parallel sample partitioning platform
Bio-Rad Experion LabChip Platform automated electrophoresis system
PRIMER Data Science Core - direct connection to a high-speed network switch for data

Biomedical Instrumentation Center (BIC)

Flow Cytometry
Cytek Aurora 5-laser, 64-channel Spectral Analyzer
Becton-Dickinson 5-laser, 30-parameter FACSymphony A5
Becton-Dickinson 4-laser, 15-parameter LSRII FACS analyzer
BD FACSAria Fusion 4-laser, 18-parameter flow sorter
BD FACSAria IIIu 4-laser, 17-parameter flow sorter
Amnis Image Stream X Mark II imaging flow cytometer with IDEAS statistical image analysis software
Bio-Rad Bio-Plex 200 multi-analyte bioassay detection system
Agilant Seahorse XPe96 Analyzer
Offline data analysis stations (3) with software packages including: FlowJo, WinList, and ModFit

Microscopy
Zeiss 980 Confocal with Airyscan2
Zeiss 700 confocal system with 405/458/488/514/561/633 laser lines
Zeiss 7 MP Multi-photon Confocal Electrophysiology System
Zeiss Elyra PS.1 Superresolution microscope for superresolution structured illumination microscopy (SR-SIM), photoactivated localization microscopy (PALM), and direct stochastic optical reconstruction microscopy (dSTORM). System is fully equipped, including the 3D-PALM/dSTORM option.
Zeiss Lightsheet Z.1
Zeiss Axioscan Z1 Slide Scanner
Zeiss AxioImager M2 upright microscope and MicroBrightField Stereo Investigator software (stereology analysis).
Leica AF6000 system, including an inverted microscope equipped with a fully motorized 3-axis stage plus atmosphere and temperature control
Transmission electron microscope (JEOL JEM-1011)
Ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment)
Grid stainer (Leica EM AC20)
Wide-field fluorescence microscopes (3)
Offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize; Metamorph.

Genomics
3500 XL Applied Biosystems Genetic Analyzer
Gene Forge 3900 and ABI 3900 and DNA Synthesizer
U-3000 Thermo-Scientific HPLC system
Agilent 1200 HPLC
QX200 Droplet Digital PCR System (ddPCR)
Roche LightCycler 480 Real-Time PCR System
Agilent Capillary Electrophoresis Fragment Analyzer

Structural Biology/Proteomics
AB Sciex 4000 QTRAP LC/MS/MS System
Agilent 1200 HPLC
Wyatt DynaPro NanoStar Dynamic Light Scattering
GE Healthcare Biacore 3000 Surface Plasmon Resonance
Art Robbins Instruments Phoenix Crystallization Robot
JANSi UVEX Fluorescence Microscope

Histopathology
Leica Autostainer XL
Leica CV 5030 Robotic Coverslipper
Sakura Tissue-Tek VIP tissue Processor
Leica CM 3050 S Cryostat
Microm HM 325 Microtome

Washington State University

Paul G. Allen School of Global Health, Washington State University in Pullman, WA

- Laminar Flow Hoods
- Fume Hoods
- Inverted Microscopes
- Microcentrifuges
- Nucleocounters
- LN2 Storage Freezers
- Biorad Thermal Cycler (PCR)
- ThermoCycler (real-time PCR)
- Digital PCR
- Spinning disc fluorescent microscope
- Spectrophotometers
- Tissue Culture Incubators
- Sequencing Power Pack and Gel Apparatus
- Beckman Centrifuges
- Thermo floor ultracentrifuges
- Microplate shakers
- New Brunswick shaking cell culture incubator (S41i)
- Cytek Biosciences Aurora Spectral Flow Cytometer equipped with 5 lasers (UV, Violet, Blue, YellowGreen, and Red), 60 fluorescent detector channels, and a high throughput sampler.
- Molecular Devices AquaMax 4000 Magnetic Plate Washer equipped with a 96 well head.
- Luminex MagPix
- Octet Red Biolayer Interferometer
- Tecan multifluorescent plate reader
- BioTek Synergy HTX Reader
- Analytical scales
- AKTA Avlant HPLC
- -80ºC Freezers
- Nanodrop 8000
- Autoclaves
- Illumina MiSeq

School of Molecular Biosciences, Washington State University in Pullman, WA

- NanoString nCounter System
- NanoString GeoMx Digital Spatial Profiler (DSP)
- Becton-Dickenson FACSCalibur flow cytometer
- Sony Biotechnology SH800 and SY3200 High Speed Sorter
- Illumina NextSeq 2000 sequencer (December 2022)
- PacBio Sequel II
- Agilent Tape Station and Bioanalyzer
- 10X Genomics ChromiumTM Controller
- Nexcelom cell counter
- Computational: 64-core node (Intel Xeon Scalable 6338) with 512GB, 3200 MT/s DDR4 of memory); 40 TB storage equipped with a GPFS file system
- The storage system is backed up by WSU’s KAMIAK Supercomputer center and backed up in time snap shots on a server powered by different power sources and physically located in a different part of the building. For security reasons all servers are only accessible via SSH within the WSU network or the VPN.

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

NAME: Schaefer, Brian Charles

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: Professor of Microbiology and Immunology, and Director (Interim), Emerging Infectious Diseases Graduate Program

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology	B.S.	1989	Biology
Harvard University	PhD	1995	Immunology
Harvard Univ/Dana Farber Cancer Institute	(Postdoc)	1995-1996	Tumor Virology
National Jewish Medical and Research Center	(Postdoc)	1996-2002	Immunology

A. Personal Statement

My group has historically focused on two major research programs. Our longest-standing program involves elucidation of mechanisms of signal transduction in immune cells and regulation of immune cell effector function, with a particular emphasis on the pathway that connects the T cell receptor (TCR) signaling to the transcription factor, NF-κB. This work has also stimulated our studies of the function of an understudied CARD protein, CARD19, which was originally (incorrectly) identified as a negative regulator of TCR signaling to NF-κB. These studies have resulted in numerous publications that define novel signaling mechanisms in the CARD11-BCL10-MALT1 signaling cascade and additional pathways. Our second major area of research is centered on immune responses in the central nervous system. Our initial work in this area involved elucidation of the role of the immune response initiated by traumatic brain injury, particularly NF-κB-mediated inflammatory responses. However, we have now shifted our emphasis to translational research on immune responses to neurotropic viruses, with an overall goal of developing immunotherapy strategies that are broadly effective against this dangerous class of human pathogens. This work is focused on lyssaviruses and henipaviruses, due to their neurotropic nature and propensity to cause severe human disease with a very high fatality rate. Our use of bioluminescence imaging to track viral replication has been a particularly important innovation for this work, as it allows us to collect highly granular data regarding viral spread and amplification from individual animals in a longitudinal manner. Specific current projects include a DoD-funded translational study in which we are defining the utility of a novel, mAb-based anti-lyssavirus immunotherapy against rabies strains circulating in Europe and Africa, and a study to develop novel henipavirus therapies (both are collaborations with Dr. Christopher Broder's group).

I am ideally suited to be PI of RP3: *Cedar henipavirus animal model*, as my group has established the only mouse model of Cedar virus infection (to our knowledge), and we now have several years of experience in the development of this very important BSL-2 animal model to investigate mechanisms of henipavirus pathogenesis, and to test efficacy of novel therapies. The following are notable ongoing projects:

HU00011920118 (**PI: Schaefer**), USU Center for Global Health Engagement
09/30/2019 – 09/29/2022 (NCE to 9/30/23)
"Efficacy testing of a novel human monoclonal antibody therapy for late-stage rabies/lyssavirus infection"

U19 AI142764-01 (PI:Broder; **Schaefer Co-I**), NIH/NIAID
03/01/2019 – 02/29/2024
"Advancement of Vaccines and Therapies for Henipaviruses"

R01 AI125552 (**PI: Schaefer**). NIH/NIAID
06/01/2017 – 05/31/2022 (NCE to 5/31/2024)
“T cell receptor-activated autophagy as a regulator of T cell effector responses”

HU00011921048 (**PI: Schaefer**), USU Multi-domain Operations Research (MDO)
02/28/2023 - 09/30/2025
“Defining the mechanism of enhancement of the pathogen-directed host adaptive immune response following anti-lyssavirus monoclonal antibody therapy”

B. Positions, Scientific Appointments, and Honors

Positions

2017 - current	Professor (with tenure) in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
2009 - 2017	Associate Professor (with tenure) in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
2002 - 2009	Assistant Professor in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
1996 - 2002	Post-doctoral Research Associate, Howard Hughes Medical Research Institute, National Jewish Medical and Research Center, Denver, CO.
1995 - 1996	Research Fellow in Pathology, Harvard University, Dana-Farber Cancer Institute

Professional Memberships and Service

2011 - 2016	NIH CMIB (Cell and Molecular Immunology-B) study section for review of R01/R21 grants (Ad hoc member Feb & May 2011, Feb & May 2013, Feb 2014, Feb 2015, May 2016)
2008, 2021	ZRG1 CB-D 30 Confocal Microscopy Shared Instrumentation NIH Study Section (Ad hoc member)
2006 - current	American Society for Cell Biology (ASCB)
2006 - current	American Association of Immunologists (AAI)
2006 - 2007	ZRG1 IMM-L 29 L, DBBD Minority and Disability Predoctoral Fellowship NIH Study Section (Ad hoc member)
2005-2006	ZRG1 F07 Immunology Fellowship and AREA NIH Study Section (Ad hoc member)

Honors

2016	Uniformed Services University Henry Wu Award for excellence in basic science research
2005 - 2008	Dana Foundation Program in Brain and Immuno-Imaging
2004 - 2006	Kimmel Scholar - Sidney Kimmel Foundation for Cancer Research
2000 - 2002	Leukemia and Lymphoma Society Special Fellowship
1989 - 1992	Office of Naval Research Graduate Research Fellowship
1989	Phi Beta Kappa
(1989)	National Science Foundation Graduate Research Fellowship (Declined)

Patents

2007	US Patent 7,169,570: *Method to Identify Regulators of Cellular Activation Using Bcl10* – BC Schaefer, JW Kappler and P Marrack

Mouse strains developed

2001	C57BL/6-Tg(UBC-GFP)30Scha/J: Ubiquitously expressed EGFP transgene on a pure C57BL/6 background. Repository strain at Jackson Laboratory (stock# 004353). This strain has been used and cited in hundreds of publications.

C. Contributions to Science

1. My Ph.D. thesis studies involved identification of the molecular mechanisms controlling distinct latency-associated Epstein Barr virus (EBV) transcriptional programs. Prior to my dissertation work, it was known that there were three forms of EBV latency which displayed two distinct phenotypes with regard to expression of EBV nuclear antigens (EBNAs): Type III latency is characterized by expression of all six EBV nuclear antigens (EBNA1-EBNA6) whereas Type I and Type II latency is characterized by expression of EBNA1 only. The molecular basis restricted EBNA expression in Types I and II latency was not known. My work demonstrated the existence of a novel EBV promoter "Qp," responsible for driving exclusive expression of EBNA1 in Types I and II latency. My studies also showed that the choice between latency programs was contingent on whether the Type III latency promoter, Cp, is methylated in the host cell. Collectively, this work contributed significantly to the current understanding of regulation of EBV latency, which is relevant to elucidating how EBV persists for life in immunocompetent individuals, how EBV emerges as a life-threatening pathogen in specific immunodeficiencies, and how specific programs of EBV latency may be related to EBV-associated tumors, such as Burkitt lymphoma.

    a. [#]**Schaefer BC**, Woisetschlaeger M, Strominger JL, and Speck SH. Exclusive expression of Epstein-Barr virus nuclear antigen 1 in Burkitt lymphoma arises from a third promoter, distinct from the promoters used in latently infected lymphocytes. Proc. Natl. Acad. Sci. USA 1991; 88:6550-6554.

    [#]Cited >130 times.

    b. [*]**Schaefer BC,** Strominger JL, and Speck SH. Redefining the Epstein-Barr virus-encoded nuclear antigen EBNA1 gene promoter and transcription initiation site in group I Burkitt's lymphoma cell lines. Proc. Natl. Acad. Sci. USA 1995; 92:10560-10564

    [*]Cited >190 times.

    c. [†]**Schaefer BC**, Strominger JL, and Speck SH. Host-cell-determined methylation of specific Epstein-Barr virus promoters regulates the choice between distinct viral latency programs. Mol. Cell Biol. 1997; 17:364-377.

    [†]Cited >100 times.

    d. [‡]**Schaefer BC**, Paulson E, Strominger JL, and Speck SH. Constitutive activation of Epstein-Barr virus (EBV) nuclear antigen 1 gene transcription by IRF1 and IRF2 during restricted EBV latency. Mol. Cell Biol. 1997; 17:873-886

    [‡]Cited >85 times.

2. My early work as an independent investigator involved determining whether NF-κB signaling proteins undergo molecular redistribution in response to TCR signaling. Previous work had shown that several different signaling molecules are recruited to "supramolecular activation clusters (SMACs)," membrane-proximal enrichments of signaling proteins that form on the cytoplasmic face of the antigen-engaged TCR. An assumption in the field, prior to our work, was that SMAC recruitment would be a shared feature of all cytoplasmic proteins important in TCR signaling. Surprisingly, our investigations demonstrated that NF-κB signaling proteins, including Bcl10 and Malt, redistribute to punctate cytoplasmic structures, which we named POLKADOTS. Our ongoing signal transduction work has involved mechanistic studies connecting the POLKADOTS signalosome to TCR activation of NF-κB. Key findings include demonstration that (i) TCR activation of NF-κB is *digital*. In other words, in contrast to pharmacological stimulation of NF-κB (PMA + ionomycin), which results in graded activation of NF-κB as the stimulus is increased, increasing TCR ligand changes only the *number* of T cells that respond, but not the *intensity* of the response; (ii) polyubiquitination of Bcl10 leads to recruitment to pre-existing p62 "speckles," forming the POLKADOTS signalosome; (iii) the POLKADOTS signalosome is required for activation of the IKK complex; (iv) the POLKADOTS signalosome contemporaneously directs a signal dampening activity that involves a highly novel regulatory mechanism: *selective* autophagy of Bcl10, which results in degradation of Bcl10, but not its direct binding parther, Malt1; (v) effector T cells form POLKADOTS signalosomes and use autophagy to degrade Bcl10, whereas naïve T cells use a distinct mechanism of NF-κB activation that appears to be independent of p62, involving recruitment of signaling proteins to SMACs. Collectively, these contributions provide key mechanistic details regarding how signals are transmitted from the TCR to the NF-κB transcription factor. Most recently, we have collaborated with Drs. Wolfgang Losert and Arpita Upadhyaya, biophysicists at the

University of Maryland, to define biophysical features of TCR-mediated activation and degradation of Bcl10. Our study with Dr. Losert in PLOS Computational biology has shown that the regulated polymerization and degradation of Bcl10 has characteristics of an excitable system, providing new insights regarding the digital behavior of this signaling module.

   a. #Kingeter LM, Paul S, Maynard SK, Cartwright NG, and **Schaefer BC**. Cutting Edge: T cell receptor ligation triggers digital activation of NF-κB. Journal of Immunology. 2010; 185:4520-4.
      #Cited >90 times.
   b. *Paul S, Kashyap AK, Jia W, He Y-W, and **Schaefer BC**. Selective autophagy of the adaptor protein Bcl10 modulates T cell receptor activation of NF-κB. Immunity. 2012; 36:947-58.
      ***Cited >200 times; *Faculty of 1000* recommended**
   c. Paul S, Traver MK, Kashyap AK, Washington MA, Latoche JR, and **Schaefer BC**. T cell receptor signals to NF-κB are transmitted by a cytosolic p62-Bcl10-Malt1-IKK signalosome. Science Signaling. 2014; 7:ra45.
   d. Campanello L, Traver MK, Shroff H, †**Schaefer BC**, †Losert W. Signaling through polymerization and degradation: Analysis and simulations of T cell activation mediated by Bcl10. PLOS Computational Biology. 2021 May 20;17(5):e1007986.
      †co-corresponding authors

3. During our studies on TCR signaling to NF-κB, we became interested in the understudied CARD protein, CARD19. Although CARD19 was initially described as a cytoplasmic negative regulator of TCR signaling to NF-κB, we found that this work was based on analysis of a partially spliced cDNA clone, and that the conclusions of this initial study were therefore spurious. Our work with a properly spliced CARD19 cDNA showed that CARD19 is a mitochondrial protein that plays no role in TCR activation of NF-κB. Our latest work has shown that CARD19 interacts with the mitochondrial cristae organizing system (MICOS), and that it plays a role in determining cristae morphology. Notably, 3 of the 7 current publications focused on CARD19 were generated by my laboratory or as a collaboration with my group. We are currently performing further studies to determine whether CARD19 regulates other aspects of mitochondrial function via its interaction with MICOS components.

   a. Rios KE, Kashyap AK, Maynard SK, Washington M, Paul S and **Schaefer BC**. CARD19, the protein formerly known as BinCARD, is a mitochondrial protein that does not regulate Bcl10-dependent NF-κB activation after TCR engagement. Cell. Immunol., 2020; 356:104179.
   b. Bjanes E, Sillas RG, Matsuda R, Demarco B, Fettrelet T, DeLaney AA, Rodriguez Lopez EM, Grubaugh D, Wynosky-Dolfi M, Philip NH, Krespan E, Tovar D, Joannas L, Beiting DP, Henao-Meija J, **Schaefer BC**, Chen KW, Broz P, and Brodsky I. Genetic targeting of Card19 is linked to disrupted NINJ1 expression, impaired cell lysis, and increased susceptibility to Yersinia infection. PLoS Pathog. 2021;17(10):e1009967.
   c. Rios KE, Zhou M, Lott N, Beauregard C, McDaniel DP, Conrads TP, and **Schaefer BC**. CARD19 interacts with mitochondrial contact site and cristae organizing system constituent proteins and regulates cristae morphology. Cells, 2022 11(7):1175.

4. A second major research focus in my lab is the study of immune responses in the central nervous system. Our initial studies focused on inflammatory responses in traumatic brain injury (TBI), using mice as a model system: The first study demonstrated that the gene expression program induced by mild vs. severe TBI is remarkably similar, with the main distinction being that the expression of certain inflammatory genes remains persistently elevated in severe TBI. The second study presented evidence that the anti-inflammatory drug salsalate is a candidate therapy for TBI, reducing inflammation and improving functional recovery, as assessed by behavior assays. Our more recent work has focused on developing novel animal models and immunotherapies for neurotropic viral infections. Our published work on this project includes a description of our methodology for longitudinal tracing of lyssavirus infections and a description of isolation and in vitro characterization of two anti-lyssavirus neutralizing mAbs.

   a. *Lagraoui M, Latoche JR, Cartwright NG, Sukumar G, Dalgard CL, **Schaefer BC**. Controlled cortical impact and craniotomy induce strikingly similar profiles of inflammatory gene expression, but with distinct kinetics. Front Neurol. 2012; 3:155.
      ***Cited >100 times**

b. Lagraoui M, Sukumar G, Latoche JR, Maynard S, Dalgard CL, **Schaefer BC**. Salsalate treatment following traumatic brain injury reduces inflammation and promotes a neuroprotective and neurogenic transcriptional response with concomitant functional recovery. Brain, Behavior and Immunity. 2017; 61:96-109.

c. Mastraccio KE, Huaman C, Warrilow D, Smith GA, Craig SB, Weir DL, Laing ED, Smith IL, Broder CC, and **Schaefer BC**. Establishment of a longitudinal pre-clinical model of lyssavirus infection. J. Virol. Methods. 2020; 281:113882.

d. Weir DL, Coggins SA, Vu B, Coertse J, Yan L, Smith I, Laing ED, Markotter S, Broder CC and **Schaefer BC**. Isolation and Characterization of Cross-Reactive Human Monoclonal Antibodies That Potently Neutralize Australian Bat Lyssavirus Variants and Other Phylogroup 1 Lyssaviruses. Viruses. 2021; 13:391.

5. A distinct area of emphasis in my career has been the development of new tools for research and the optimization of existing tools. Among many publications in this area, I have edited a protocols book and written a review on methodology for identification of cDNA ends by RACE (which included an optimized protocol). I produced a C57BL/6 transgenic mouse strain ubiquitously expressing GFP, which has been a highly utilized tool for cell transfer studies. I also generated a set of retroviral vectors for rapid and reliable creation of stable cell lines. My lab has published optimized protocols for visualizing NF-κB signaling intermediates via confocal microscopy, and for quantifying NF-κB activation using imaging flow cytometry. We have most recently described our methodology for using bioluminescence imaging to semi-quantitatively trace lyssavirus infection in laboratory mice. A central goal of this work is to facilitate progress across the biomedical research community, through the development and distribution of better tools and clear explanation of how to reliably execute challenging techniques.

a. [†]**Schaefer BC**. Revolutions in rapid amplification of cDNA ends: new strategies for polymerase chain reaction cloning of full-length cDNA ends. Anal Biochem. 1995 May 20;227(2):255-73.

[†] **Cited >475 times**

b. [§]**Schaefer BC**, Schaefer ML, Kappler JW, Marrack P, Kedl RM. Observation of antigen-dependent CD8 + T-cell/ dendritic cell interactions in vivo. Cell Immunol. 2001 Dec 15;214(2):110-22.

[§] **Describes creation and use of Jackson stock strain 004353 - C57BL/6-Tg(UBC-GFP)30Scha/J. Publication cited >400 times, strain cited many additional times.**

c. Traver MK, Paul S, and **Schaefer BC**. T Cell Receptor Activation of NF-κB in Effector T Cells: Visualizing Signaling Events Within and Beyond the Cytoplasmic Domain of the Immunological Synapse. Methods in Molecular Biology, 2017; 1584:101-27.

d. Mastraccio KE, Huaman C, Laing ED, Broder CC, and **Schaefer BC**. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. In: *Bioluminescence*. Methods in Molecular Biology, Kim, SB. (ed.), 2022; **2524**:369-394.

Complete publications from My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/brian.schaefer.1/bibliography/public/

VERTEBRATE ANIMALS - RP3 (Cedar Henipavirus Animal Model)

1. **Description of Procedures**

   The following mouse strains will be used in this proposal:

   i) **B6-albino-Ifnar1-/- mice**. We maintain a breeding colony of B6-albino Ifnar1-/- mice at Charles River Labs (CRL). We generated these mice by crossing the B6-albino strain (Jackson Laboratory B6(Cg)-Tyrc-2J/J, Strain #:000058) with the Ifnar1-/- strain (Jackson Laboratory B6(Cg)-Ifnar1<tm1.2Ees>/J, Strain # 028288), followed by crossing F1 mice back to B6-albino and then intercrossing white progeny that carried the Infar1- allele. This colony is now producing only albino mice that are homozygous Ifnar1-/-.

   ii) **B6-albino-STAT1-/- mice.** We are currently establishing a breeding colony of B6-albino STAT1-/- mice at CRL. We are currently crossing the B6-albino strain (Jackson Laboratory B6(Cg)-Tyrc-2J/J, Strain #:000058) with the STAT1-/- strain (Jackson Laboratory B6.129S(Cg)-Stat1tm1Dlv/J, Strain # 012606), followed by crossing F1 mice back to B6-albino and then intercrossing white progeny that carry the STAT1- allele. We anticipate establishing a colony of the desired genotype by early 2024..

   We will use mice for in vivo infection with rCedVs, with many experiments including bioluminescent imaging. Animals are housed in an approved ABSL-2 room and all laboratory and animal care staff follow BSL-2 safety precautions. The total number of required mice (including all mice produced during breeding) is 5440. Experiments will be performed with male and female mice. The PI has an IACUC-approved experimental protocol and an IBC-approved biosafety protocol that details the use of the above strains of mice for the described experiments.

2. **Justifications for Use**

   The goal of this project is to validate the mouse as a BSL-2 model for henipavirus pathogenesis, and as a platform for development and testing of countermeasures. For this work, we must use an experimental animal model to allow the course of in vivo infection and the host immune response to be studied. There is no in vitro model which can model the complex in vivo dynamics of viral infection, moving between distinct cell types and anatomical locations, or the host immune response to infection. We have chosen to utilize mice due to their ease of care, the existence of well-characterized inbred strains and knockout models, and our preliminary data with rCedVs showing robust and reproducible infection of mice which is highly amenable to statistical analysis. The number of animals to be used is based on power analyses, as detailed in our approved IACUC protocols.

3. **Minimization of Pain and Distress**

   Experiments will involve the euthanasia of infected animals experiencing pain or distress. Our IACUC protocols also define a weight loss threshold or a combined disease score criterion as end points for virus-infected animals requiring euthanasia. Animals not euthanized for the above reasons will be euthanized for tissue collection, at time points described in our IACUC protocol. Euthanasia will be performed as described in part 4, below. Mice reaching criteria/endpoints described in 3, above, will be euthanized by ketamine or ketamine-xylazine overdose. This method is consistent with AMVA guidelines.

DEPARTMENT OF DEFENSE · UNITED STATES OF AMERICA

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

June 1st, 2023

Dr. Schaefer,

It is with great enthusiasm that I can confirm support for your proposed research study: *Research Project 3 (RP3), Cedar henipavirus animal model*, that is part of the multi-site center application, Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX). This project is a sound representation of your research expertise in cellular, histological and molecular methodologies to understanding the modulation of infectious viral consequences in the mammalian brain and central nervous system, using mice as a model system. This proposal is centered on exciting preliminary data suggesting that infection of STAT1-/- mice with chimeric recombinant Cedar viruses (rCedVs) causes neurovirulence and mortality (whereas immunocompetent animals rapidly control infection). Through your proposed transcriptomics studies, we can facilitate your experimental plan to define gene expression changes across cell types in the brains of infected animals. Moreover, through your use of distinct recombinant viruses and different immunocompromised mouse strains, we can identify those gene expression changes that correlate with neurovirulence and mortality. The proposed unbiased approach to defining pathogenic mechanisms will represent a truly important step in establishing, validating and gaining biological insights from this novel BSL-2 model of henipavirus disease.

We are well poised to collaboratively support the high complexity molecular workflows from the well curated tissue specimens your team will provide. At the laboratories of The American Genome Center (TAGC), we have model system experience in transcriptomic and proteomic profiling for experimental models using whole rodent brain (see PubMed Id (PMID): 21190398, 22291617 and 23118733). Specifically, we will assist your laboratory in bulk transcriptomics, single cell RNA-sequencing and Visium spatial transcriptomics using 10x Genomics workflows. We have current standard operating procedures for your experiments using the 10x Genomics CytAssist platform and tissue compatibility for fixed tissues. We will also provide data analysis through validated pipelines and data storage from our compute infrastructure and storage area network. Importantly, we will build cross-compatible protocols with your laboratory team, including a key liason in your laboratory, who has previously trained in our genome center. Our laboratory and Data Science division computational biology associates will be available for analysis and interpretation of the data, with your leadership.

I look forward to working with you on this exciting project!

Sincerely,

(b) (6)

Clifton Lee Dalgard, PhD
Professor
Department of Anatomy, Physiology & Genetics
Director, Center for Military Precision Health
Director, The American Genome Center
Uniformed Services University of the Health Sciences
USU is "America's Medical School"

RESOURCE SHARING PLAN - RP3 (Cedar Henipavirus Animal Model)

Sharing Model Organisms: No model organisms will be generated from this research. Strains of mice to be used in this proposal are directly available from Jackson labs, or will be derived via breeding of these commercial strains. As these strains are readily available to the scientific community, no further action on our part is required.

All genomic data resulting from this project will be deposited in NCBI GEO (Gene Expression Omnibus) prior to or concurrent with publication.

Sharing Research Tools: In general, we will adhere to NIH policies regarding sharing of resources and data. Our lab has demonstrated its commitment to sharing by providing published reagents upon request for the last 20+ years. Regarding any novel materials and resources that may be generated in this proposal, the general Resource Sharing Plan developed by the Henry M. Jackson Foundation and USUHS is as follows: Unique research resources, research tools, novel materials, including model organisms or modified strains, and related protocols, genetic and phenotypic data (including data from Genome-Wide Association Studies) that may arise out of the funded studies will be made available to others in the private and public sector as soon as appropriate agreements covering such transfer can be executed. In the case of project data, findings shall be published as soon as possible or otherwise shared with other researchers pursuant to NIH policies regarding data sharing. The USUHS-HJF Joint Office of Technology Transfer (JOTT) will manage all technology transfer activities for data and resources generated by researcher of either USUHS or HJF. The JOTT will adhere to all applicable policies, principles, guidelines and procurement rules in making unique research resources readily available for research purposes to qualified individuals and entities, and in ensuring that there is no more than a short–term restriction on publication or public dissemination of data in order for the JOTT to evaluate and file for patent protection on any subject invention that may arise from the funded study. Dissemination of unpublished data that is considered to be proprietary or confidential shall occur pursuant to a suitable confidential disclosure agreement prepared by the JOTT. Patient data shall be de-identified and shared in accordance with USU and HJF policies and applicable laws and regulations. Data associated with Genome-Wide Association Studies (GWAS) will be submitted to the NIH-designated GWAS data repository. Material transfer agreements, license agreements, and Cooperative Research and Development Agreements (CRADAs), as appropriate, will be used to transfer material property. Material transfer agreements will be established to share resources among the research community for noncommercial research use.

AUTHENTICATION OF KEY BIOLOGICAL AND CHEMICAL RESOURCES - RP3 (Cedar Henipavirus Animal Model)

USUHS

Project Lead: Brian Schaefer, Ph.D.

The reagents that are used in the Schaefer lab are routinely authenticated as follows:

Expression constructs: All new expression constructs are verified by (1) restriction digest and electrophoretic separation of fragments to ensure fragments of the predicted size are generated and (2) sequencing to ensure the correct sequence is present with no spurious mutations.

Antibodies: Antibodies are tested for specificity as follows: (1) For antibodies used repeatedly, the staining/blotting performance of new antibody lots are compared against previous lots; (2) For new antibodies, banding/staining patterns are compared against published data; (3) Whenever possible, new antibodies are also validated using objective controls, such as cells known to lack the protein of interest (e.g., knockout cells) or cells engineered to over-express an epitope-tagged form of the molecule (i.e., staining by the new antibody is compared side-by-side with staining by the epitope-tag antibody).

Cell lines: Cell lines are routinely validated using flow cytometry for key cell surface markers and/or functional tests. Cell lines transfected for virus production are validated via measuring titers of virus in the resultant supernatants.

Viruses: Viruses are validated through a combination of genome sequencing, infectivity and functional testing. Rescued recombinant viruses are validated through genome sequencing, with comparison to the original cloned full length viral genomes.

Mice: Every new litter of knockout animals is PCR screened for both the wild-type and inactivated allele, with validated wild-type and knockout control DNAs included in every screen. For transgenic animals, presence of the transgene is PCR verified in every litter, with validated controls included in the screen. Additional screens include periodic western blotting and/or FACS analysis of tissues/cells from knockout animals, to validate that PCR-identified knockouts indeed fail to express the protein product of the inactivated gene.

Commercial chemical reagents: Such reagents are validated by one or both of the following: (1) performance comparison to previous lots of the same reagent; (2) use in an established assay that incorporates one or more validated performance controls.

BUDGET JUSTIFICATION - RP3 (The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. in partnership with the Uniformed Services University of the Health Sciences)

KEY PERSONNEL

Brian. C. Schaefer, Ph.D., Project Lead (1.2 calendar months, 10% effort) is a Professor of Microbiology and Immunology at USUHS. His expertise is in cellular immunology, animal models of immune responses in the central nervous system and applying advanced imaging methods to biological questions. He and Dr. Broder have been collaborating for nearly a decade on creating animal models and investigating therapeutic interventions for neurotropic viral infections. The preliminary data in this application resulted from this collaboration, through an NIH-funded U19 grant (with aims that do not overlap the aims of this proposal). Dr. Schaefer will direct all animal studies in this project, managing and advising staff and overseeing data collection and data analysis. Dr. Schaefer will manage the overall experimental direction, the writing and editing of manuscripts for publication and the preparation of progress reports and other administrative functions related to this project, with assistance from the Senior Staff Scientist. (Dr. Schaefer is a full-time Federal employee. Hence, no salary is requested for this project).

OTHER PERSONNEL

†**To be named** (Scientist II, 9 calendar months, 75% effort). A Scientist with experience in animal models and transcriptomics analysis will be recruited for this project. This Scientist will supervise animal research studies, coordinate, conduct and supervise data analyses for transcriptomics studies. The scientist will also coordinate overall data analysis and will participate with Dr. Schaefer in preparation of manuscripts for funding and in preparing progress reports and performing other administrative tasks related to the conduct of this project. The Scientist II will be employed by the Henry M. Jackson Foundation, and salary support is requested on this project.

†**To be named** (Postdoctoral fellow, 12 calendar months, 100% effort). A Postdoctoral fellow with experience in animal research and virology will be recruited for this project. The Postdoctoral fellow will perform approximately half of the animal experiments proposed for this study. The Postdoctoral fellow will be employed by the Henry M. Jackson Foundation, and salary support is requested on this project.

†**Celeste Huaman** (Graduate Student, 12 calendar months,100% effort) will perform approximately half of the proposed in vivo experiments described in this proposal. Ms. Huaman is a senior graduate student in Dr. Schaefer's lab, who has been working collaboratively with members of the Broder lab on this project. Notably, Ms. Huaman generated the majority of preliminary data in this proposal and has extensive experience in all procedures and analysis techniques required for the execution of the proposed experiments. Ms. Huaman worked in Dr. Schaefer's lab as a Research Assitant II for 5 years before joining the USU Emerging Infectious Diseases graduate program, and she acquired a very deep technical skill set during that time. Ms. Huaman completed the CEEZAD BSL-3 training course at the Biosecurity Research Institute of Kansas State University in 2021, and she has trained most current laboratory staff in proper BSL-2 technique. Ms. Huaman is currently employed by the Henry M. Jackson Foundation, and salary support is requested for her on this project.

†**Caitlyn Clouse** (Research Assistant II, 9 calendar months, 75% effort) will provide general technical support, performing routine tissue culture, ordering reagents, and maintaining databases, as required for the project. Ms. Clouse will also perform genotyping of animals breeding at Charles River Lab and she will maintain detailed records of animal genotypes. She will also assist the postdoctoral fellow and Ms. Huaman with all animal experiments. Ms. Clouse completed the CEEZAD BSL-3 training course at the Biosecurity Research Institute of Kansas llent technical support, including assistance to Ms. Huaman in gathering the preliminary data for this project. Ms. Clouse is an employee of the Henry M. Jackson Foundation and we are requesting salary support for her on this project.

†**Camille Alba** (Research Associate, The American Genome Center (TAGC), 1.85 calendar months, 15.4% effort) will be responsible for leading the collaborative performance task for this study. She will oversee performance of library preparation, library quality assessment, library clustering and whole genome sequencing of the samples across the entire project. She will report Illumina NovaSeq platform performance metrics to the collaborating staff scientist and oversee data transfer batches. Ms. Alba is employed as a Henry Jackson Foundation employee therefore salary support is requested.

†A merit increase has been added to years 2-5 of the personnel budget to account for salary increases for Henry M. Jackson Foundation personnel.

EQUIPMENT
An ultralow (-80 C) freezer, ThermoFisher Scientific Model TSX60086A ($12,123), is needed for storage of the many RNA samples, viral stocks and frozen tissues (for sectioning) and slides with frozen sections. Dr. Schaefer's current -80 freezers are filled to capacity.

TRAVEL
Funds are requested to attend two domestic scientific conferences or one international conference in Year 1. This travel is for data presentation, exchange of ideas with colleagues, and for keeping up-to-date regarding the latest research findings relevant to the proposed project. Meeting attendance and data presentation is a key component of postdoctoral and graduate student training.

MATERIALS AND SUPPLIES
For the laboratory to successfully complete the experiments, the project will need to procure the following reagents and supplies:

General Laboratory Supplies
Serological pipettes, microscope slides and coverslips, pipet tips, microcentrifuge tubes, sterile conical tubes, ultracentrifuge tubes, syringes, needles, gloves, respirators, gowns, buffers, flasks, plates, etc.

Materials
Trizol reagents, RNAse-free DNAse, RNAse-free water and buffers, Reverse Transcriptase, Taq Polymerase, qPCR master mix, RNAse-free consumables for tissue homogenization, magnetic beads for cell sorting, tissue culture media, fetal bovine serum, antibiotics, etc

Real-time PCR probes DNA oligonucleotide
Labeled antibodies for histology and flow cytometry

Animal Costs (procurement and husbandry):

Animal Breeding at Charles River Labs
The breeding of experimental mice at USU is contracted to Charles River Labs (CRL). All aspects of animal care and breeding are managed by CRL, while genotyping of progeny is performed by Dr. Schaefer's group, using biosamples collected and shipped by CRL. We maintain a colony of B6-albino Ifnar1-/- mice and B6-albion STAT1-/- mice in our breeding colony at CRL. For this project, we expect to maintain 12 breeding cages and 12 progeny cages to supply the animals needed for the proposed experiments. ($1.80/cage/day x 24 cages x 365 days = $15,768 + Transportation charges $1,500 = $20,768).

Animal Husbandry at USU
Animal facility (DLAR) mouse per diems:
USU DLAR FY24 per diem for biohazardous mice is $4.78/cage/day. We expect to have an average of 8 experimental cages housed in the facility (8 cages x 365 days x $4.78/cage/day = $13,957.60/yr)

Translational Imaging Core (BRIC):
BRIC – IVIS Spectrum CT bioluminescence imaging (9 hr/week x 52 weeks/yr x $50/hr = $23,400/yr)

Biomedical Instrumentation Core (BIC):
BIC – flow cytometry (4 hr/week x 52 weeks/yr x $36/hr = $7,488/year).
BIC – confocal microscopy (2 hr/week x 52 weeks/yr x $30/hr =$3,120/year)

The American Genome Center (TAGC):
Transcriptomics studies performed at the USU American Genome Center

Total RNAseq on brain slices from 32 animals/year x $300 per animal = $9,600/year

Supplies for Visium Spatial Gene Expression:

Visium CytAssist Spatial Gene Expression for FFPE, Mouse Transcriptome 6.5mm slides (quantity of 16 per year) $24,500/year
Visium CytAssist Tissue Slide Cassettes = $1,800/year Dual INdex Kit TS set A = $620/year
S1 Novaseq Flow cells (for spatial transcriptomics sequencing), 2 flow cells/year = $8,600/year

Supplies for Single-cell RNAseq:
Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1 = $27,130/year Chromium Next GEM Automated Chip G Single Cell Kit = $630/year
S1 Novaseq Flow cells (for single-cell RNAseq), 1 flow cell/year = $4,300/year

Publication Costs: (Year 2)

We estimate 2 manuscripts per year for an average cost of $2,500 per publication. This cost estimate is based on our average fee paid per open access journal publication for the last 3 years.

INDIRECT COSTS

HJF indirect cost (IDC) is calculated based on the value-added cost base overhead rates. The fringe rate used is 29.42 % for Tier 1 employees and 7.29% for Tier 2 employees (all employees in this proposal are Tier 1).

The HJF indirect cost is calculated based on the value-added cost base overhead rates. The IDC rate is 36.74% USU Onsite Overhead. Additionally, 16.70% Companywide G&A and applied on the total direct cost less subaward plus the USU Onsite Overhead. For proposals including subawards, an additional 1.93% is applied on total external subaward costs.

The above fringe benefits and indirect cost rates for FY2023 were approved by the U.S. Army Medical Research Acquisition Activity on September 1, 2022.

From: Haller, Sherry on behalf of Haller, Sherry <shhaller@UTMB.EDU>
To: Boxley, Kimberly; Brian Schaefer
Cc: Geisbert, Thomas W.; CHRISTOPHER BRODER; Stutz, Sonja J.
Subject: RP3 Files for Review
Date: Sunday, June 4, 2023 11:56:03 AM
Attachments: RP3 - Abstract 06032023.docx
SCHAEFER - RP3 - Facilities 06032023.pdf
SCHAEFER - RP3 EQUIPMENT 06032023.pdf
BIOSKETCH - SCHAEFER.pdf
SCHAEFER - VERTEBRATE ANIMALS.pdf
SCHAEFER - LOS DALGARD TAGC.pdf
SCHAEFER - RESOURCE SHARING PLAN.pdf
SCHAEFER - AUTHENTICATION KEY.pdf
Budget justification-Schaefer-RP3 5-24-2023 v4 sjs.docx
image001.png

Hello,
We have made a pass through the files that have been sent over from both of you for RP5 on the UTMB/USUHS U19 (PABVAX). Please let me know if you have any last changes or comments for the files attached before we upload them into the application. Also, please send over to us as soon as possible the items listed below by the end of the day **Monday, June 5.**

- Final Specific Aims – 1 pg
- Final Research Strategy – 12 pgs plus bibliography

Thank you,

Sherry L. Haller, PhD
Associate Director
Center for Biodefense and Emerging Infectious Diseases
The University of Texas Medical Branch
Keiller Bldg., Rm. 1.104D
301 University Blvd., Galveston, TX 77555-0609

C: (785) 554-1713 O: (409) 747-0766
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E: shhaller@utmb.edu
utmb Health
Center for Biodefense &
Emerging Infectious Diseases

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Xu, Jianliang

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE	FIELD OF STUDY
Nanjing Agriculture University, China	BS	06/2004	Bioengineering
Nanjing University, China	MS	06/2007	Biochemistry and Molecular Biology
Nanjing University, China	PHD	06/2010	Biochemistry and Molecular Biology
Kyoto University, Japan	Postdoctoral Fellow	11/2014	Antibody Diversification
National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH	Postdoctoral Fellow	08/2018	Antibody Engineering
	Research Fellow	06/2022	Antiviral Nanobody Development
Vaccine Research Center, NIAID/NIH	Research Fellow	05/2023	Antiviral Antibody/Nanobody

A. Personal Statement

I am an assistant professor with expertise in advanced nanobody and antibody technologies in the Department of Biology at Georgia State University. The SARS-CoV-2 pandemic has highlighted the urgent need for new antiviral drugs and for rapidly deployable novel drug development technologies that are prepositioned to respond rapidly when emergent pathogens arise. My expertise can contribute to this important goal. Nanobodies are antigen-binding entities derived from the heavy chain-only antibodies of camelid animals, and they retain full antigen specificity at a very small size (15 kDa). They can penetrate tissues and recognize epitopes that are often inaccessible to conventional antibodies. Such "hidden" epitopes also have the potential to be conserved across viral strains, making nanobodies ideal antiviral drugs with great cross-reactivity to multiple viral pathogens. More interestingly, some nanobodies can naturally cross blood-brain barrier (BBB), and more can passively cross BBB through receptor and adsorptive-mediated transcytosis, enabling them to fulfill therapeutic, diagnostic, and theragnostic promises for the benefit of patients suffering from central nervous system (CNS) pathologies.

I have recently engineered a novel nanobody-producing mouse model (nanomouse, NIH invention), and demonstrated the power of both nanomouse and nanobody by developing potent cross neutralizing nanobody molecules against SARS, SARS-CoV-2 and Bat WIV 16, utilizing structure-based engineering approach (Xu et al., *Nature*, 2021). For this work, I was awarded the 23rd Annual Norman P. Salzman Memorial Award in Basic and Clinical Virology. *Nature News and Views* commented that "*Just as the development of mice with antibodies containing human variable domains (such as Regeneron's VelocImmune mouse) has helped to deliver the 100th FDA-approved monoclonal antibody, perhaps the nanomouse will give nanobody-based therapeutics a push in the same direction.*". In addition to the nanomouse nanobody-development platform, I have recently cloned the CDR regions of human antibodies (IgM, IgD, IgG1-4, IgE, IgA1-2) heavy chain from 60 healthy donors of various demographic backgrounds, assembled them on Ighv3 family framework, and generated a human nanobody library that is close to natural human antibody heavy chain variable domain. The potentially low immunogenicity of such a library will enable us to quickly identify nanobody leads that need minimum optimization for clinical use. My ongoing work on HIV-1 has further highlighted the power of nanobody engineering in developing broad and potent antiviral drugs, with the best nanobody molecule neutralizing 96% of 208 HIV-1 strains at half maximal inhibitory concentration (geometric mean IC50) of 0.016 ug/ml. Structure guided engineering results in ultra-potent HIV-1 bispecific antibodies that target CD4 binding site and V2-Apex of HIV envelope protein simultaneously.

My postdoctoral training with Professor Tasuku Honjo at Kyoto University focused on the mechanism of antibody maturation and diversification. I then extended this work in Dr. Rafael Casellas's lab at NIAMS, where I studied transcription factor dynamics in primary B cells, engineered nanomouse for nanobody development. The one-year training at Dr. Peter Kwong's lab at Vaccine Research Center/NIAID afforded me great opportunity to learn and practice structure-based immunogen design and antibody improvement.

My long-term research goal is to establish efficient nanobody development platforms that will result in novel biological molecules across multiple therapeutic areas, including infectious diseases and immuno-oncology. My research builds upon three nanobody platforms: 1) conventional camelid animals; 2) the nanomouse model that I have established and newer versions that I am going to engineer; 3) human nanobody libraries with low immunogenicity that I am currently constructing and optimizing. The focus of my research will be on developing broad and potent neutralizing nanobodies against SARS-CoV-2, HIV-1, human metapneumovirus (HMPV), respiratory syncytial virus (RSV) and henipavirus (HNV), but the underlying principle can be adapted for other viruses, as well as cancer targets. These studies have the potential to result in life-saving therapeutics, and also will broaden our understanding of the mechanisms of viral infection, guide vaccine development, thereby advancing our societal goal of better public health crisis preparedness.

1. Xu J, Xu K, Jung S, Conte A, Lieberman J, Muecksch F, Lorenzi JCC, Park S, Schmidt F, Wang Z, Huang Y, Luo Y, Nair MS, Wang P, Schulz JE, Tessarollo L, Bylund T, Chuang GY, Olia AS, Stephens T, Teng IT, Tsybovsky Y, Zhou T, Munster V, Ho DD, Hatziioannou T, Bieniasz PD, Nussenzweig MC, Kwong PD, Casellas R. Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. Nature. 2021 Jul;595(7866):278-282. PubMed Central PMCID: PMC8260353.
2. Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, Schaefer-Babajew D, Cipolla M, Gaebler C, Lieberman JA, Oliveira TY, Yang Z, Abernathy ME, Huey-Tubman KE, Hurley A, Turroja M, West KA, Gordon K, Millard KG, Ramos V, Da Silva J, Xu J, Colbert RA, Patel R, Dizon J, Unson-O'Brien C, Shimeliovich I, Gazumyan A, Caskey M, Bjorkman PJ, Casellas R, Hatziioannou T, Bieniasz PD, Nussenzweig MC. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature. 2021 Apr;592(7855):616-622. PubMed Central PMCID: PMC8503938.
3. Kieffer-Kwon KR, Nimura K, Rao SSP, Xu J, Jung S, Pekowska A, Dose M, Stevens E, Mathe E, Dong P, Huang SC, Ricci MA, Baranello L, Zheng Y, Tomassoni Ardori F, Resch W, Stavreva D, Nelson S, McAndrew M, Casellas A, Finn E, Gregory C, St Hilaire BG, Johnson SM, Dubois W, Cosma MP, Batchelor E, Levens D, Phair RD, Misteli T, Tessarollo L, Hager G, Lakadamyali M, Liu Z, Floer M, Shroff H, Aiden EL, Casellas R. Myc Regulates Chromatin Decompaction and Nuclear Architecture during B Cell Activation. Mol Cell. 2017 Aug 17;67(4):566-578.e10. PubMed Central PMCID: PMC5854204.
4. Xu J, Husain A, Hu W, Honjo T, Kobayashi M. APE1 is dispensable for S-region cleavage but required for its repair in class switch recombination. Proc Natl Acad Sci U S A. 2014 Dec 2;111(48):17242-7. PubMed Central PMCID: PMC4260585.

B. Positions, Scientific Appointments and Honors

Positions and Scientific Appointments

2023 -	Assistant Professor, Georgia State University, Atlanta, GA
2022 - 2023	Research Fellow, Vaccine Research Center, NIAID/NIH, Bethesda, MD
2018 - 2022	Research Fellow, NIAMS/NIH, Bethesda, MD
2014 - 2018	Postdoctoral Fellow, NIAMS/NIH, Bethesda, MD
2010 - 2014	Postdoctoral Fellow, Kyoto University, Kyoto, KY, Japan

Honors

2023	NIH Earl Stadtman Investigator (Semi-finalists)
2021	23rd Annual Norman P. Salzman Memorial Awards in Basic and Clinical Virology, Foundation for the National Institutes of Health
2016	Fellows Award for Research Excellence (FARE), NIH
2014	JMF Young Scholar Award, Frontiers in Immunology
2004	Excellent Undergraduate Student, Nanjing Agriculture University
2003	Award of National Scholarship, Nanjing Agriculture University

C. Contribution to Science

1. Identification of potent neutralizing nanobody against SARS-CoV-2

   Equipped with systematic knowledge in nanobody development and the ready to use platform, I identified potent SARS-CoV-2 neutralizing nanobodies from nanomouse and llama. These nanobodies could potently neutralizing all variants of concern tested, including Alpha, Beta, Gamma and Delta variants. More importantly, with the help of structural analysis I demonstrated two distinct mechanisms of the enhanced neutralization breadth of nanobody trimers: by increasing avidity and by targeting conserved region on RBD. These nanobodies have very good potential of becoming a treatment for COVID19. I also participated in a project led by Dr. Michel Nussenzweig and demonstrated that mRNA-vaccines (Moderna and Pfizer) can elicit neutralizing antibodies against SARS-CoV-2 circulating variants.

   a. Xu J, Xu K, Jung S, Conte A, Lieberman J, Muecksch F, Lorenzi JCC, Park S, Schmidt F, Wang Z, Huang Y, Luo Y, Nair MS, Wang P, Schulz JE, Tessarollo L, Bylund T, Chuang GY, Olia AS, Stephens T, Teng IT, Tsybovsky Y, Zhou T, Munster V, Ho DD, Hatziioannou T, Bieniasz PD, Nussenzweig MC, Kwong PD, Casellas R. Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. Nature. 2021 Jul;595(7866):278-282. PubMed Central PMCID: PMC8260353.
   b. Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, Schaefer-Babajew D, Cipolla M, Gaebler C, Lieberman JA, Oliveira TY, Yang Z, Abernathy ME, Huey-Tubman KE, Hurley A, Turroja M, West KA, Gordon K, Millard KG, Ramos V, Da Silva J, Xu J, Colbert RA, Patel R, Dizon J, Unson-O'Brien C, Shimeliovich I, Gazumyan A, Caskey M, Bjorkman PJ, Casellas R, Hatziioannou T, Bieniasz PD, Nussenzweig MC. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature. 2021 Apr;592(7855):616-622. PubMed Central PMCID: PMC8503938.

2. Establishment of a mouse model for nanobody development

   With deep understanding of the mechanism of antibody development and maturation, I wondered whether one could produce heavy chain-only antibodies in mouse instead of their original host, camelid animals. With bioinformatic analysis of camelid immunoglobulin variable genes, sophisticated design and extensive genome editing of ES cells by CRISPR/Cas9, I successfully established a Igh camelized mouse strain (Nanomouse, NIH invention) in which B cells express heavy chain-only IgM and IgG1 of camelid origin instead of the conventional heavy and light chain-paired IgM and IgG1. Dr. James E. Voss in the Department of Immunology and Microbiology at The Scripps Research Institute commented in Nature News and Views that “Just as the development of mice with antibodies containing human variable domains (such as Regeneron’s VelocImmune mouse) has helped to deliver the 100th FDA-approved monoclonal antibody, perhaps the nanomouse will give nanobody-based therapeutics a push in the same direction.”.

   a. Xu J, Xu K, Jung S, Conte A, Lieberman J, Muecksch F, Lorenzi JCC, Park S, Schmidt F, Wang Z, Huang Y, Luo Y, Nair MS, Wang P, Schulz JE, Tessarollo L, Bylund T, Chuang GY, Olia AS, Stephens T, Teng IT, Tsybovsky Y, Zhou T, Munster V, Ho DD, Hatziioannou T, Bieniasz PD, Nussenzweig MC, Kwong PD, Casellas R. Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. Nature. 2021 Jul;595(7866):278-282. PubMed Central PMCID: PMC8260353.

3. Regulation of chromatin dynamics and nuclear architecture during B cell activation

   I generated two knock-in mouse strains (CTCF-Halo, Jund-Halo) and used the novel single molecule imaging microscope technique to study transcription factor dynamic in primary B cells. I demonstrated that activated B cells decondense chromatin and the chromatin architectural change facilitates promoter-enhancer contacts and requires Myc and continual ATP production. These novel findings defined the nature and transcriptional impact of chromatin decondensation and revealed an unexpected role for Myc in the establishment of nuclear topology in mammalian cells.

   a. Kieffer-Kwon KR, Nimura K, Rao SSP, Xu J, Jung S, Pekowska A, Dose M, Stevens E, Mathe E, Dong P, Huang SC, Ricci MA, Baranello L, Zheng Y, Tomassoni Ardori F, Resch W, Stavreva D, Nelson S, McAndrew M, Casellas A, Finn E, Gregory C, St Hilaire BG, Johnson SM, Dubois W, Cosma MP, Batchelor E, Levens D, Phair RD, Misteli T, Tessarollo L, Hager G, Lakadamyali M, Liu Z, Floer M, Shroff

H, Aiden EL, Casellas R. Myc Regulates Chromatin Decompaction and Nuclear Architecture during B Cell Activation. Mol Cell. 2017 Aug 17;67(4):566-578.e10. PubMed Central PMCID: PMC5854204.

4. Role of critical molecules in antibody diversification

   I demonstrated that, although APE1 is important for CSR, it is dispensable for SHM as well as IgH/c-myc translocation. I further proved that during CSR process, APE1 repairs rather than induces DNA breaks and function as a DNA end-processing enzyme to facilitate the joining of broken ends. I also demonstrated that the C-terminal region of activation induced deaminase (AID) is required for efficient generation of DNA breaks in CSR and gene conversion (GC) and for the pairing/synapse formation of cleaved DNA ends. To identify chromatin components that bind to the antibody gene locus after AID-induced DNA breaks, I genetically modified antibody gene to enable locus-specific proteomic study. Mass spectrometry analysis led to the identification of 39 chromatin-binding proteins, many with known functions in DNA break repair. Among them I found that a protein called sterile alpha motif and histidine-aspartic acid domain-containing protein 1 (SAMHD1), serves as a novel DNA repair regulator of antibody class switch recombination as well as aberrant genomic rearrangements via regulating the cellular dNTP level. My findings have deepened our understanding of the fundamental mechanisms of AID-induced antibody diversification.

   a. Husain A, Xu J, Fujii H, Nakata M, Kobayashi M, Wang JY, Rehwinkel J, Honjo T, Begum NA. SAMHD1-mediated dNTP degradation is required for efficient DNA repair during antibody class switch recombination. EMBO J. 2020 Aug 3;39(15):e102931. PubMed Central PMCID: PMC7396875.
   b. Xu J, Husain A, Hu W, Honjo T, Kobayashi M. APE1 is dispensable for S-region cleavage but required for its repair in class switch recombination. Proc Natl Acad Sci U S A. 2014 Dec 2;111(48):17242-7. PubMed Central PMCID: PMC4260585.
   c. Nguyen T, Xu J, Chikuma S, Hiai H, Kinoshita K, Moriya K, Koike K, Marcuzzi GP, Pfister H, Honjo T, Kobayashi M. Activation-induced cytidine deaminase is dispensable for virus-mediated liver and skin tumor development in mouse models. Int Immunol. 2014 Jul;26(7):397-406. PubMed PMID: 24569264.
   d. Sabouri S, Kobayashi M, Begum NA, Xu J, Hirota K, Honjo T. C-terminal region of activation-induced cytidine deaminase (AID) is required for efficient class switch recombination and gene conversion. Proc Natl Acad Sci U S A. 2014 Feb 11;111(6):2253-8. PubMed Central PMCID: PMC3926049.

5. Transcriptional regulation of oncogene PRL-3

   My doctoral training centered on the transcriptional regulation of oncogene PRL-3. I demonstrated MEF2C as a VEGF-dependent transcription factor that controls PRL-3 gene expression in epithelia cells. The work was recommended by Faulty 1000 as “must read”.

   a. Xu J, Cao S, Wang L, Xu R, Chen G, Xu Q. VEGF promotes the transcription of the human PRL-3 gene in HUVEC through transcription factor MEF2C. PLoS One. 2011;6(11): e27165. PubMed Central PMCID: PMC3206935.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/jianliang.xu.1/bibliography/public/

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2021 Approved Through 02/28/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Xu, Kai

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: Assistant Professor of Immunology, Structural biology and Emerging Infectious Diseases

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Xiamen University	B.S	06/1998	Biochemistry
Cornell University Medical College	Ph.D.	05/2009	Neuroscience
Sloan-Kettering Institute	Postdoctoral	04/2014	Structural Biology

A. Personal Statement

My research primarily focuses on investigating the intricate mechanisms of viral-host interaction during viral entry. I study the humoral immune response triggered by natural infections and vaccinations, and apply this knowledge to inform the design of vaccines and antiviral therapeutics. Throughout my interdisciplinary research endeavors, I have honed my expertise in conducting both structural and functional analyses of viral envelope proteins. Notably, I have utilized advanced techniques such as X-ray protein crystallography and Electron Microscopy single particle analysis to gain a comprehensive understanding of their properties. By employing a structure-based approach, I have made significant contributions to vaccine design and the development of protective antibodies.

Furthermore, I possess extensive experience in conducting animal immunization studies involving various species, including mice, guinea pigs, and NHPs. This has provided valuable insights into the immune response generated by these animals. Additionally, I have conducted rigorous studies to identify antibodies using mice and NHPs, resulting in crucial findings. In line with recent advancements, I have successfully isolated nanobodies from "Nanomice," thereby expanding my repertoire of techniques and contributing to the field of antibody research.

In the current U19 proposal, I have been appointed as the co-PI for project 5, which focuses on the development of nanobody therapeutics against Henipaviruses and Bunyaviruses. In this role, I will utilize my expertise to structurally and functionally characterize nanobody epitopes and understand the viral neutralization mechanism. Additionally, I will employ a structure-based rational design approach to improve the efficacy of protection and the delivery of nanobody therapies to the brain.

I have established long-term collaborations with several members of the team, namely Dr. Christopher Broder, Dr. Jianliang Xu, Dr. Brian Schaefer, Dr. Moushimi Amaya, and Dr. Eric Laing. Notably, I have closely collaborated with Dr. Broder on glycoprotein structures, antigenicity, and entry mechanisms, resulting in the co-publication of many high-impact papers over the years. Additionally, I have collaborated closely with Dr. Jianliang Xu in the past on the identification of potent and broad nanobodies against SARS-CoV2 variants, leading to a co-published Nature paper in 2021.

Ongoing projects that I would like to highlight:

Path to K Grant. Office of the Chancellor for Health Sciences at The Ohio State University, (PI: Xu) 10/01/2021-09/30/2023. *"Identification and characterization of protective antibodies against SARS-CoV-2 variants for therapeutic development"*

U01 AI173348-01. NIAID, (PI: Xu) 03/01/2023-02/29/2028 NIH/NIAID “Structure, function, and antigenicity of emerging henipavirus surface glycoproteins”

UH2 AI171611-01A1. NIAID, (PI: Xu) 03/07/2023-02/28/2025 NIH/NIAID “Targeting glycoprotein (G) domain-III for pan-lyssavirus nanobody therapeutics

Key publications:

1. Wang Z*, Dang HV*, Amaya M*, Xu Y*, et al, **Xu K**[#], Broder CC[#], Veesler D[#]. Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. ***Proc Natl Acad Sci U S A***. (2022) May 31;119(22)
2. Laing ED, Navaratnarajah CK, Cheliout Da Silva S, et al, Broder CC[#] and **Xu K**[#]. *Structural and functional analyses reveal promiscuous and species-specific use of ephrin receptors by Cedar virus*. (2019) ***Proc Natl Acad Sci U S A.*** Oct 8;116(41):20707-20715.
3. Xu J*, **Xu K***, et al, Kwong PD, Casellas R. *Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants*. (2021) ***Nature***. Jun 7.
4. **Xu K*,** Acharya P*, Kong R*, Cheng C*, et al, Mascola JR, Kwong PD. *Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1*. (2018) ***Nat Med.*** Jun;24(6):857-867.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2021-pres	Assistant Professor, Department of Veterinary Biosciences, The Ohio State University, Columbus, OH
2014-2021	IRTA fellow, Senior Scientist, Project Team Lead and Senior Scientific Project Manager, Vaccine Research Center, National Institute of Health, Bethesda, MD
2014-2014	Senior Research Scientist, Structural Biology Program, Sloan-Kettering Institute, New York, NY

Scientific Appointments

2021-pres	Member, Infectious Diseases Institute, The Ohio State University
2021-pres	Member, Cancer Biology Group, The Comprehensive Cancer Center, The Ohio State University

Honors

2021	NIH Director's Award (Outstanding efforts in the pursuit of efficacious vaccines to prevent COVID-19)
2019	NIH Earl Stadtman Investigator (Semi-finalists)
2019	NIAID Merit Awards (Advancing HIV Vaccine Research)
2019	MSC Award for Excellence in HIV-1 Vaccine Research
2010	Sloan-Kettering Institute Annual Award for the Outstanding Postdoctoral Research
2009	Gordon Conference Travel Award (Chemical and Biological Terrorism Defense)
2009	MSKCC Award for Excellent Graduate Students
2009	Cornell University Medical College Award for the Excellent Graduate Students

C. Contributions to Science

1. **Henipavirus glycoproteins’ structure, function and protective immune recognition mechanism.** I have a long track record in the study of HNV glycoproteins through collaboration with Dr. Christopher Broder (USU). Our structural and functional studies uncovered the functional role of G and F proteins in viral entry process, and how they can be recognized by protective antibodies, which paves the way for further vaccine and therapeutic design.
    a. Laing ED, Navaratnarajah CK, Cheliout Da Silva S, et al, Broder CC[#] and **Xu K**[#]. *Structural and Functional Analyses Reveal Promiscuous and Species-Specific Use of Ephrin Receptors by Cedar Virus*. (2019) *Proc Natl Acad Sci U S A.* Oct 8;116(41):20707-20715. ([#]corresponding author)

b. **Xu K***, Chan YP*, Bradel-Tretheway B*, Akyol-Ataman Z*, Zhu Y*, Dutta S, Yan L, Feng Y, Wang LF, Skiniotis G, Lee B, Zhou ZH, Broder CC, Aguilar HC, Nikolov DB. *Crystal Structure of the Pre-fusion Nipah Virus Fusion Glycoprotein Reveals a Novel Hexamer-of-Trimers Assembly*. (2015) PLoS Pathog. Dec 8;11(12)

c. **Xu K**, Rockx B, Xie YH, DeBuysscher BL, Fusco DL, Zhu ZY, Chan YP, Xu Y, Luu T, Cer RZ, Feldmann H, Mokashi V, Dimitrov DS, Bishop-Lilly KA, Broder CC, Nikolov DB. *Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody*. (2013) PLoS Pathog., 9(10):e1003684.

d. **Xu K**, Rajashankar KR, Chan YP, Himanen JP, Broder CC, Nikolov DB. *Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3*. (2008) Proc Natl Acad Sci U S A; 105(29):9953-8.

2. **HIV vaccine research.** Through structural and functional characterization of broadly HIV neutralizing antibodies, I revealed HIV fusion peptide as promising vaccine target. With antibody-guided and structure-based approach I designed fusion peptide-based HIV vaccine, and validated vaccine efficacy in mouse, guinea pig and NHP immunization studies. Jointly I isolated FP-directed broadly neutralizing antibodies (up to 59% overall breadth) from FP vaccinated NHPs and defined their developmental pathways.

a. **Xu K***, Acharya P*, Kong R*, Cheng C*, et al, Mascola JR, Kwong PD. *Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1*. (2018) Nat Med. Jun;24(6):857-867. (*Co-first author)

b. Kong R*, Duan H*, Sheng Z*, **Xu K***, Acharya P*, Chen X*, Cheng C*, Dingens AS*, Gorman J*, Sastry M*, Shen CH*, Zhang B*, Zhou T*, et al, Kwong PD, Mascola JR. *Antibody Lineages with Vaccine-Induced Antigen-Binding Hotspots Develop Broad HIV Neutralization.* (2019) Cell. Jul 25; 178(3): 567-584. (*Co-first author)

c. Kong R*, **Xu K***, Zhou T*, et al, Kwong PD, Mascola JR. *Fusion peptide of HIV-1 as a site of vulnerability to neutralizing antibody*. (2016) Science. May 13;352(6287):828-33. (*Co-first author)

d. Zhou T, **Xu K**. *Structural Features of Broadly Neutralizing Antibodies and Rational Design of Vaccine*. (2018) Adv Exp Med Biol. 1075:73-95.

3. **Other infectious diseases.** In COVID-19 related research, I co-developed and characterized a series of protective nanobodies against SARS-CoV2 and its variants, and identified conserved neutralizing targets for vaccine design. In malaria-related study, I revealed the mechanism of *Plasmodium* RIFIN-mediated immune suppression as well as *Plasmodium* recognition by recently discovered domain-inserted antibodies. In paramyvovirus research, I co-developed a tetravalent subunit of hPIV1-4 vaccine using structure-based design to stabilize the F glycoproteins in their prefusion status.

a. Xu J*, **Xu K***, Jung S, Conte A, Lieberman J, Muecksch F, Lorenzi JCC, Park S, Schmidt F, Wang Z, Huang Y, Luo Y, Nair M, Wang P, Schulz JE, Tessarollo L, Bylund T, Chuang GY, Olia AS, Stephens T, Teng IT, Tsybovsky Y, Zhou T, Munster V, Ho DD, Hatziioannou T, Bieniasz PD, Nussenzweig MC, Kwong PD, Casellas R. *Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants*. (2021) Nature. Jun 7. (*Co-first author)

b. **Xu K#,** Wang Y, Shen CH, Chen Y, Zhang B, Liu K, Tsybovsky Y, Wang S, Farney SK, Gorman J, Stephens T, Verardi R, Yang Y, Zhou T, Chuang GY, Lanzavecchia A, Piccoli L, Kwong PD#. *Structural basis of LAIR1 targeting by polymorphic Plasmodium RIFINs.* (2021) Nat Commun. Jul 9;12(1):4226. (#corresponding author)

c. Chen Y*, **Xu K***, Piccoli L, Foglierini M, Tan J, Jin W, Gorman J, Tsybovsky Y, Zhang B, Traore B, Silacci-Fregni C, Daubenberger C, Crompton PD, Geiger R, Sallusto F, Kwong PD, Lanzavecchia A. *Structural basis of malaria RIFIN binding by LILRB1-containing antibodies* (2021) Nature Apr;592(7855):639-643. (*Co-first author)

d. Stewart-Jones GBE*, Chuang GY*, **Xu K***, Zhou T*, Acharya P*, Tsybovsky Y*, et al, Mascola JR, Lanzavecchia A, Kwong PD. *Structure-based design of a quadrivalent fusion glycoprotein vaccine for human parainfluenza virus types 1-4*. (2018) Proc Natl Acad Sci U S A. (2018) Nov 27;115(48):12265-12270. (*Co-first author)

4. **Ligand-receptor interaction and receptor activation in neuronal development and degenerative diseases.** Axons travel long range to reach their final destination during neuronal development and neuron circuit formation. This process depends on the precise control assisted by a few classes of molecules named guidance cues. We used structural and functional analyses to reveal ligand-receptor interaction and signa activation mechanism underlying axon guidance.
    a. Xu Y, Robev D, Saha N, Wang B, Dalva MB, **Xu K**, Himanen JP, Nikolov DB. *The Ephb2 Receptor Uses Homotypic, Head-to-Tail Interactions within Its Ectodomain as an Autoinhibitory Control Mechanism*. (2021) Int J Mol Sci. Sep 28;22(19).
    b. **Xu K***, Wu Z*, Renier N*, et al, Tessier-Lavigne M, Nikolov DB. *Structures of netrin-1 bound to two receptors provide insight into its axon guidance mechanism*. (2014) Science. 344(6189):1275-9.
    c. **Xu K**, Olsen O, Tzvetkova-Robev D, Tessier-Lavigne M, Nikolov DB. *The crystal structure of DR6 in complex with the amyloid precursor protein provides insight into death receptor activation*. (2015) Genes Dev. 29(8):785-90
    d. **Xu K***, Tzvetkova-Robev D*, Xu Y, Goldgur Y, Chan YP, Himanen JP, Nikolov DB. *Insights into Eph receptor tyrosine kinase activation from crystal structures of the EphA4 ectodomain and its complex with ephrin-A5*. (2013) Proc Natl Acad Sci U S A; 110(36):14634-9.
5. **Patents issued and significant technology licenses**
    - "HIV-1 env fusion peptide immunogens and their use", WO2018067582A3
    - "Prefusion PIV F immunogens and their use", WO2018081289A3, licensed to Moderna
    - "Neutralizing antibodies to nipah and hendra virus", WO2012149536A1
    - "HIV fusion peptide immunogens based on self-assembling nanoparticles and strong T cell help", PCT/US19/52419

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/kai.xu.1/bibliography/public/

BUDGET JUSTIFICATION – RP5 (The Ohio State University)

KEY PERSONNEL

Kai Xu, Ph.D., Project Lead. Dr. Xu will dedicate 3.6 calendar months for this grant, which includes 2.7 months of academic year and 0.9 months of summer. Dr. Xu will be responsible for the design and implementation of all experiments, interpretation of study results, and preparation of progress reports and publications. He will oversee the entire project and supervise other personnel (two postdoctoral researchers and a graduate student) to carry out the experiments proposed in this grant.

OTHER PERSONNEL

We are seeking to hire two postdoctoral researchers for this grant project. Both postdoctoral researchers will work collaboratively and contribute to the overall success of the project.

Postdoctoral Researcher to be hired (100% effort, 12 calendar months). He or She will have extensive training in molecular biology, cell biology, and combinatorial biology. The postdoc will also have experience in mouse work and phage-display technology. His or her primary responsibilities will include immunizing nanomice and identifying nanobodies against the listed viruses in this project. He or She will conduct experiments, collect and analyze data, prepare research reports and manuscripts, and provide assistance to the graduate student researcher.

Postdoctoral Researcher to be hired (100% effort, 12 calendar months). He or She will have strong training in molecular biology, cell biology, and structural biology, with specific experience in protein purification, crystallographic techniques, and electron microscopic structural analysis. His or her main role will involve the structural and functional characterization of the nanobodies identified in the proposed project. He or She will conduct experiments, collect and analyze data, and contribute to the preparation of research reports and manuscripts.

MATERIALS AND SUPPLIES

$98,375 per year is requested to help defray costs for general research supplies, including reagents and supplies for cell culture, and materials for biological assays, antibodies, supplies for cloning. The funds requested is based on current operating costs in the laboratory.

Cell Culture Supplies:	$12,000
Nanobody Phage Library Construction and Screening:	$15,000
Supplies and Reagent for Protein Purification:	$10,000
Supplies and Reagents for structural determination (EM and crystal):	$25,000
Reagents and Chips for affinity measurement:	$9,000
Plasticware:	$5,000
Nanobody Production:	$8,000
Molecular Biology Reagents and Enzyme:	$5,000
General Lab Supplies:	$3,875
DNA and RNA Purification Kits:	$3,000
Chemicals:	$2,500
Subtotal	$98,375

EQUIPMENT COST

Mosquito® Xtal3 - liquid handler for high-throughput crystal screening	$113,109

This expense occurs in Year-1 only. Nanobodies, being small in size, are often amenable to high-resolution structure determination using X-ray crystallography. The proposed equipment, the Mosquito® Xtal3, will be utilized to efficiently screen protein crystals in a high-throughput manner, thereby reducing the expenses associated with protein production and crystallization reagents. This liquid handler will enhance our capabilities for crystal screening, allowing us to optimize the conditions for obtaining high-quality crystals suitable for structural analysis. The acquisition of this equipment is crucial for facilitating our research on nanobodies and their structural characterization.

TRAVEL

$8,000 per year is requested to support travel and meeting registration related expenses to attend and present research findings at relevant national/international scientific conferences such as, Keystone Symposia, Gordon Research Conferences. The PI and postdoctoral researchers will attend these meetings. These meetings provide an opportunity to gain feedback from other experts in the field, which will help guide the direction of future research.

PUBLICATION COSTS

$6,000 is requested to support costs associated with publishing results in peer-reviewed scientific journals.

SERVICES and SHARED RESOUCES COSTS

Funds in the amount of $50,500 per year is requested to support user fees for the following Shared Resources and Services:

Mouse Facility:	$15,000
Electron Microscopy:	$20,000
Gene Synthesis (Glycoproteins and Nanobodies)	$8,000
Sanger Sequencing: (Nanobody and Regular)	$7,500
Subtotal	$50,500

FACILITIES AND ADMINISTRATIVE COSTS

In accordance with the Ohio State University’s Federally-negotiated F&A rate agreement, indirect costs are calculated at 57.5% MTDC.

EQUIPMENT – RP5 (Nanobody Discovery and Structural Biology)

Ohio State University, Columbia OH (OSU)

Laboratory and major equipment: Dr. Xu's laboratory is fully equipped for structural, cellular, molecular and virological studies in the newly renovated Center for Retrovirus Research at The Ohio State University (OSU). It includes a fully equipped cell-culture room of about 120 sq. ft. and a lab space of ~1,100 sq. ft. with multiple benches. The following shared rooms and equipment are available for Xu laboratory use, a virus containment BLS-2 room (370 sq. ft. shared by four PIs) that is equipped with four laminar flow hoods and four CO2 incubators, a shared storage room, a cold room, a freezer room, a PCR-prep room, a service room, and a conference room (shared by 4 laboratories). Major equipment includes: a Octet Red instrument for affinity measurement of protein-protein interaction with Bio-Layer Interferometry, a Thermo Fisher Sorvall LYNX 6000 Superspeed Centrifuge for media clearance in glycoprotein production, two INFORS HT CO2 incubator shakers for high yield suspension mammalian cell culture, a Sorvall X4R Pro-MD benchtop centrifuge for cell culture and protein concentration, multiple -80 and -20 freezers, two CO2 incubators, a bacterial culture shaker, a Leica cell-culture microscope, a stereo microscopy, Bio-Rad cell counter, a GE DeltaVision Elite imaging system, a Nanodrop spectrometer, a PerkinElmer multifunctional Victor X3 plate reader, a Bio-Rad CFX96 Real-Time PCR Detection System, a mini PCR machine, a liquid nitrogen tank, a Cytiva Go FPLC system for protein purification, an automatic plate washer, various gel apparatus and power supplies for electrophoresis, as well as other standard small equipment for molecular biology research.

Computer and office: Dr. Xu's lab has six computers, two printers and a scanner in his laboratory and offices. All computers are installed with software for protein structural processing and analysis and sequence analysis and database searching. Dr. Xu has a workstation with 4GPUs, as well as access to a remote cryo-EM data processing server with 10 GPUs and 200TB data storage. The department and college have strong support of computer and information systems. Dr. Xu has over 160 sq. ft. of office space near his laboratory. Dr. Xu's lab also has an office for postdoctoral fellows and students (~145 sq. ft.). Built-in desks, bookshelves, file cabinets and telephones are installed in all offices.

Access to shared equipment: Dr. Xu's laboratory has access to various campus-wide Shared Resource Facilities available at OSU' Columbus campus and Nationwide Children's Hospital, which is accessible by 10-minute drive from the main campus. In addition, as a member Dr. Xu has full access to the OSU Comprehensive Cancer Center (OSUCCC) Shared Resources and Cores. The OSUCCC has 20 Shared Resources and Cores that provide specialized services to researchers. Collectively, at the institutional level, there are core services for genomics, proteomics, flow cytometry, microscopy, and biostatistics among others.

Georgia State University, Atlanta GA (GSU)

Major Equipment: The Biology and Chemistry Departments share the state-of-the-art Advanced Biotechnology Core (ABC Core) Facilities under the auspices of the Center of Biotechnology and Drug Design http://biology200.gsu.edu/core directed by the Chairs of Biology and Chemistry. The ABC Core is supervised by Dr. Houghton, an Associate Professor in the Biology Department. There are 10 full time staff members with different expertise, including 4 Research Scientists with Ph.D. degrees in Chemistry or Biology, who provide the consultation, services and maintenance of the facilities.

DNA/Protein Core Facility: This facility is staffed by five full-time technicians. The core facilities and instruments are located mainly on the 5th floor of the 4-year old Petit Science Center, close to the laboratory space on the 5th floor. Equipment housed in this facility includes three DNA sequencers (two ABI 3100 and one Beckman CEQ 8000), a Genomic Gene-Chip analysis system with Fluidics/Hybridization unit (Affymetrix), an Ettan DALTII Proteomics 2-D gel electrophoresis system - including Typhoon 9400 Variable Mode Imager, MD Personal Densitometer SI, automated spot picker and automated digestion platform (Amersham), three additional automated workstations (two Beckman Biomek 2000 and one ABI Symbiot work station), N2 generator (Parkin-Balston), Ciphergen SELDI-mass spectrometry system (Ciphergen), Prism 7500 RT-PCR Systems (ABI), two densitometers (BioRad), four capillary electrophoresis units (MDQ/P/ACE 5010 + LIF detector,P/ACE 5510, 2 P/ACE 5500 Beckman), fluoroimager (Molecular Dynamics), FACSCanto Cell sorter (Becton Dickinson), XLA-analytical ultracentrifuge (Beckman-Coulter), four AKTA Automated FPLC Systems (Pharmacia), and 2100

Bioanalyzer (Agilent), Affimetrix GeneChip Analyger. The core has recently added a DNA 454 Junior genome sequencer.

The core facility also includes: Analytical Centrifuge, a Biacore 3000 for analysis of protein interactions by plasmon resonance spectroscopy, and a MicroCal Isothermal Titration Calorimeter (VP-ITC) and Differential Scanning Calorimeter (VP-DSC).

Jianliang Xu Lab: The lab is newly established in June 2023. By the end of 2023, the lab will be equipped with Electroporator, incubator shakers (for bacteria and mammalian cells separately), CO2 incubators, cell counter, nanodrop, photometer, thermocyclers, protein/DNA electrophoresis system, Orbital shaker, AKTA Automated FPLC System, Octet® R8 Protein Analysis System, fridges, freezers, balance, fluorimeter/spectrophotometer microplate reader, biosafety cabinets, superspeed centrifuges, water purifiers, Gel documentation system, and microscopes. As part of University System of Georgia (USG connect), all lab members have also access to all molecular imaging and sequencing core facilities at Emory University and Georgia Institute of Technology.

FACILITIES AND OTHER RESOURCES - RP5 (Nanobody Discovery and Structural Biology)

Ohio State University

Scientific environment:

Dr. Xu is a member of the **Center for Retrovirus Research (CRR)** at OSU. The Center is directed by Dr. Patrick Green. The membership of the CRR includes more than 20 principal investigators at OSU, Nationwide Children's Hospital Research Institute, Cincinnati Children's Hospital Research Institute, and Wright State University. The research interests of Center members are diverse and form a highly interdisciplinary network of collaboration for research initiatives. This multi-disciplinary team is available to collaborate and to help design and carryout comprehensive research studies on retrovirus diseases and related topics that would not be possible by individual laboratories.

Dr. Xu is a member of the newly established **Infectious Diseases Institute (IDI)** of Ohio State. The mission of the IDI is to embrace the broad biological studies applicable to the complex analysis of microbe-host interactions, including the interface between viruses and their hosts. There are currently 170 faculty members in the IDI from throughout OSU. The collective IDI faculty is involved in and represents the fields of immunology, cell biology, pathology, biochemistry and pharmacology, microbiology, genetics, structural biology, and bioinformatics.

Dr. Xu actively participates in **interdisciplinary graduate programs** at OSU, including Molecular, Cellular, and Developmental Biology (MCDB) graduate program, and Microbiology and Biomedical Science Graduate Program (MBS). These training programs prepare students for careers as research scientists and educators in an academic or industrial environment. These programs are interdisciplinary in nature and include the combined talents of more than 130 Ph.D. students and 170 faculty members from more than 25 departments in six colleges.

Taken together, these unique features of the collaborative scientific environment and intellectual rapport will strongly enhance the proposed studies and contribute to the probability of success of the project.

Departmental and college shared research facilities:

The Department of Veterinary Biosciences and College of Veterinary Medicine Biochemistry and Molecular Biology Core provide and maintain selected instrumentation and provide sufficient training to facilitate end- user operation. Major pieces of supported equipment include: GloMax® – Multi Detection System, Real-time PCR (Roche LightCycler 480), Typhoon Imager (GE), Phosphorimager (Molecular Dynamics), PTC-225 PCR Tetrad (MJ Research/BioRad), two AlphaImagers (Alpha Innotech), Wallac MicroBeta TriLux. Other shared-use equipment includes: UV/Vis spectrometer (Lambda 45, Perkin Elmer), Luminescence spectrometer (LS55, Perkin Elmer), High pressure liquid chromatograph (Waters), Medium pressure liquid chromatograph (Pharmacia Biosciences), Lyopholyzer (Virtis), French pressure cell (Aminco), hydraulic press (Carver), and Attune NxT Flow Cytometer (Thermo Fisher).

The Imaging Core is a college-wide service, supported by the Department of Veterinary Biosciences, which offers state-of-the-art instrumentation for flow cytometry analysis, high-resolution confocal microscopy (Leica TCS SP2 AOBS Confocal Laser Scanning Microscope), and magnetic cell sorting. The autoMACS from Miltenyi Biotec is an automated bench-top magnetic cell sorter. It is capable of sorting up to 10 million cells per second from samples of up to $4x10^9$ total cells. Instruments are staffed with full time technicians who are responsible for processing samples for examination by flow cytometry and confocal microscopy. The Department has a 3-laser Coulter EPICS Elite flow cytometer operated by experienced staff. The Flow Cytometry Service can simultaneously analyze up to five fluorescent parameters plus forward scatter and time for cell samples provided by the investigator. Analysis possibilities include cell surface phenotype markers, gene expression reporters, cell cycle analysis, cytokine production, apoptosis, cell viability, rare cell detection, and calcium metabolism. Assistance with experimental design and data analysis is available. The core also has a BD Biosciences FACSCalibur, which is a 2-laser flow cytometer that can be used to simultaneously analyze up to 4 fluorescent parameters. Moreover, a high-speed, state-of-the-art flow cytometry sorter (i-Cyt Reflection) is available in a biosafety cabinet in a BSL-II facility of the Flow Cytometry Core, which can be used to sort virus-infected cells. In addition, the Immunospot ELISPOT reader (Cellular Technology) permits fully automated, high-throughput ELISPOT plate scanning and analysis.

Campus-wide Shared Resources:

Dr. Xu has access to various campus-wide shared resources available at OSU' Columbus campus and Nationwide Children's Hospital, which is accessible by 10 minute drive from the main campus. In addition, Dr.

Xu has full access to the **OSU Comprehensive Cancer Center (OSUCCC) Shared Resources and Cores**. The OSUCCC has 20 Shared Resources and Cores that provide specialized services to researchers. They offer cost-effective, state-of-the-art technology and instrumentation; expert guidance and training; and clinical, administrative and technical support. Collectively, at the institutional level, there are core services for genomics, proteomics, flow cytometry, microscopy, and biostatistics among others. Relevant to this proposal:

Core facility of Chemistry and Biochemistry includes Biophysical Interaction and Characterization Facility that provide equipment for protein-protein interaction measurement, Chemical and Biophysical Dynamics provides various biophysical analysis platforms, and X-ray Crystallography Laboratory provides instruments and tools for X-ray crystallographic study.

The **Analytical Cytometry Shared Resource** provides state-of-the-art flow cytometry analysis and sorting of cell populations. They assist investigators with experimental design and assay development and provide individual training and 24-hour access to flow cytometry instrumentation. The facility provides researchers access to five cell analyzers and two cell sorters. Detection capabilities on these instruments range to five excitation lasers and eighteen color emission.

The **Genomics Shared Resource** provides both Nucleic Acid services and Microarray services. It offers instrumentation and expertise for DNA and RNA analysis using sequencing, genotyping, real-time PCR, Affymetrix GeneChips, nCounter Analysis, next-generation sequencing, DNA synthesis support and genome-wide analysis using the Illumina NGS platform and Affymetrix and customizable gene chips.

The **Center for Electron Microscopy and Analysis (CEMAS)** is located in a custom-designed facility at The Ohio State University's main campus. All cryo-EM instruments at CEMAS meet or exceed manufacturer performance specifications. CEMAS has a Thermo Scientific Glacios Cryo-TEM with a Falcon III direct electron detector and a Ceta-D camera for MicroED analysis. In addition, CEMAS has a Thermo Scientific Krios G3i Cryo-TEM equipped with a Bioquantum imaging filter and a K3 direct electron detector, imaging phase plates, STEM/TEM tomography, and an image spherical aberration corrector. CEMAS is able to assist users in all aspects of the cryo-EM workflow, which may include initial negative stain analysis, cryo sample preparation, grid screening, high resolution data collection, and preliminary data analysis.

The **Center for Biostatistics and Bioinformatics** provide collaborative support in all areas of biostatistical expertise, including study design, grant proposal development, data management, manuscript preparation, and statistical analysis of clinical, epidemiological, and laboratory research data. The Center's team consists of over 20 biostatisticians, each of whom has developed expertise in specific areas through hands on collaboration with investigators.

The Ohio State University's large consolidated **animal care program** supports an extensive and comprehensive research program including the Colleges of Medicine, Public Health, Veterinary Medicine, Dentistry, Pharmacy, Biological Sciences, and Social and Behavioral Sciences. The university's centralized animal vivaria consist of over 14 buildings located primarily within the Health Sciences complex. Approximately 70,000 sq ft of animal housing space consists primarily of rodent barrier housing utilizing individually ventilated caging systems. In addition, facilities for accommodating large animals, rabbits, and nonhuman primates are available. Support facilities include an experimental surgery suite, small animal imaging core, and biohazard housing at the ABSL2 and ABSL3 levels.

Summary:

All of the equipment, scientific environment, facility and research resources required for Dr. Xu's research are in place at the OSU, which will ensure the continued success of the Xu lab, including the project described in this application.

GEORGIA STATE UNIVERSITY, ATLANTA GA (GSU)

Scientific Environment at GSU: With total annual research awards of $128.1 million received in the fiscal year 2019 (the fifth year in a row that the University has earned more than $100 million in external investments), GSU is one of the nation's premier urban public research universities. The University ranks among the top 115 public and private universities in the Carnegie Foundation's elite category of R1: Highest Research Activity. The scientific environment in which the research will be carried out at GSU is highly conducive to the success of the proposed project. The University and its Departments are expanding via faculty recruited into new University Research Centers. We collaborate with a number of faculty in the Departments of Biology, Chemistry, and Computer Science and participate in several University Centers and Interdisciplinary Programs. The presence of these colleagues, both new and old, offers opportunities to discuss projects and results in both informal and formal collaboration. In addition, the scientific community at GSU has the privilege of working in the Atlanta area, where other important institutions, such as Emory University, Georgia State University (GSU), University of Georgia, Georgia Institute of Technology, and the Centers for Disease Control and Prevention (CDC) are located. These institutions work collaboratively for advancements in scientific research with special emphasis on infectious diseases.

Jianliang Xu Laboratory: The Xu lab has an 800-sq.ft. BSL-2 laboratory with bench space for eight investigators in the Petit Science Center (the building was completed in June 2010). The lab is newly established in June 2023. By the end of 2023, the lab will be equipped with Electroporator, incubator shakers (for bacteria and mammalian cells separately), CO2 incubators, cell counter, nanodrop, photometer, thermocyclers, protein/DNA electrophoresis system, Orbital shaker, AKTA Automated FPLC System, Octet® R8 Protein Analysis System, fridges, -20°C and -80°C freezers, balance, fluorimeter/spectrophotometer microplate reader, biosafety cabinets, superspeed centrifuges, water purifiers, Gel documentation system, and microscopes. The laboratory is fully prepared for microbial, molecular genetics, biochemistry, protein production, purification and characterization work. As part of University System of Georgia (USG connect), all lab members have also access to all molecular imaging and sequencing core facilities at Emory University and Georgia Institute of Technology. The department provides excellent office facilities for staff and students and provides numerous seminars and journal clubs which staff are encouraged to attend.

Animals: GSU has in place two full-time university veterinarians, veterinarian technicians, animal facilities manager, and six full-time animal caretaker staff, many of whom are certified by the American Association of Laboratory Animal Science. An AAALAC-approved, state-of-the-art 25,000 sq. ft. facility is housed in the PSC building. It is composed of 28 housing rooms, eight procedural rooms, a full surgical suite, full BSL-2 and BSL-3 suites. The facility is equipped with high-capacity autoclave and cage-washers, an imaging room with bioluminescent and fluorescent imaging machine. The facility has automated temperature, humidity, illumination, and air flowing monitoring that remotely alerts personnel of any system failures. A security officer is located inside the building 24/7 to monitor and secure the building. All animal rooms are under the supervision of animal facility personnel.

Computer: In addition to computers that are running the Octet protein analysis system, the protein purification system, and the gel imagine system, the Xu lab also has computer workstations that are available for his personnel. Each lab member has an individual workstation providing access to extensive computational resources, including access to ARCTIC (Advanced Research Computing Technology & Innovation Core) at GSU.

Office: Dr. Xu has a ~100 sq. ft. office with iMac and printer. His students use two shared office rooms. The laboratory and all office spaces are nearby on the same floor.

RESOURCE SHARING PLAN

All nanobody sequence data, nanobody constructs, and reagents generated in this application will be made available to the scientific community upon request following publication. All data sets will be submitted to public databases, such as Sequence Read Archive (SRA), allowing access to researchers in the field.

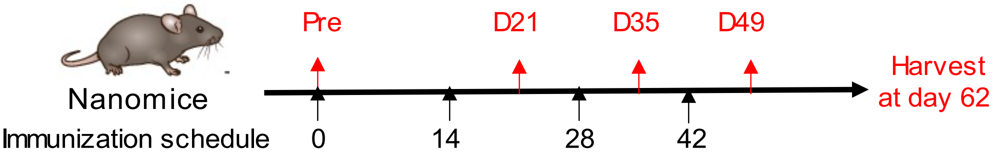
Sharing Research Tools: We routinely present data derived from our studies at scientific meetings such as the Gordon Conferences, Keystone Symposia among others. When components are completed, invention disclosures filed, and following consultation with the OSU technology transfer offices, we publish and disclose our findings. Material transfer agreements will be established to share resources among the research community for noncommercial research use. If research findings we deem sensitive are requested by outside parties, prior to publication mutual non-disclosure agreements will be put in place. In addition, we will have a free exchange of findings and data with all our collaborators. We will adhere to all institutional, state and federal resource sharing guidelines and agree to share knowledge, research materials, and any other resources necessary and relevant to the particular focus of the grant, recognizing that final authorization will be cleared with all appropriate organizational parties.

Sharing Model Organisms: All generated expression vectors, cell lines and experimental protocols will be made available to the public. Results will be presented at scientific meetings in the form of posters and oral presentations. All sequencing data and structural data will be deposited to public databases, such as GenBank or Protein Structural Data Bank and these data will be available for all to use.

VERTEBRATE ANIMALS SECTION - RP5 (Nanobody Discovery and Structural Biology)
Ohio State University

The following sections of the proposal will use mice and/or camelid animals: **Aim 1** (To identify cross-reactive nanobodies to HNVs); **Aim 2** (Identification of nanobodies to ARVs.); and **Aim 5** (Expanding to other members of the viral groups in Center Phase 2.). The Jianliang Xu lab at Georgia State University, Atlanta, and Kai Xu lab at the Ohio State University will perform the experiments using nanomice to develop neutralizing nanobodies (Aim 1, 2 and 5). When needed, contracting company (Capralogics, etc.) will immunize camelid animals housed in their farm and provide PBMC to Xu labs (Aim 1, 2 and 5).

1. Description of Procedures:



1.1 Nanomice immunization: Male and female nanomice (B6.129P2-Ighem1Rcas/J) at 8-12 weeks old will be used. Each virus protein nanobody development will involve 8 gender balanced mice, and considering potential different designs of some immunogens, in total about 300 mice will be used in the study. Mice will be injected with recombinant proteins purified through mammalian cell culture system. Protein antigens include HNV G and F proteins, and ARV GPC proteins. All proteins are obtained by transfecting 293 cells with plasmids that express these recombinant proteins, and then purified from cell culture supernatant and dissolved in PBS. Mice immunization will be performed over a 62-day immunization protocol. Specifically, mice will be immunized intraperitoneally (*i.p.*) with 50 ug of recombinant protein in the presence of Complete Freund's Adjuvant (CFA) on day 0, and boost immunized intraperitoneally with 25 ug of protein in the presence of Incomplete Freund's Adjuvant (IFA) on days 14, 28 and 42. Two more immunizations with 25 ug of protein in PBS will be performed on day 56 and 59, intraperitoneally and intravenously (to stimulate memory B cell expansion), respectively. Bone marrow, spleen and blood samples will be collected on day 62. On day 0, 21, 35 and 49, per ARAC guidelines, about 200ul of blood samples will be drawn and analyzed by ELISA for antibody titer. Typically, experiment ends at day 62, but for certain immunogens, more boost injections may be performed to induce stronger antigen-specific antibody response. In that case, after the fourth immunization on day 42, mice will be boost immunized once every 2 weeks and blood samples collected one week after each immunization. Immunization will end when antigen-specific antibodies in serum is detected or maximum of 8 injections reached. For the extended experiments, about 200ul of blood samples will be drawn every 4 weeks or longer. In total, starting from the first blood draw on day 0, no more than 6 times of blood draw will be performed. Mice immunization will be performed under pathogen-free conditions at an AAALAC accredited animal facility in the PSC building and housed in accordance with the procedures outlined in the Guide for the Care and Use of Laboratory Animals under an animal study proposal approved by the GSU or OSU Animal Care and Use Committee. Animals used in this study are either generated and maintained by Xu labs or purchased directly from Jackson Laboratory. The animal protocol is approved by the Institutional Laboratory Animal Care and Use Committee (ILACUC) at GSU or OSU.

1.2 Camelid animal immunization: For immunogens that fail to elicit strong immune response in nanomice, company (Capralogics, etc.) housing camelid animals will be contracted to perform llama or alpaca immunization and provide PBMC to Xu labs. Animal will be immunized subcutaneously (*s.c.*) with 1 mg of recombinant protein in the presence of CFA on day 0 and boost immunized (*s.c.*) with 0.5 mg of protein in the presence of IFA on days 14, 28, 42, 56 and 70. Test blood/serum will be collected 10 days after each injection for monitoring antibody response by ELISA. On day 80, about 500 ml of whole blood will be collected for library preparation. All procedures will be done with the approval from company’s Animal Care and Use Committee.

2. Animal use justifications:

Nanobody was traditionally obtained by vaccinating camelids. Nanomouse is a camelid antibody knock-in mouse model in which B cells express single domain nanobody instead of conventional antibody. It has been demonstrated robust in our recent work of generating potent and broadly protective nanobodies against variants of SARS-CoV2. The introduction of nanomice has replaced the sacrifice of large animals. To obtain affinity-matured and antigen-specific nanobodies against various virus envelope proteins, nanomice model is necessary for the immunization and nanobody identification procedure described in our study. The number of animals used in each group is a calculation based on past experience of the variable immune responses rate. For most immunogens, it can range from 25 to 75% of nanomice in a group who showed strong antigen-specific immune response. To promote nanobody epitope diversity, we assign eight animals in each group to ensure at least two high responders from which nanobodies will be identified. In the case of ideal antibody response not achieved from nanomice immunization, camelid animal immunization will be tried. When possible, multiple immunogens will be pooled for injection in one llama or alpaca.

3. Minimization of Pain and Distress:

Our procedures include *i.p.* injections of protein immunogens adjuvanted with or without CFA/IFA. Injection (*i.p.*) of small volume of non-viscous solution is generally not considered a painful procedure by the USDA and are therefore done without anesthesia. Animals may experience minor distress related to the immunization protocol. Complications are not expected. No adverse reactions to the immunizations are anticipated. The animal appearance and behavior post infection will be observed twice daily, and their weight will be recorded daily. Procedure time and laboratory personnel schedules will be arranged to allow for this frequent mouse monitoring. Mice showing severe stress signs such as being hunched, lethargy, head tilt, paralysis, labored breathing, or show ~20% weight deviation from age matched control mice will be euthanized. The project trained personnel, the veterinarian staff, and or the veterinarian may determine the animal comfort level and make the decision regarding humane endpoints and euthanasia. In general, inflammation at immunization sites subsides 6 - 7 days post- injection. At this point, the immune response concentrates in spleen for intraperitoneal injections. This "acquired" response peaks approximately 16 days in intraperitoneal immunizations. In case excessive inflammation (swollen severely that mice cannot move freely) occurs in some mice from lateral tarsal immunizations, those mice will be treated with the analgesic Buprenorphine HCl at a dose of 2.0 mg/kg BW, SQ, every 12 hrs. while inflammation is visible (or until the inflammation resolves). Anesthesia may be induced by inhalation of isoflurane. Topical anesthetic (proparacaine or tetracaine) or general anesthesia will be used for retro-orbital sinus blood collection depending upon technician preference. No surgical procedures will be done. No restraint devices will be required for the proposed studies. Animals will be euthanized with CO2 followed by cervical dislocation in accordance with American Veterinary Medical Association.

Georgia State University

The following sections of the proposal will use mice and/or camelid animals: **Aim 1** (To identify cross-reactive nanobodies to HNVs); **Aim 2** (Identification of nanobodies to ARVs.); and **Aim 5** (Expanding to other members of the viral groups in Center Phase 2.). The Xu lab at Georgia State University, Atlanta, will perform the experiments using nanomice to develop neutralizing nanobodies (Aim 1, 2 and 5). When needed, contracting company (Capralogics, etc.) will immunize camelid animals housed in their farm and provide PBMC to Xu lab (Aim 1, 2 and 5).

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Nanobody was traditionally obtained by vaccinating camelids. Nanomouse is a camelid antibody knock-in mouse model in which B cells express single domain nanobody instead of conventional antibody. It has been demonstrated robust in our recent work of generating potent and broadly protective nanobodies against variants of SARS-CoV2. The introduction of nanomice has replaced the sacrifice of large animals. To obtain affinity-matured and antigen-specific nanobodies against various virus envelope proteins, nanomice model is necessary for the immunization and nanobody identification procedure described in our study. The number of animals used in each group is a calculation based on past experience of the variable immune responses rate. For most immunogens, it can range from 25 to 75% of nanomice in a group who showed strong antigen-specific immune response. To promote nanobody epitope diversity, we assign eight animals in each group to ensure at least two high responders from which nanobodies will be identified. In the case of ideal antibody response not achieved from nanomice immunization, camelid animal immunization will be tried. When possible, multiple immunogens will be pooled for injection in one llama or alpaca.

3. Minimization of Pain and Distress:

Our procedures include *i.p.* injections of protein immunogens adjuvanted with or without CFA/IFA. Injection (*i.p.*) of small volume of non-viscous solution is generally not considered a painful procedure by the USDA and are therefore done without anesthesia. Animals may experience minor distress related to the immunization protocol. Complications are not expected. No adverse reactions to the immunizations are anticipated. The animal appearance and behavior post infection will be observed twice daily, and their weight will be recorded daily. Procedure time and laboratory personnel schedules will be arranged to allow for this frequent mouse monitoring. Mice showing severe stress signs such as being hunched, lethargy, head tilt, paralysis, labored breathing, or show ~20% weight deviation from age matched control mice will be euthanized. The project trained personnel, the veterinarian staff, and or the veterinarian may determine the animal comfort level and make the decision regarding humane endpoints and euthanasia. In general, inflammation at immunization sites subsides 6 - 7 days post- injection. At this point, the immune response concentrates in spleen for intraperitoneal injections. This "acquired" response peaks approximately 16 days in intraperitoneal immunizations. In case excessive inflammation (swollen severely that mice cannot move freely) occurs in some mice from lateral tarsal immunizations, those mice will be treated with the analgesic Buprenorphine HCl at a dose of 2.0 mg/kg BW, SQ, every 12 hrs. while inflammation is visible (or until the inflammation resolves). Anesthesia may be induced by inhalation of isoflurane. Topical anesthetic (proparacaine or tetracaine) or general anesthesia will be used for retro-orbital sinus blood collection depending upon technician preference. No surgical procedures will be done. No restraint devices will be required for the proposed studies. Animals will be euthanized with CO2 followed by cervical dislocation in accordance with American Veterinary Medical Association.

BUDGET JUSTIFICATION – RP5 (Georgia State University)

KEY PERSONNEL

Jianliang Xu, Ph.D., Co-Project Lead (*5% effort or 0.45 person months during the academic year – years 1 & 2 12.5% effort or 1.13 person months during the academic year – years 3-5 100% effort or 3 person months during summer – years 1 & 2; 75% effort or 2.25 person months during summer – years 3-5).* Dr. Xu will serve as the co-Lead for research project 5 (RP5). He is an expert in antibody and nanobody research, especially in the areas of nanobody engineering and antiviral nanobody discovery. He has engineered a novel nanobody-producing mouse model, nanomouse, and demonstrated the power of both nanomouse and nanobody by developing potent cross neutralizing nanobody molecules against SARS- CoV-2, SARS and Bat WIV 16. He has recently generated a human nanobody library that is close to natural human antibody heavy chain variable domain. The potentially low immunogenicity of such a library will enable quick identification of nanobody leads that need minimum optimization for clinical use. Dr. Xu will be responsible for overall project direction, program administration and reporting. In addition, he will oversee the experiments conducted at GSU; these include the animal immunization, nanobody phage library construction and screening, nanobody production and characterization (Aim 1 and 2), support for structure analysis (Aim 3), nanobody optimization, engineering, production and functional assay (Aim 4), and work for additional viral targets (Aim 5). Dr. Xu will super vise the experimental design, data analysis and manuscript preparation. He will hire and train graduate students and postdocs, and participate in collaborative meetings to discuss the project.

OTHER PERSONNEL

Postdoctoral Fellow, TBN (*100% effort or 12 person months during the calendar year – years 1-5).* The postdoctoral fellow will be responsible for the project aspects including immunizing animals, constructing nanobody phage libraries, identifying and characterizing nanobody candidates that bind to henipavirus F and G proteins, arenaviruses GPC proteins (Aim 1 and 2), nanobody engineering, small- scale production and characterization (Aim 3 and 4), and work on additional viral targets (Aim 5). She/he will manage the laboratory, perform experiments, record and collect data, write manuscripts and supervise the students.

Graduate Students, TBN, Total of 2 Grad students (*100% effort or 12 person months, each, during the calendar year – years 1-5)* Two students will be recruited for the project. They will be responsible for the project aspects including screening nanobody phage libraries, preparing nanobody deep sequencing libraries, producing and charactering nanobodies (Aim 1-5). They will perform experiments, collect and record data and write manuscripts, and work closely with the postdoctoral fellow to help achieving all aim goals.

FRINGE BENEFITS

The negotiated fringe benefits rate for both the academic year and for summer is 36.0% for faculty and full-time staff. The fringe benefits rate for part-time faculty and temporary staff is 4.0% and for graduate students is 3.40%. A 2% salary increase is included on all personnel salaries for years 2-5.

TRAVEL

Domestic travel support is requested for the following: The domestic travel budget includes expenses for Dr. Xu, Post-Doctoral associates, and/or graduate students to travel to relevant national conferences that are significant to the project and scope of work and to visit the Co-PI Dr. Kai Xu at Ohio State University, and PD/PI Dr. Christopher Broder at Uniformed Service University.

Foreign Conference travel support is requested for the following: The foreign travel budget includes expenses for Dr. Xu and/or Post-Doctoral associates to travel to relevant international national conferences that are significant to the project and scope of work.

OTHER DIRECT COSTS

Materials & Supplies – Year 1: $168,949, Year 2: $165,148, Year 3: $162,591, Year 4: $158,707, Year 5: $154,769

The supplies will include:

1) Media, chemicals, antibiotics, growth supplements, and disposables - $23,949 (Year 1), $25,148 (Year 2), $22,591 (Year 3), $ 23,707 (Year 4) and 24,769 (Year 5)

2) Molecular and biochemical reagents and supplies, including molecular cloning enzymes, kits and reagents, antibodies for ELISA and FACS staining, TG1 competent cells for nanobody library construction and screening, protein purification resin and column, protein buffer exchange and desalt columns, FPLC affinity and ion exchange columns, and kits for site specific mutagenesis, DNA and RNA preparation - $50,000 (Year 1-3), $45,000 (Year 4-5).

3) Nanobody sanger sequencing and deep sequencing - $20,000 per year.

4) Nanobody DNA synthesis - $20,000 per year.

5) Octet biosensors for nanobody affinity and epitope mapping test - $20,000 (Year 1-4), $15,000 (Year 5).

6) Mice purchasing and housing - $5,000 per year.

7) Camelid animal immunization and maintenance - $20,000 (Year 1), $15,000 (Year 2-5).

8) Funds for equipment maintenance, and small equipment items. $5,000 per year.

9) Computer costs including software licenses, replacement of aging computers, backup equipment - $5,000 per year.

Publication Costs – Years 1-5: $3,000 per year.

Other Costs - Conference Registration Fees - Years 1-5: $3,000 per year for Dr. Xu, Post-Doctoral associates, and/or graduate student.

INDIRECT COSTS: The Georgia State University negotiated F&A/Indirect Cost rates are calculated at the federally approved rate of 56%. Indirect Costs are calculated on a modified total direct costs basis. Equipment and Participant Support are excluded from the calculation. Only the first $25,000 of each subaward is included in the calculation.

From: Haller, Sherry on behalf of Haller, Sherry <shhaller@UTMB.EDU>
To: Jianliang Xu; xu.4692@osu.edu
Cc: workman.45@osu.edu; Lindsey Marie Hornsby; Geisbert, Thomas W.; CHRISTOPHER BRODER; Stutz, Sonja J.
Subject: RP5 Files for Review
Date: Sunday, June 4, 2023 11:37:54 AM
Attachments: Biosketch -JianliangXu 2023-FINAL.pdf
biosketch-XuKai U19 UTMB-USU.pdf
OSU Budget Justification sjs.docx
RP5 - Equipment 06042023.docx
RP5 - Facilities 06042023.pdf
RP5 - Resource Sharing Plan - 06042023.docx
RP5 - Vertebrate Animals OSU-GSU 06042023.pdf
RP5 - GSU Xu-Budget Justification.docx
image001.png
Importance: High

Hello,

We have made a pass through the files that have been sent over from both of you for RP5 on the UTMB/USUHS U19 (PABVAX). Please let me know if you have any last changes or comments for the files attached before we upload them into the application. Also, please send over to us as soon as possible the items listed below by the end of the day **Monday, June 5.**

- Abstract/Summary – 30 lines (Word doc)
- Multi-PI Plan – (Word doc) - *if either Kai or Jianliang prefer to be Project Lead and the other Co-I, we will not need this. If Co-Leading, we will need a plan to describe how the responsibilities will be shared and the justification for the shared leadership.
- Authentication of Key Biologicals and Chemical Resources
- Resource Sharing Plan – (Word doc attached; please be sure to add information about sharing research tools and model organisms)
- Final Specific Aims (Word doc) – 1 pg
- Final Research Strategy (Word doc) – 12 pgs plus bibliography

Thank you,

Sherry L. Haller, PhD
Associate Director
Center for Biodefense and Emerging Infectious Diseases
The University of Texas Medical Branch
Keiller Bldg., Rm. 1.104D
301 University Blvd., Galveston, TX 77555-0609

C: (785) 554-1713 O: (409) 747-0766
Teams: Call Chat
E: shhaller@utmb.edu

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

NAME: Schaefer, Brian Charles

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: Professor of Microbiology and Immunology, and Director (Interim), Emerging Infectious Diseases Graduate Program

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology	B.S.	1989	Biology
Harvard University	PhD	1995	Immunology
Harvard Univ/Dana Farber Cancer Institute	(Postdoc)	1995-1996	Tumor Virology
National Jewish Medical and Research Center	(Postdoc)	1996-2002	Immunology

A. Personal Statement

My group has historically focused on two major research programs. Our longest-standing program involves elucidation of mechanisms of signal transduction in immune cells and regulation of immune cell effector function, with a particular emphasis on the pathway that connects the T cell receptor (TCR) signaling to the transcription factor, NF-κB. This work has also stimulated our studies of the function of an understudied CARD protein, CARD19, which was originally (incorrectly) identified as a negative regulator of TCR signaling to NF-κB. These studies have resulted in numerous publications that define novel signaling mechanisms in the CARD11-BCL10-MALT1 signaling cascade and additional pathways. Our second major area of research is centered on immune responses in the central nervous system. Our initial work in this area involved elucidation of the role of the immune response initiated by traumatic brain injury, particularly NF-κB-mediated inflammatory responses. However, we have now shifted our emphasis to translational research on immune responses to neurotropic viruses, with an overall goal of developing immunotherapy strategies that are broadly effective against this dangerous class of human pathogens. This work is focused on lyssaviruses and henipaviruses, due to their neurotropic nature and propensity to cause severe human disease with a very high fatality rate. Our use of bioluminescence imaging to track viral replication has been a particularly important innovation for this work, as it allows us to collect highly granular data regarding viral spread and amplification from individual animals in a longitudinal manner. Specific current projects include a DoD-funded translational study in which we are defining the utility of a novel, mAb-based anti-lyssavirus immunotherapy against rabies strains circulating in Europe and Africa, and a study to develop novel henipavirus therapies (both are collaborations with Dr. Christopher Broder's group).

I am ideally suited to be PI of RP3: *Cedar henipavirus animal model*, as my group has established the only mouse model of Cedar virus infection (to our knowledge), and we now have several years of experience in the development of this very important BSL-2 animal model to investigate mechanisms of henipavirus pathogenesis, and to test efficacy of novel therapies. The following are notable ongoing projects:

HU00011920118 (**PI: Schaefer**), USU Center for Global Health Engagement
09/30/2019 – 09/29/2022 (NCE to 9/30/23)
"Efficacy testing of a novel human monoclonal antibody therapy for late-stage rabies/lyssavirus infection"

U19 AI142764-01 (PI:Broder; **Schaefer Co-I**), NIH/NIAID
03/01/2019 – 02/29/2024
"Advancement of Vaccines and Therapies for Henipaviruses"

R01 AI125552 (**PI: Schaefer**). NIH/NIAID
06/01/2017 – 05/31/2022 (NCE to 5/31/2024)
“T cell receptor-activated autophagy as a regulator of T cell effector responses”

HU00011921048 (**PI: Schaefer**), USU Multi-domain Operations Research (MDO)
02/28/2023 - 09/30/2025
“Defining the mechanism of enhancement of the pathogen-directed host adaptive immune response following anti-lyssavirus monoclonal antibody therapy”

B. Positions, Scientific Appointments, and Honors

Positions

2017 - current	Professor (with tenure) in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
2009 - 2017	Associate Professor (with tenure) in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
2002 - 2009	Assistant Professor in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
1996 - 2002	Post-doctoral Research Associate, Howard Hughes Medical Research Institute, National Jewish Medical and Research Center, Denver, CO.
1995 - 1996	Research Fellow in Pathology, Harvard University, Dana-Farber Cancer Institute

Professional Memberships and Service

2011 - 2016	NIH CMIB (Cell and Molecular Immunology-B) study section for review of R01/R21 grants (Ad hoc member Feb & May 2011, Feb & May 2013, Feb 2014, Feb 2015, May 2016)
2008, 2021	ZRG1 CB-D 30 Confocal Microscopy Shared Instrumentation NIH Study Section (Ad hoc member)
2006 - current	American Society for Cell Biology (ASCB)
2006 - current	American Association of Immunologists (AAI)
2006 - 2007	ZRG1 IMM-L 29 L, DBBD Minority and Disability Predoctoral Fellowship NIH Study Section (Ad hoc member)
2005-2006	ZRG1 F07 Immunology Fellowship and AREA NIH Study Section (Ad hoc member)

Honors

2016	Uniformed Services University Henry Wu Award for excellence in basic science research
2005 - 2008	Dana Foundation Program in Brain and Immuno-Imaging
2004 - 2006	Kimmel Scholar - Sidney Kimmel Foundation for Cancer Research
2000 - 2002	Leukemia and Lymphoma Society Special Fellowship
1989 - 1992	Office of Naval Research Graduate Research Fellowship
1989	Phi Beta Kappa
(1989)	National Science Foundation Graduate Research Fellowship (Declined)

Patents

2007	US Patent 7,169,570: *Method to Identify Regulators of Cellular Activation Using Bcl10* – BC Schaefer, JW Kappler and P Marrack

Mouse strains developed

2001	C57BL/6-Tg(UBC-GFP)30Scha/J: Ubiquitously expressed EGFP transgene on a pure C57BL/6 background. Repository strain at Jackson Laboratory (stock# 004353). This strain has been used and cited in hundreds of publications.

C. Contributions to Science

1. My Ph.D. thesis studies involved identification of the molecular mechanisms controlling distinct latency-associated Epstein Barr virus (EBV) transcriptional programs. Prior to my dissertation work, it was known that there were three forms of EBV latency which displayed two distinct phenotypes with regard to expression of EBV nuclear antigens (EBNAs): Type III latency is characterized by expression of all six EBV nuclear antigens (EBNA1-EBNA6) whereas Type I and Type II latency is characterized by expression of EBNA1 only. The molecular basis restricted EBNA expression in Types I and II latency was not known. My work demonstrated the existence of a novel EBV promoter "Qp," responsible for driving exclusive expression of EBNA1 in Types I and II latency. My studies also showed that the choice between latency programs was contingent on whether the Type III latency promoter, Cp, is methylated in the host cell. Collectively, this work contributed significantly to the current understanding of regulation of EBV latency, which is relevant to elucidating how EBV persists for life in immunocompetent individuals, how EBV emerges as a life-threatening pathogen in specific immunodeficiencies, and how specific programs of EBV latency may be related to EBV-associated tumors, such as Burkitt lymphoma.

   a. #**Schaefer BC**, Woisetschlaeger M, Strominger JL, and Speck SH. Exclusive expression of Epstein-Barr virus nuclear antigen 1 in Burkitt lymphoma arises from a third promoter, distinct from the promoters used in latently infected lymphocytes. Proc. Natl. Acad. Sci. USA 1991; 88:6550-6554.
   #Cited >130 times.
   b. ***Schaefer BC,** Strominger JL, and Speck SH. Redefining the Epstein-Barr virus-encoded nuclear antigen EBNA1 gene promoter and transcription initiation site in group I Burkitt's lymphoma cell lines. Proc. Natl. Acad. Sci. USA 1995; 92:10560-10564
   ***Cited >190 times.**
   c. †**Schaefer BC**, Strominger JL, and Speck SH. Host-cell-determined methylation of specific Epstein-Barr virus promoters regulates the choice between distinct viral latency programs. Mol. Cell Biol. 1997; 17:364-377.
   †Cited >100 times.
   d. ‡**Schaefer BC**, Paulson E, Strominger JL, and Speck SH. Constitutive activation of Epstein-Barr virus (EBV) nuclear antigen 1 gene transcription by IRF1 and IRF2 during restricted EBV latency. Mol. Cell Biol. 1997; 17:873-886
   ‡Cited >85 times.

2. My early work as an independent investigator involved determining whether NF-κB signaling proteins undergo molecular redistribution in response to TCR signaling. Previous work had shown that several different signaling molecules are recruited to "supramolecular activation clusters (SMACs)," membrane-proximal enrichments of signaling proteins that form on the cytoplasmic face of the antigen-engaged TCR. An assumption in the field, prior to our work, was that SMAC recruitment would be a shared feature of all cytoplasmic proteins important in TCR signaling. Surprisingly, our investigations demonstrated that NF-κB signaling proteins, including Bcl10 and Malt, redistribute to punctate cytoplasmic structures, which we named POLKADOTS. Our ongoing signal transduction work has involved mechanistic studies connecting the POLKADOTS signalosome to TCR activation of NF-κB. Key findings include demonstration that (i) TCR activation of NF-κB is *digital*. In other words, in contrast to pharmacological stimulation of NF-κB (PMA + ionomycin), which results in graded activation of NF-κB as the stimulus is increased, increasing TCR ligand changes only the *number* of T cells that respond, but not the *intensity* of the response; (ii) polyubiquitination of Bcl10 leads to recruitment to pre-existing p62 "speckles," forming the POLKADOTS signalosome; (iii) the POLKADOTS signalosome is required for activation of the IKK complex; (iv) the POLKADOTS signalosome contemporaneously directs a signal dampening activity that involves a highly novel regulatory mechanism: *selective* autophagy of Bcl10, which results in degradation of Bcl10, but not its direct binding parther, Malt1; (v) effector T cells form POLKADOTS signalosomes and use autophagy to degrade Bcl10, whereas naïve T cells use a distinct mechanism of NF-κB activation that appears to be independent of p62, involving recruitment of signaling proteins to SMACs. Collectively, these contributions provide key mechanistic details regarding how signals are transmitted from the TCR to the NF-κB transcription factor. Most recently, we have collaborated with Drs. Wolfgang Losert and Arpita Upadhyaya, biophysicists at the

University of Maryland, to define biophysical features of TCR-mediated activation and degradation of Bcl10. Our study with Dr. Losert in PLOS Computational biology has shown that the regulated polymerization and degradation of Bcl10 has characteristics of an excitable system, providing new insights regarding the digital behavior of this signaling module.

a. [#]Kingeter LM, Paul S, Maynard SK, Cartwright NG, and **Schaefer BC**. Cutting Edge: T cell receptor ligation triggers digital activation of NF-κB. Journal of Immunology. 2010; 185:4520-4.
   [#]Cited >90 times.
b. [*]Paul S, Kashyap AK, Jia W, He Y-W, and **Schaefer BC**. Selective autophagy of the adaptor protein Bcl10 modulates T cell receptor activation of NF-κB. Immunity. 2012; 36:947-58.
   [*]Cited >200 times; *Faculty of 1000* recommended
c. Paul S, Traver MK, Kashyap AK, Washington MA, Latoche JR, and **Schaefer BC**. T cell receptor signals to NF-κB are transmitted by a cytosolic p62-Bcl10-Malt1-IKK signalosome. Science Signaling. 2014; 7:ra45.
d. Campanello L, Traver MK, Shroff H, [†]**Schaefer BC**, [†]Losert W. Signaling through polymerization and degradation: Analysis and simulations of T cell activation mediated by Bcl10. PLOS Computational Biology. 2021 May 20;17(5):e1007986.
   [†]co-corresponding authors

3. During our studies on TCR signaling to NF-κB, we became interested in the understudied CARD protein, CARD19. Although CARD19 was initially described as a cytoplasmic negative regulator of TCR signaling to NF-κB, we found that this work was based on analysis of a partially spliced cDNA clone, and that the conclusions of this initial study were therefore spurious. Our work with a properly spliced CARD19 cDNA showed that CARD19 is a mitochondrial protein that plays no role in TCR activation of NF-κB. Our latest work has shown that CARD19 interacts with the mitochondrial cristae organizing system (MICOS), and that it plays a role in determining cristae morphology. Notably, 3 of the 7 current publications focused on CARD19 were generated by my laboratory or as a collaboration with my group. We are currently performing further studies to determine whether CARD19 regulates other aspects of mitochondrial function via its interaction with MICOS components.

a. Rios KE, Kashyap AK, Maynard SK, Washington M, Paul S and **Schaefer BC**. CARD19, the protein formerly known as BinCARD, is a mitochondrial protein that does not regulate Bcl10-dependent NF-κB activation after TCR engagement. Cell. Immunol., 2020; 356:104179.
b. Bjanes E, Sillas RG, Matsuda R, Demarco B, Fettrelet T, DeLaney AA, Rodriguez Lopez EM, Grubaugh D, Wynosky-Dolfi M, Philip NH, Krespan E, Tovar D, Joannas L, Beiting DP, Henao-Meija J, **Schaefer BC**, Chen KW, Broz P, and Brodsky I. Genetic targeting of Card19 is linked to disrupted NINJ1 expression, impaired cell lysis, and increased susceptibility to Yersinia infection. PLoS Pathog. 2021;17(10):e1009967.
c. Rios KE, Zhou M, Lott N, Beauregard C, McDaniel DP, Conrads TP, and **Schaefer BC**. CARD19 interacts with mitochondrial contact site and cristae organizing system constituent proteins and regulates cristae morphology. Cells, 2022 11(7):1175.

4. A second major research focus in my lab is the study of immune responses in the central nervous system. Our initial studies focused on inflammatory responses in traumatic brain injury (TBI), using mice as a model system: The first study demonstrated that the gene expression program induced by mild vs. severe TBI is remarkably similar, with the main distinction being that the expression of certain inflammatory genes remains persistently elevated in severe TBI. The second study presented evidence that the anti-inflammatory drug salsalate is a candidate therapy for TBI, reducing inflammation and improving functional recovery, as assessed by behavior assays. Our more recent work has focused on developing novel animal models and immunotherapies for neurotropic viral infections. Our published work on this project includes a description of our methodology for longitudinal tracing of lyssavirus infections and a description of isolation and in vitro characterization of two anti-lyssavirus neutralizing mAbs.

a. *Lagraoui M, Latoche JR, Cartwright NG, Sukumar G, Dalgard CL, **Schaefer BC**. Controlled cortical impact and craniotomy induce strikingly similar profiles of inflammatory gene expression, but with distinct kinetics. Front Neurol. 2012; 3:155.
   [*]Cited >100 times

   b. Lagraoui M, Sukumar G, Latoche JR, Maynard S, Dalgard CL, **Schaefer BC**. Salsalate treatment following traumatic brain injury reduces inflammation and promotes a neuroprotective and neurogenic transcriptional response with concomitant functional recovery. Brain, Behavior and Immunity. 2017; 61:96-109.
   c. Mastraccio KE, Huaman C, Warrilow D, Smith GA, Craig SB, Weir DL, Laing ED, Smith IL, Broder CC, and **Schaefer BC**. Establishment of a longitudinal pre-clinical model of lyssavirus infection. J. Virol. Methods. 2020; 281:113882.
   d. Weir DL, Coggins SA, Vu B, Coertse J, Yan L, Smith I, Laing ED, Markotter S, Broder CC and **Schaefer BC**. Isolation and Characterization of Cross-Reactive Human Monoclonal Antibodies That Potently Neutralize Australian Bat Lyssavirus Variants and Other Phylogroup 1 Lyssaviruses. Viruses. 2021; 13:391.

5. A distinct area of emphasis in my career has been the development of new tools for research and the optimization of existing tools. Among many publications in this area, I have edited a protocols book and written a review on methodology for identification of cDNA ends by RACE (which included an optimized protocol). I produced a C57BL/6 transgenic mouse strain ubiquitously expressing GFP, which has been a highly utilized tool for cell transfer studies. I also generated a set of retroviral vectors for rapid and reliable creation of stable cell lines. My lab has published optimized protocols for visualizing NF-κB signaling intermediates via confocal microscopy, and for quantifying NF-κB activation using imaging flow cytometry. We have most recently described our methodology for using bioluminescence imaging to semi-quantitatively trace lyssavirus infection in laboratory mice. A central goal of this work is to facilitate progress across the biomedical research community, through the development and distribution of better tools and clear explanation of how to reliably execute challenging techniques.

   a. †**Schaefer BC**. Revolutions in rapid amplification of cDNA ends: new strategies for polymerase chain reaction cloning of full-length cDNA ends. Anal Biochem. 1995 May 20;227(2):255-73.
   †Cited >475 times
   b. §**Schaefer BC**, Schaefer ML, Kappler JW, Marrack P, Kedl RM. Observation of antigen-dependent CD8+ T-cell/ dendritic cell interactions in vivo. Cell Immunol. 2001 Dec 15;214(2):110-22.
   §Describes creation and use of Jackson stock strain 004353 - C57BL/6-Tg(UBC-GFP)30Scha/J. Publication cited >400 times, strain cited many additional times.
   c. Traver MK, Paul S, and **Schaefer BC**. T Cell Receptor Activation of NF-κB in Effector T Cells: Visualizing Signaling Events Within and Beyond the Cytoplasmic Domain of the Immunological Synapse. Methods in Molecular Biology, 2017; 1584:101-27.
   d. Mastraccio KE, Huaman C, Laing ED, Broder CC, and **Schaefer BC**. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. In: *Bioluminescence*. Methods in Molecular Biology, Kim, SB. (ed.), 2022; **2524**:369-394.

Complete publications from My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/brian.schaefer.1/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: Amaya, Moushimi

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6) [redacted]

POSITION TITLE: Research Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Kwa-Zulu Natal, Durban, South Africa	B.S.	12/2004	Biological Science
George Mason University, Manassas, VA	M.S.	12/2012	Molecular Biology
George Mason University, Manassas, VA	Ph.D.	12/2015	Cell and Molecular Bio
Uniformed Services University, Bethesda, MD	Postdoctoral Fellow	02/2019	Molecular Virology
Uniformed Services University, Bethesda, MD	Scientist	09/2022	Molecular Virology

A. Personal Statement

As an active researcher in the development of novel therapeutics and vaccine candidates against emerging and re-emerging henipaviruses in relation to pandemic preparedness, I am uniquely qualified to participate as a Co-Investigator in **Research Project 2: Vaccines and antibodies to henipaviruses** of the present U19 application (ReVAMPP). The extensive experience in henipavirus reverse genetics platforms necessary to conduct the proposed studies began in my postdoctoral training, under the guidance and mentorship of **Dr. Christopher Broder**, a leading expert in henipavirus biology. During my fellowship, I rescued and fully characterized a replication competent recombinant Cedar virus (rCedV) as non-reporter and luciferase expressing reporter versions that can safely be utilized at BSL-2. This work resulted in a first co-author publication (see below). In addition, I generated replication competent chimeric viruses as non-reporter and reporter versions that express the envelope glycoproteins of either Nipah virus Bangladesh (NiV-B) (rCedV-NiV-B) or Hendra virus (HeV) (rCedV-HeV) instead of those of CedV. All 6 chimeric viruses were fully characterized in in vitro studies. Together with **Dr. Thomas Geisbert**, at the University of Texas Medical Branch (UTMB), with whom I have a 5-year collaboration, we demonstrated that the chimeras can serve as suitable surrogate viruses for authentic NiV-B or HeV as determined by plaque reduction neutralization tests. Moreover, the fluorescent reporter chimeric viruses were further characterized in a high throughput fluorescence reduction neutralization test (FRNT) to analyze neutralization potencies of sera from henipavirus immunized animals and monoclonal antibodies. This work resulted in a first author manuscript that was very recently published (see below). The FRNT has also been implemented in several neutralization and synergistic neutralization tests that resulted in 2 co-author publications (see below). The highly productive collaborations between Drs. Broder, Geisbert and I underscore the feasibility of the proposed project. The research proposed in this application will not only build on my work developed thus far but also strengthen my scientific portfolio to achieve my long-term goal of vaccine development for priority pathogens.

Citations:

1. **Amaya, M.**, Yin, R., Yan, L., Borisevich, V., Adhikari, B.N., Bennett, A., Malagon, F., Cer, R.Z., Bishop-Lilly, K.A., Dimitrov, A.S., Cross, R.W., Geisbert, T.W., Broder, C.C. (2023). A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses*, *15*(5). https://doi.org/10.3390/v15051077
2. Wang, Z.*, Dang, H. V.*, **Amaya, M.***, Xu, Y., Yin, R., Yan, L., Hickey, A. C., Annand, E. J., Horsburgh, B. A., Reid, P. A., Smith, I., Eden, J. S., Xu, K., Broder, C. C., & Veesler, D. (2022).

Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. *Proc Natl Acad Sci U S A*, *119*(22), e2122769119. https://doi.org/10.1073/pnas.2122769119 (*equal contribution)

3. Wang, Z., **Amaya, M.**, Addetia, A., Dang, H. V., Reggiano, G., Yan, L., Hickey, A. C., DiMaio, F., Broder, C. C., & Veesler, D. (2022). Architecture and antigenicity of the Nipah virus attachment glycoprotein. *Science*, *375*(6587), 1373-1378. https://doi.org/10.1126/science.abm5561
4. **Amaya, M.***, Cheng, H.*, Borisevich, V., Navaratnarajah, C. K., Cattaneo, R., Cooper, L., Moore, T. W., Gaisina, I. N., Geisbert, T. W., Rong, L., & Broder, C. C. (2021). A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res*, *193*, 105084. https://doi.org/10.1016/j.antiviral.2021.105084 (*equal contribution)

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022-pres	Research Assistant Professor, Department of Microbiology and Immunology, USU, Bethesda, MD.
2019-2022	Scientist, Henry M. Jackson Foundation Inc., Department of Microbiology and Immunology, USU, Bethesda, MD.
2016-2019	Postdoctoral Research Fellow, Department of Microbiology and Immunology, USU, Bethesda, MD.
2016	Adjunct Professor for BIOL 306 Biology of Microorganisms Lab, George Mason University, Fairfax, VA.
2012-2015	Graduate Teaching Assistant for BIOL 306 Biology of Microorganisms Lab, George Mason University, Fairfax, VA.
2011-2012	Graduate Teaching Assistant for BIOL 104 Introduction to Biology I and II Lab, George Mason University, Fairfax, VA.

Other Experience and Professional Memberships

2019-2020	Member, American Society of Cell Biology
2018-2019	Ad hoc reviewer - Emerging Microbes & Infections
2012-2015	Member, American Society of Microbiology

Honors and Awards

2015	College of Science Summer Fellowship Recipient, George Mason University.
2014	College of Science Summer Fellowship Recipient, George Mason University.
2014-2015	Outstanding Doctoral Student Award, George Mason University, School of Systems Biology.
2013-2015	Bioscience Fellowship Recipient, George Mason University, School of Systems Biology.
2013	PhD Impact Award, George Mason University, School of Systems Biology.

Patents issued

- Recombinant Cedar Virus Chimeras. Provisional. Atty. Dkt. No. 103783-0276.

C. Contributions to Science

1. **Graduate career:** My Ph.D. thesis research involved characterizing the host viral interactions in human cells with a focus on the least characterized non-structural protein, nsP3, from VEEV. I initially constructed a tagged version of nsP3 both as an overexpression construct and in the context of the viral genome. Mass spectrometry analyses revealed that VEEV-nsP3 interacted with multiple host factors. Those of particular interest were chosen for additional studies, namely the kinase IKKβ and the RNA helicases DDX1 and DDX3. I validated that VEEV-nsP3 interacted and complexed with IKKβ, DDX1 and DDX3 and furthermore, siRNA knockdown studies indicated a reliance of these host factors for productive and continuous viral replication. These findings formed the basis of the functional model where VEEV-nsP3 interacts with the host translational machinery via DDX3 to potentially aid in translation of viral proteins that could be part of the replication complex, and that this interaction could be essential in the viral life cycle. This contribution will aid in elucidating the functionality of nsP3 in the viral life cycle as well as to promote the development of host based therapeutic options to treat RNA viral infections or disease. Results from my research were highly relevant as they provided new details and insight in understanding the role of the uncharacterized VEEV protein, nsP3, in

the new world alphavirus life cycle. As such, this work resulted in two first author research publications and one review article (see below). My Ph.D. research on host viral interactions was expanded to include host pathways that were altered during viral infection by utilizing small molecule inhibitors. Such pathways included the host phospho-signaling and the ubiquitin proteasome pathways. I validated, as a collaboration, that the MAPK/ERK signaling pathway was activated and necessary for the infectious process in VEEV infected human cells through utilization of the small molecule inhibitor, Ag-126. This joint effort resulted in a second author publication. In a different study, I had revealed with the use of an FDA approved proteasome inhibitor, Bortezomib that VEEV requires the ubiquitin proteasome pathway in the viral life cycle. My results indicated the likely mechanism was that K48 ubiquitination of VEEV capsid protein may be necessary for the early stages of infection. This study was the first to demonstrate that the capsid protein was post translationally modified with ubiquitin and this resulted in a first author publication (see below). In addition, I had also collaborated to investigate the requirement of the ubiquitin proteasome pathway in the negative stranded RNA virus, RVFV infection. We showed that with Bortezomib treatment, the formation of the characteristic nuclear filaments formed by the virulence factor NSs was abolished, thereby preventing NSs interaction with host repressor proteins SAP30 and mSin3A. This study resulted in a second author publication. Cumulatively, these studies would pave the way for developing host-based therapies and/or repurposing FDA approved drugs to treat these re-emerging diseases.

1. **Amaya, M.**, Brooks-Faulconer, T., Lark, T., Keck, F., Bailey, C., Raman, V., & Narayanan, A. (2016). Venezuelan equine encephalitis virus non-structural protein 3 (nsP3) interacts with RNA helicases DDX1 and DDX3 in infected cells. *Antiviral Res*, *131*, 49-60. https://doi.org/10.1016/j.antiviral.2016.04.008
2. **Amaya, M.**, Keck, F., Bailey, C., & Narayanan, A. (2014). The role of the IKK complex in viral infections. *Pathog Dis*, *72*(1), 32-44. https://doi.org/10.1111/2049-632X.12210
3. **Amaya, M.**, Keck, F., Lindquist, M., Voss, K., Scavone, L., Kehn-Hall, K., Roberts, B., Bailey, C., Schmaljohn, C., & Narayanan, A. (2015). The ubiquitin proteasome system plays a role in venezuelan equine encephalitis virus infection. *PLoS One*, *10*(4), e0124792. https://doi.org/10.1371/journal.pone.0124792
4. **Amaya, M.**, Voss, K., Sampey, G., Senina, S., de la Fuente, C., Mueller, C., Calvert, V., Kehn-Hall, K., Carpenter, C., Kashanchi, F., Bailey, C., Mogelsvang, S., Petricoin, E., & Narayanan, A. (2014). The role of IKKbeta in Venezuelan equine encephalitis virus infection. *PLoS One*, *9*(2), e86745. https://doi.org/10.1371/journal.pone.0086745

2. **Postdoctoral career:** My postdoctoral training involved the development and characterization of a recombinant henipavirus platform that can safely be used at BSL-2 to investigate and understand henipavirus infection with respect to pathogenesis, neutralization, drug discovery, therapeutics and vaccine potential. The reverse genetics system for the rCedV platform resulted in a first co-author publication (see below). A collaborative research project entailed the optimization of a replication competent rCedV-Luc for high throughput screening assays to identify small drug like molecules that can target multiple stages in the henipavirus life cycle, which resulted in a first co-author publication (see below). Building on the rCedV platform, I designed and characterized a robust recombinant chimeric henipavirus reverse genetics system to be used as a surrogate system for pathogenic henipaviruses. The envelope glycoproteins of rCedV were replaced with those of either NiV-B (rCedV-NiV-B) or HeV (rCedV-HeV) and non-reporter and reporter versions were generated. I optimized and implemented a rapid high throughput cell-based fluorescence reduction microneutralization assay using rCedV chimeras to determine monoclonal antibody neutralization potencies and detect the presence of neutralizing antibodies and measure neutralizing titers in henipavirus immunized animal sera. This work resulted in a first author manuscript (see below). In a collaborative effort, I generated recombinant henipavirus chimeric virus escape mutants to understand the structure of henipavirus envelope glycoproteins. This collaboration resulted in a co-author manuscript (see below). In addition, my work has been recently presented at multiple meetings such as the Keystone Symposia, Bat Infectious Diseases Symposium, and the World One Health Congress.

1. **Amaya, M.**, Yin, R., Yan, L., Borisevich, V., Adhikari, B.N., Bennett, A., Malagon, F., Cer, R.Z., Bishop-Lilly, K.A., Dimitrov, A.S., Cross, R.W., Geisbert, T.W., Broder, C.C. (2023). A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses*, *15*(5). https://doi.org/10.3390/v15051077
2. Wang, Z., **Amaya, M.**, Addetia, A., Dang, H. V., Reggiano, G., Yan, L., Hickey, A. C., DiMaio, F., Broder, C. C., & Veesler, D. (2022). Architecture and antigenicity of the Nipah virus attachment glycoprotein. Science, 375(6587), 1373-1378. https://doi.org/10.1126/science.abm5561
3. **Amaya, M.**, Cheng, H., Borisevich, V., Navaratnarajah, C. K., Cattaneo, R., Cooper, L., Moore, T. W., Gaisina, I. N., Geisbert, T. W., Rong, L., & Broder, C. C. (2021). A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral Res, 193, 105084. https://doi.org/10.1016/j.antiviral.2021.105084
4. Laing, E. D., **Amaya, M.**, Navaratnarajah, C. K., Feng, Y. R., Cattaneo, R., Wang, L. F., & Broder, C. C. (2018). Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. Virol J, 15(1), 56. https://doi.org/10.1186/s12985-018-0964-0

Complete list of published work in MyBibliography: MAmaya Bibliography.

BIOGRAPHICAL SKETCH

NAME: Broder, Christopher C.

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: Professor of Microbiology, Immunology and Emerging Infectious Diseases

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Florida Institute of Technology, Melbourne, FL	B.S.	1983	Biological Science
Florida Institute of Technology, Melbourne, FL	M.S.	1985	Molecular Biology
University of Florida, Gainesville, FL	Ph.D.	1989	Immunology and Med-Micro

A. Personal Statement

The objectives of this ReVAMPP Center proposal are to support Pandemic Preparedness through the study and advancement of a broadly applicable viral subunit immunogen vaccine delivery platform and antibody-based countermeasures for bunyavirales and paramyxovirus agents. I will serve as Co-PI with Thomas Geisbert (Center PI) of the **Administrative Core A**; and as Project Leader of **Research Project 2 (RP2): Vaccines and antibodies against henipaviruses.** I have researched enveloped virus-host cell interactions for 34 years, funded primarily by multiple NIAID and DoD grants on HIV and zoonotic viral agents. 1999, I established a collaborative international group of research experts in Hendra and Nipah virus biology, and later over a 14-year period I was the PI of two multi-component, international, NIAID, U01 awards, and also the Co-PI of a third U01 with the Center PI (Geisbert) all focused on henipavirus research, and have collaborated with Dr. Geisbert for the past 16 years. I also currently serve as PI on a NIAID CETR U19 focused on henipavirus countermeasures. Some of my collaborative henipavirus research contributions include: structural biochemistry on the viral G and F glycoproteins; animal model development and *in vivo* pathogenesis studies in the feline and ferret (with the AAHL-CSIRO, Australia), and the African green monkey (with Geisbert); development and testing of vaccines and therapeutics; discovery of the entry receptors ephrin-B2/B3; development of antiviral mouse and human monoclonal antibodies (mAb) including m102.4 that successfully completed a Phase 1 trial in Australia (2020) and since used by emergency protocol in 18 people in Australia and the US as a post-exposure treatment; developed and patented the Hendra/Nipah soluble G (sG) and soluble F (sF) subunit vaccine candidates, including HeV-sG for horses called Equivac® HeV (Zoetis, Inc.), the first commercialized vaccine to a BSL-4 agent; and now in Phase I trial in the US (Auro Vaccines/PATH/CEPI). More recently my lab developed a reverse genetics platform for generating recombinant Cedar henipavirus (rCedV) that can be utilized at BSL-2 for both high-throughput antiviral drug discovery and rCedV chimeras for performing rapid neutralization tests for Nipah and Hendra. I have the expertise in many aspects of the henipavirus-related project goals proposed here, and I have led both basic and translational research projects in a broad area of study on Nipah and Hendra. Here **RP2** will be focused on the implementation of a new, rapid and dissolvable, microneedle patch (MNP) vaccine technology with the 'prototype' viruses Nipah and Hendra; develop reporter virus platforms for the divergent Langya and Angavokely henipaviruses; assess vaccine responses and test neutralizing antibodies and nanobodies to be developed by the Center's **Core D** and **RP4** and **RP5**. We will also pivot and adapt the MNP approach and new tools in Phase 2 of the Center to another virus.

Ongoing projects that I would like to highlight:

U19 AI142764. NIAID, Center of Excellence for Translational Research (CETR): Role: PI
03/20/19-02/29/24. "Advancement of Vaccines and Therapies for Henipaviruses"

U19 AI171413. NIAID AViDD: "UTMB-Novartis Alliance for Pandemic Preparedness": PIs: Diagana and Shi.
05/01/2022-04/30/2027; Sub-project 3: Henipaviruses. Role: Co-PI with Geisbert

CRADA; Auro Vaccines; CEPI (Coalition for Epidemic Preparedness Innovations): Role: Co-PI with Hamm
07/01/18-06/30/2023; "A Subunit Vaccine (HeV-sG) to Protect against Nipah and Hendra Diseases"

1. Amaya M, Cheng H, Borisevich V, Navaratnarajah CK, Cattaneo R, Cooper L, Moore TW, Gaisina IN, Geisbert TW, Rong L, **Broder CC***. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral Res. 2021 May 30:105084. doi: 10.1016/j.antiviral.2021.105084. PMCID: PMC8631057
2. Playford EG, Munro T, Mahler SM, Elliott S, Gerometta M, Hoger KL, Jones ML, Griffin P, Lynch KD, Carroll H, El Saadi D, Gilmour ME, Hughes B, Hughes K, Huang E, de Bakker C, Klein R, Scher MG, Smith IL, Wang LF, Lambert SB, Dimitrov DS, Gray PP, **Broder CC**. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. Lancet Infect Dis. 2020 Apr;20(4):445-454. PMID: 32027842
3. Geisbert TW, Bobb K, Borisevich V, Geisbert JB, Agans KN, Cross RW, Prasad AN, Fenton KA, Yu H, Fouts TR, **Broder CC***, Dimitrov AS*. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. NPJ Vaccines. 2021 Feb 8;6(1):23. doi: 10.1038/s41541-021-00284-w. PMCID: PMC6789926
4. Amaya, M, Yin R, Yan L, Borisevich V, Adhikari BN, Bennett A, Malagon F, Cer RZ, Bishop-Lilly KA, Dimitrov AS, Cross RW, Geisbert TW, **Broder CC***. A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. Viruses 2023, 15(5), 1077.

B. Positions, Scientific Appointments

Positions and Employment

2018-pres.	Chair, Department of Microbiology and Immunology, USU, Bethesda, Maryland.
2006-2018	Director, Emerging Infectious Diseases Graduate Program, USUHS, Bethesda, Maryland.
2005-pres.	Professor, Department of Microbiology and Immunology, Joint appointment, Emerging Infectious Diseases Graduate Program, USUHS, Bethesda, Maryland.
2000-2005	Associate Professor, Department of Microbiology and Immunology, Joint appointment, Emerging Infectious Diseases Graduate Program, USUHS, Bethesda, Maryland.
1996-2000	Assistant Professor, Department of Microbiology and Immunology, Joint appointment, Molecular and Cell Biology Graduate Program, Uniformed Services University, Bethesda, Maryland.
1993-1996	IRTA Fellow, LVD, NIAID, NIH, Bethesda, Maryland.
1990-1992	National Research Council, Research Associate, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

Other Experience and Professional Affiliations

2022-	Member, Virus Expert Panel for Disease X Vaccine Libraries (CEPI).
2018-	Nipah Task Force (CEPI).
2018-	Nipah Therapeutics Protocol Team; ICMR, NIAID, WHO.
2011	Member, Discontools Nipah Virus Infection Panel Expert Group. Gap analysis. International Federation for Animal Health Europe, Brussels, Belgium.
	Editorial boards, dates since joining: *Journal of Virology-07'*, *Virology-10'*, *Viruses-11'*, *Pathogens-11;* V*irologica Sinica-12'*
2009	Member, National Veterinary Stockpile Nipah virus Countermeasures Workshop; United States Department of Agriculture. Geelong, Australia.

Honors and Awards

2022	The Carol Johns Medal (highest honor USU faculty can bestow on a fellow faculty member).
2021	Military Health System Research Symposium (MHSRS) 2021 Outstanding Research Accomplishment Team Award. (COVID-19 / SARS-CoV-2 serology research).
2020	Military Health System Research Symposium (MHSRS) 2020 Outstanding Individual Research Accomplishment by an Individual Senior Researcher. (Hendra and Nipah virus countermeasures).
2020	Inaugural, 2020 Federal Laboratory Consortium (FLC) Impact Award.
2019	USU Outstanding Biomedical Graduate Educator Award.
2019	The University of Florida, College of Medicine, “Wall of Fame”.
2019	Dean’s Award for Leadership, University of Florida, College of Medicine.

2019 Federal Laboratory Consortium (FLC) Award for Excellence in Technology Transfer.
2016 The James J. Leonard Award for Excellence in Translational/Clinical Research.
2014 The Cinda Helke Award for Excellence in Graduate Student Advocacy.
2013 The 3rd Sidney Pestka Lecture; 22nd Annual Philadelphia Infection & Immunity Forum.
2013 Federal Laboratory Consortium (FLC) Award for Excellence in Technology Transfer.
2013 Second Finalist for the Australian Infectious Diseases Research Centre Eureka Prize.
2013 The CSIRO Chairman's Medal. The Commonwealth Scientific and Industrial Research Organisation (CSIRO); Australia's national science agency.
2008 The Henry Wu Award for Excellence in Basic Science Research.
1996 The Fellows Award for Research Excellence, Office of Science Education, NIH.
1996-1997 The Newcomb Cleveland Prize - AAAS: and Breakthrough of the Year, Science Magazine.
1990-1992 National Research Council Research Associateship Award.
1989 Medical Guild Graduate Research Award, University of Florida College of Medicine.

Patents issued and significant technology licenses

- Bacterial plasmin receptors as fibrinolytic agents: U.S. Pat. No. 5,237,050.
- Oligomeric HIV-1 envelope glycoproteins (gp140): U.S. Pat. Nos. 6,039,957 and 6,171,596.
- HIV-1 Envelope Glycoprotein Oligomer and Methods of Use. U.S. Pat. No. 8,597658.
- CCR5 DNA, new animal models and therapeutic agents for HIV infection: U.S. Pat. No. 7,151,087.
- Cells expressing both human CD4 and CXCR4 associated with HIV infection: U.S. Pat. No. 6,197,578.
- Compositions and methods for the inhibition of membrane fusion by paramyxoviruses: U.S. Pat. Nos. 7,666,431 and 8,114,410.
- Soluble Forms of Hendra and Nipah Virus G Glycoprotein. Australian Pat. No. 2005327194. U.S. Pat. Nos. 8,865,171; 9,045,532; 9,056,902; 9,533,038, 10,053,495.
- Soluble Forms of Hendra and Nipah Virus F Glycoprotein and Uses Thereof: Australian Patent No. 2013276968. U.S. Pat. Nos. 10,040,825; 10,590,172.
- Human monoclonal antibodies against Hendra and Nipah viruses. U.S. Pat. Nos. 7,988,971; 8,313,746, 8,858,938.
- Antibodies against F glycoprotein of Hendra and Nipah viruses. U.S. Pat. Nos. 9,982,038 and 10,738,104
- Cedar virus and Methods of Use. U.S. Patent No., 10,227,664.
- Recombinant Cedar Virus Chimeras. Provisional. Atty. Dkt. No. 103783-0276.
- Hendra sG: Licensed to Zoetis, Inc. (Pfizer Animal Health). Equivac ® HeV; Nov, 2012, Australia.
- Hendra sG: Licensed to AuroVaccines, Aurobindo Pharma USA., Nipah/Hendra human vaccine candidate.
- Monoclonal antibodies to Henipaviruses. Absolute Antibody Ltd, United Kingdom.
- Soluble Nipah and Hendra virus F and G glycoproteins. The Native Antigen Company Ltd, United Kingdom.

C. Contributions to Science

My Ph.D. thesis studies centered on the discovery and characterization of a specific receptor for human plasmin on Group A Streptococci during my rotation project as a first year student. My studies revealed that certain group A streptococci elaborated surface receptors that could bind selectively a key fibrinolytic enzyme, plasmin. The bacterium-bound plasmin remained enzymatically active including its ability to hydrolyze a fibrin clot and could not be inhibited by its physiological regulator, alpha 2-plasmin inhibitor. Since these organisms produced streptokinase producing an active enzyme that can convert plasminogen to plasmin, they could accelerate the destruction of the extracellular matrix environment: a model for the "flesh-eating streptococci".

a. Lottenberg, R., **C.C. Broder**, and M.D.P. Boyle. Identification of a Specific Receptor for Plasmin on a Group A Streptococcus. Infection and Immunity. 55(8):1914-1918, 1987.
b. **Broder, C.C.**, R. Lottenberg, and M.D.P. Boyle. Mapping of the Domain of Human Plasmin Recognized by its Unique Group A Streptococcal Receptor. Infection and Immunity. 57(9): 2597-2605, 1989.
c. **Broder, CC**, R Lottenberg, GO vonMering, K. Johnston and MDP Boyle. Isolation of a prokaryotic plasmin receptor: relationship to a plasminogen activator produced by the same microorganism. J. Biol. Chem. 266:4922-28, 1991.

d. Lottenberg, R., **C.C. Broder**, M.D.P. Boyle, S.J. Kain, B.L. Schroeder, and R. Curtiss III. Cloning, Sequence Analysis, and Expression in *Escherichia coli* of a Streptococcal Plasmin Receptor. J. Bacteriology. 174:5204-5210, 1992.

My independent work as a postdoctoral fellow focused on the early stages of HIV-1 envelope glycoprotein mediated membrane fusion as a surrogate model of HIV-1 entry. I established a vaccinia virus-based reporter gene assay for measuring viral (HIV-1) glycoprotein-mediated membrane fusion and generated the first panel of T-cell tropic and Macrophage-tropic HIV-1 envelope glycoprotein (Env) encoding recombinant vaccinia virus vectors and I used these tools to be the first to hypothesize that the cellular tropism of HIV-1 could be explained by specific membrane fusion factors required for the different classes of HIV-1 Envs. I also developed the first soluble and secreted full-length oligomeric HIV-1 gp140 glycoprotein and explored the importance of its native oligomeric structure in terms of its presentation of conformational and virus-neutralizing epitopes through the development and characterization of more than 100 murine monoclonal antibodies.

a. **Broder, C.C.,** D.S. Dimitrov, R. Blumenthal, and E.A. Berger. The block to HIV-1 envelope glycoprotein-mediated membrane fusion in animal cells expressing human CD4 can be overcome by a human cell component(s). Virology. 193:483-491, 1993.
b. Nussbaum, O., **C.C. Broder,** and E.A. Berger. HIV-1 Envelope Glycoprotein/CD4 Mediated Cell Fusion: A Novel Recombinant Vaccinia Virus-Based Assay Measuring Activation of a Reporter Gene by Bacteriophage T7 RNA Polymerase Selectively In Fused Cells. J.Virol. 68:5411-5422, 1994.
c. **Broder, C.C.,** P.L. Earl, D. Long, B. Moss, and R.W. Doms. Antigenic Implications of HIV-1 Envelope Glycoprotein Quaternary Structure: Oligomer-Specific and -Sensitive mAbs. PNAS. 91:11699-11703, 1994.
d. **Broder, C.C.** and E.A. Berger. Fusogenic Selectivity of the Envelope Glycoprotein is a Major Determinant of HIV-1 Tropism for CD4+ T-Cell Lines vs. Macrophages. PNAS. USA. 92:9004-08, 1995.

My early studies on the cellular and viral membrane fusion tropism of HIV-1 and the development of a sensitive and specific reporter gene assay of cell-cell membrane fusion facilitated the discovery of the first membrane fusion accessory factor (“fusin”; CXCR4) that I earlier hypothesized existed, and this rapidly led to the discovery by us and others of the second factor for macrophage-tropic Envs (CCR5); the HIV-1 coreceptors. These findings were a significant breakthrough in HIV research leading to numerous new directions in understanding HIV-1 pathogenesis as well as new therapeutic strategies.

a. Feng, Y., **C.C. Broder**, P.E. Kennedy, and E.A. Berger. HIV-1 Entry Cofactor: Functional cDNA Cloning of a Seven-Transmembrane, G Protein-Coupled Receptor. Science. 272:872-877, 1996.
b. Alkhatib*, G., C. Combadiere*, **C.C. Broder***, Y. Feng*, P.E. Kennedy*, P.M. Murphy, and E.A. Berger. CC CKR5: a RANTES, MIP-1α , MIP-1β Receptor as a Fusion Cofactor for Macrophage-Tropic HIV-1. Science. 272:1955-1958, 1996. (*equal contribution).
c. Rucker, J., M. Samson, B. J. Doranz, F. Libert, J. F. Berson, Y. Yi, R. G. Collman, **C. C. Broder**, G. Vassart,, R. W. Doms, and M. Parmentier. Regions in β-chemokine Receptors CCR-5 and CCR-2b that Determine HIV-1 Cofactor Specificity. Cell. 87:1-10, 1996.
d. Edinger, A.L., A. Amedee, K. Miller, B.J. Doranz, M. Endres, M. Sharron, M. Samson, Z-h. Lu, J.E. Clements, M. Murphey-Corb, S.C. Peiper, M. Parmentier, **C.C. Broder**, and R.W. Doms. Differential utilization of CCR5 by macrophage and T cell tropic SIV strains. PNAS. 94:4005-4010, 1997.

My initial work on HIV-1 entry led to further independent studies which focused on follow-up investigations characterizing the roles of the HIV-1 coreceptors in the virus entry process. These studies revealed the interplay between the HIV-1 entry receptors, mapped important domains of the coreceptors involved in HIV-1 Env interaction, and also revealed possible avenues of how an HIV-1 Env might engage and differently utilize the CXCR4 and CCR5 coreceptors for infection. In addition, I also engaged in collaborative follow-up studies exploring the utility of soluble oligomeric HIV-1 envelope glycoproteins as subunit vaccine immunogens (gp140) which I initiated at NIH while a postdoctoral fellow.

a. Chabot, D.J., P-F. Zhang, G.V. Quinnan, and **C.C. Broder***. Mutagenesis of CXCR4 Identifies Important Domains for HIV-1 X4 Isolate Envelope-Mediated Membrane Fusion and Virus Entry and Reveals Cryptic Coreceptor Activity for R5 Isolates. J. Virol. 73:6598-6609, 1999.
b. Xiao, X., L. Wu, T.S. Stantchev, Y-R. Feng, S. Ugolini, H. Chen, Z. Shen, **C.C. Broder**, Q.J. Sattentau, and D.S. Dimitrov. Constitutive cell surface association between CD4 and CCR5. PNAS. 96:7496-7501, 1999.
c. Chabot, D.J., H. Chen, D.S. Dimitrov, and **C.C. Broder***. N-linked Glycosylation in CXCR4 Masks Coreceptor Function for CCR5-Dependent HIV-1 Isolates. J. Virol. 74:4404-4413, 2000.

d. Zhang, P.F., Cham, F., Dong, M., Choudhary, A., Bouma, P., Zhang, Z., Shao, Y., Feng, Y.R., Wang, L., Mathy, N., Voss, G., **Broder, C.C**., Quinnan, G.V., Jr. Extensively cross-reactive anti-HIV-1 neutralizing antibodies induced by gp140 immunization. PNAS. USA. 104(24):10193-8. 2007.

My recent efforts have focused on emerging viruses that impact human and domestic livestock populations; including lyssaviruses, filoviruses, and henipaviruses for the past 23 years. I obtained the first NIAID funded biodefense project providing fiscal support on select agent research to an overseas laboratory (2003; U01 AI056423). I developed the first henipavirus peptide-based fusion inhibitors, subunit vaccines, and neutralizing human and humanized monoclonal antibodies (mAb), and my NIAID grants supported the development of the feline, ferret and African green monkey models of Hendra and Nipah pathogenesis. Henipavirus countermeasures development is a major a focus of my lab and supported by a NIAID U19 CETR. Here, we are developing next-generation mAb therapies and next-generation vaccines and delivery methods against Nipah and Hendra for both human and livestock use. I am also heavily engaged in collaborative virus surveillance activities focusing on henipaviruses, filoviruses, coronaviruses, and others, in wildlife and human populations in more than 10 countries in South Asia, Southeast Asia, West Africa and Southern Africa; using the Luminex xMAP-based platform which has proven to be more sensitive than ELISAs for the detection of antibodies to viral infections. The key feature of our multiplex microsphere immunoassays (MMIA) are the use of my lab’s production of recombinant, soluble, native-like, oligomeric viral glycoproteins, designed similar to our HeV-sG subunit vaccine. Our MMIA array presently contains more than 20 different soluble glycoprotein ectodomains. These programs are funded by the NIAID and the Defense Threat Reduction Agency (DTRA), an agency within the US Department of Defense.

a. Bonaparte, M. I., A. S. Dimitrov, K. N. Bossart, G. Crameri, B. A. Mungall, K. A. Bishop, V. Choudhry, D. S. Dimitrov, L.-F. Wang, B. T. Eaton, and **C. C. Broder*.** Ephrin-B2 Ligand is a Functional Receptor for Hendra Virus and Nipah Virus. Proc Natl Acad Sci U S A. 102(30):10652-7. 2005. (***from the cover***).
b. Bossart, KN, Geisbert, TW, Feldmann, H, Zhu, Z, Feldmann, F, Geisbert, JB, Yan, L, Feng, Y-R, Brining, D, Scott, D, Wang, Y, Dimitrov, AS, Callison, J, Chan, Y-P, Hickey, AC, Dimitrov, DS, **Broder, CC***, and Rockx, B. A neutralizing human monoclonal antibody protects African Green monkeys from Hendra virus challenge. Sci. Transl. Med. 3, 105ra103. 2011. *corresponding author. (***from the cover***). PMID: 22013123
c. Bossart KN, Rockx B, Feldmann F, Brining D, Scott D, Lacasse R, Geisbert JB, Feng YR, Chan YP, Hickey AC, **Broder CC*,** Feldmann H, Geisbert TW. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med. 4(146):146ra107. 2012 (***from the cover***) PMID: 22875827.
d. Geisbert TW, Mire CE, Geisbert JB, Chan YP, Agans KN, Feldmann F, Fenton KA, Zhu Z, Dimitrov DS, Scott DP, Bossart KN, Feldmann H, **Broder CC***. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med. 2014, (***from the cover***) 6(242):242ra82. PMID: 24964990.

Total publications: >200; citations: >26,000; h-index 72 (google scholar).

A more complete list of published work in: MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/181k8EOf_FNkT/bibliography/public/

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Antony S. Dimitrov

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6) [redacted]

POSITION TITLE: Senior Staff Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Sofia State University, Bulgaria	M.Sci.	06/1987	Chemistry
The University of Tokyo, Japan	Ph.D.	04/1995	Physical Chemistry
NCI-Frederick, NIH, Frederick, MD	Postdoctoral Training	04/2004	Biochemistry, Viral entry

A. Personal Statement

I have been familiar with HeV-sG since its discovery. I am a co-author of the HeV-sG characterization article in JVI 2005. It shows that HeV and NiV as well G-glycoproteins, membrane attached and soluble as well, exist as dimers and dimers of dimers. Under the supervision of Dr. Broder I played a major role in the discovery of ephrin B2 as a functional receptor for Nipah and Hendra viruses, sharing an equal contribution with Dr. Bonaparte in the related PNAS article (2005). I had been also characterizing the binding site of ephrin B2 to HeV and NiV G glycoprotein and found aminoacid residues on ephrin B2 that are critical for the virus receptor binding.

I was a PI (MPI, with Dr. Fouts) of two pre-clinical development programs – one for the HeV-sG as subunit vaccine against henipavirus infections and the other for the human antibody, m102.4, as postexposure treatment against such infections. I am currently involved in the clinical investigation of the HeV-sG subunit vaccine led by Auro Vaccines LLC. I am also actively involved in coordination the work of the research projects and cores within the CETR U19- AI142764 "Advancement of Vaccines and Therapies for Henipaviruses" (Christopher Broder, PI), where I wrote the proposal for RP1 related to the next generation HeV-sG human vaccine. Although the subunit vaccine in its current formulation has an excellent efficacy, its stability at ambient condition and efficacy are expected to be significantly enhanced within RP1 of the above CETR U19, there is still room to explore the immunization efficiency by decreasing the cost of storage and application procedure. Within one of the latest awards we are developing patches of soluble microneedle arrays for intradermal delivery of HeV-sG. The vaccine is expected to be stable at ambient conditions and ready for easy self-administration by pressing the patch to a bare skin place, like on the shoulder. During the past 2 decades I accumulated relevant experience related to GLP, GMP, and partly GCP and believe that I can significantly contribute to the proposed project.

B. Positions, Scientific Appointments, and Honors

2019 – Senior Scientist, USUHS, Bethesda, MD 20814 / HJF, Bethesda, MD 20817

2006 – 2019 Senior Staff Scientist, Profectus BioSciences, Inc., 6411 Beckley Str., Baltimore, MD 21224

2004 – 2006 Research Associate, Dept. of Microbiology at USUHS, Bethesda, MD 20814

1999 – 2004 Post-Doctoral Fellow, Laboratory of Experimental and Computational Biology, CCR, NCI, NIH, Bldg. 469, Rm. 211, P.O. Box B, Frederick, MD 21702-1201

1995 – 1999 Scientist, L'ORÉAL Tsukuba Center, Tokodai 5-5, Tsukuba, Japan

1992 – 1995 Senior Research Associate, Nagayama Protein Array Project, ERATO, JRDC, Tokodai 5-5, Tsukuba, Japan

1987 – 1993 Research Specialist (Chemist), Faculty of Chemistry, Sofia State University, Sofia, Bulgaria

C. Contributions to Science

1. *Development of HeV-sG as a human subunit vaccine against henipavirus infection.*
Relates to my current activities described above in the Personal Statement.

A. Geisbert TW, Bobb K, Borisevich V, Geisbert JB, Agans KN, Cross RW, Prasad AN, Fenton KA, Yu H, Fouts TR, Broder CC, **Dimitrov AS**. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. **NPJ Vaccines**, 2021 Feb 8;6(1):23. doi: 10.1038/s41541-021-00284-w

2. *Discovery that ephrin B2 is a functional receptor for both HeV and NiV. Characterization of HeV-sG.*
Dr. Broder (USUHS, Bethesda) pioneered the research on HeV and NiV starting the beginning of the century. I joint his group in 2004. Dr. Bossart had already created soluble versions of HeV and NiV G glycoproteins and was spending time on a search for the viral receptors. It was anticipated that both viruses share common receptor indicated by immune response crossreactivity and anti-G antibody crossreactivity, including m102. Dr. Broder suggested me to join in the efforts of receptor discovery. We mainly attempted to co-immunoprecipitate the receptor with soluble G from lysates of permissive and non-permissive cells using coomassie stain, radiolabeling, and different modification. It later became clear that the S-agarose we used for co-IP with the S-tagged soluble G produces a non-specific band overlapping ephrin B2 and, thus, the band was considered false positive. When Matthew Bonaparte joint the team proposed to use gene chip technology developed at his university, SUNY. He sent lysates from permissive and non-permissive cells for analyses. Using the gene chip data he successfully narrowed the possibilities for the receptor down to low 20s. Farther on, narrowing couldn't be justified by the available data. Then, I proposed to purchase plasmids with the identified genes from Origene, transfect non-permissive cells, and find out which gene will render them permissive to HeV or NiV. Thus, ephrin B2 was discovered as receptor for HeV and NiV. Looking back into those data ephrin B3 shows a signal higher than background, but we have ignored it at that time, being excited of ephrin B2 identification.

A. Bonaparte MI, **Dimitrov AS**, Bossart KN, Crameri G, Mungall BA, Bishop KA, Choudhry V, Dimitrov DS, Wang LF, Eaton BT, Broder CC. From the Cover: Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. **PNAS** 2005; 102: 10652-10657.

B. Bossart KN, Crameri G, **Dimitrov AS**, Mungall BA, Feng YR, Patch JR, Choundhary A, Wang LF, Eaton BT, Broder CC. Receptor Binding, Fusion Inhibition, and Induction of Cross-Reactive Neutralizing Antibodies by a Soluble G Glycoprotein of Hendra Virus. **J. Virol.** 2005; 79: 6690-6702.

3. *Having a contribution in the discovery of m102.4 by identifying its parent m102 as a crossreactive neutralizer.*
Being in the laboratory of Dr. Broder I was using HeV and NiV mediated cell-cell fusion assays to study mutant G glycoproteins created by inactivating glycosylation sites. At that time, Dr. Zhu had found Fab binders to HeV-sG and asked me to test those for inhibitory activities in the cell-cell fusion assay. His Fabs showed different inhibitory potency to HeV and NiV with Fab m102, although not the best against either virus, showing a balanced inhibition to both viruses. Then, Dr. Zhu optimized m102 by in vitro maturation to m102.4.

A. Zhu Z, **Dimitrov AS**, Bossart KN, Crameri G, Bishop KA, Choudhry V, Mungall BA, Feng YR, Choudhary A, Zhang MY, Feng Y, Wang LF, Xiao X, Eaton BT, Broder CC, Dimitrov DS. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. **J. Virol.** 2006; 80: 891-9.

B. Zhu Z, **Dimitrov AS**, Chakraborti S, Dimitrova D, Xiao X, Broder CC, Dimitrov DS. Development of human monoclonal antibodies against diseases caused by emerging and biodefense-related viruses. **Expert Rev Anti Infect Ther**. 2006; 4: 57-66.

C. Zhu Z, Bossart KN, Bishop KA, Crameri G, **Dimitrov AS**, McEachern JA, Feng Y, Middleton D, Wang LF, Broder CC, and Dimitrov DS. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. **J Infect Dis** 2008; 197:846-853.

D. Prabakaran P, Zhu Z, Xiao X, Biragyn A, **Dimitrov AS**, Broder CC, and Dimitrov DS. Potent human monoclonal antibodies against SARS CoV, Nipah and Hendra viruses. **Expert Opin Biol Ther** 2009; 9:355-368.

4. *Editing a volume from the Methods of Molecular Biology series*

It was an exciting time finding appropriate chapter topics covering the discovery and preclinical development of therapeutic antibodies, then finding authors and reviewing and editing the chapters.

A. **Dimitrov AS**, Editor; Therapeutic antibodies. Methods and protocols, *Methods Mol. Biol.* v. 525; March 2009, Humana Press / Springer, New York, NY 10013. ISBN: 978-1-934115-92-3

B. **Dimitrov AS**. Methods in molecular biology. Therapeutic antibodies. Methods and protocols. Preface. Methods Mol Biol 2009; 525: vii-viii, xiii.

C. Prado I, Fouts TR, **Dimitrov AS**. (2009) Methods Mol Biol. v. 525 pp. 517-31. "*Neutralization of HIV by antibodies.*"

5. *Studying the mechanism of HIV entry. Applying mAbs to inhibit HIV entry*

While postdoc under the supervision of Dr. Blumenthal, we proposed that HIV-1 Env gp41 destabilizes target cell or its own membrane upon activation of gp120 by CD4 and respective coreceptor, CCR5 or CXCR4, complexes, resulting in fusion or viral inactivation, respectively. In relation to HIV-1 entry to cells we detected intermediates of the fusion process by using conformation specific mAb. Later, at Profectus I worked with anti-HIV antibodies as entry inhibitors. Results from the completed grant awards identify sweet epitopes that are simultaneously targeted by inhibitory antibodies show synergistic effect and an exceptional efficacy in vitro.

A. **Dimitrov AS**, Xiao X, Dimitrov DS, Blumenthal R. Early Intermediates in HIV-1 Envelope Glycoprotein-mediated Fusion Triggered by CD4 and Co-receptor Complexes. J. Biol. Chem. 2001; 276: 30335-30341.

B. **Dimitrov AS**, Rawat SS, Jiang S, Blumenthal R. The Role of the Fusion Peptide and Membrane-proximal Domain in HIV-1 Envelope Glycoprotein-mediated Membrane Fusion. Biochemistry 2003;42:14150-14158.

C. **Dimitrov AS**, Jacobs A, Finnegan CM, Stiegler G, Katinger H, Blumenthal R. Exposure of the membrane-proximal external region of HIV-1 gp41 in the course of HIV-1 envelope glycoprotein-mediated fusion. Biochemistry. 2007 Feb 6;46(5):1398-401.

D. **Dimitrov A**, Drug evaluation: Ibalizumab, a CD4-specific mAb to inhibit HIV-1 infection. Current Opinion in Investigational Drugs 2007 Aug; 8(8):653-61.

Project/Performance Site Location(s)

Project/Performance Site Primary Location

UEI: Z83LJK5NWBC1

Organization Name: Uniformed Services University of the Health Sciences

* Street1: 4301 Jones Bridge Road		Street2:
* City: Bethesda	County: Montgomery	* State: MD: Maryland
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 20814-4799
DUNS Number: 931258755	* Project/Performance Site Congressional District: MD-008	

Project/Performance Site Location 1

Organization Name: The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

UEI: UYLKBRENAPG5

* Street1: 6720-A Rockledge Drive		Street2: Suite 100
* City: Bethesda	County: Montgomery	* State: MD: Maryland
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 20817-0000
DUNS Number: 144676566	* Project/Performance Site Congressional District: MD-008	

	File Name	Mime Type
Additional Location(s)		

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Christopher	C.	Broder	Ph.D.

Position/Title: Professor and Chair
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Organization Name: Uniformed Services University of the Health Sciences
Division: School of Medicine
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*** Project Role:** Other (Specify)
Other Project Role Category: Site Principal Investigator

Degree Type: PhD
Degree Year: 1989

	File Name	Mime Type
***Attach Biographical Sketch**	Bios-ketch_Broder_TO_BE_EDITED1032988677.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

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	Brian		Schaefer	Ph.D.

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Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 20814-4799

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Credential, e.g., agency login: (b) (6) [redacted]

*** Project Role:** Other (Specify)
Other Project Role Category: Site Principal Investigator

Degree Type: Ph.D.
Degree Year: 1995

	File Name	Mime Type
***Attach Biographical Sketch**	Bios-ketch_RP3_Schaefer_v21032988678.pdf	application/pdf
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PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Moushimi		Amaya	Ph.D.

Position/Title: Research Assistant Professor
Department: Microbiology & Immunology

Organization Name: Uniformed Services University of the Health Sciences
Division: School of Medicine

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* State: MD: Maryland
Province:

* Country: USA: UNITED STATES
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*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type:
Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Amaya_v21032988679.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Antony		Dimitrov	Ph.D.

Position/Title: Senior Staff Scientist
Department: Microbiology & Immunology

Organization Name: The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.
Division: School of Medicine

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* State: MD: Maryland
Province:

* Country: USA: UNITED STATES
* Zip / Postal Code: 20814-4799

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Credential, e.g., agency login: (b) (6) [redacted]

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type:
Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Dimitrov_Broder_RP2_v11032988680.pdf	application/pdf
Attach Current & Pending Support		

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Additional Senior/Key Person Form Attachments

When submitting senior/key persons in excess of 8 individuals, please attach additional senior/key person forms here. Each additional form attached here, will provide you with the ability to identify another 8 individuals, up to a maximum of 4 attachments (32 people).

The means to obtain a supplementary form is provided here on this form, by the button below. In order to extract, fill, and attach each additional form, simply follow these steps:

- Select the "Select to Extract the R&R Additional Senior/Key Person Form" button, which appears below.
- Save the file using a descriptive name, that will help you remember the content of the supplemental form that you are creating. When assigning a name to the file, please remember to give it the extension ".xfd" (for example, "My_Senior_Key.xfd"). If you do not name your file with the ".xfd" extension you will be unable to open it later, using your PureEdge viewer software.
- Using the "Open Form" tool on your PureEdge viewer, open the new form that you have just saved.
- Enter your additional Senior/Key Person information in this supplemental form. It is essentially the same as the Senior/Key person form that you see in the main body of your application.
- When you have completed entering information in the supplemental form, save it and close it.
- Return to this "Additional Senior/Key Person Form Attachments" page.
- Attach the saved supplemental form, that you just filled in, to one of the blocks provided on this "attachments" form.

Important: Please attach additional Senior/Key Person forms, using the blocks below. Please remember that the files you attach must be Senior/Key Person Pure Edge forms, which were previously extracted using the process outlined above. Attaching any other type of file may result in the inability to submit your application to Grants.gov.

1) Please attach Attachment 1 ☐

2) Please attach Attachment 2 ☐

3) Please attach Attachment 3 ☐

4) Please attach Attachment 4 ☐

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)

Filename

MimeType

Additional Biographical Sketch(es) (Senior/Key Person)

Filename

MimeType

Additional Current and Pending Support(s)

Filename

MimeType

Tracking Number:

Research Strategy

A. Significance

The constant threat posed to global public health by emerging and reemerging pathogenic zoonotic viruses emphasizes the importance for pandemic preparedness by developing much needed countermeasures. The highly pathogenic HeV and NiV are the prototype members in the genus Henipavirus in the family Paramyxoviridae[1]. HeV was first discovered almost 30 years ago (1994), in Queensland, Australia where it was the causative agent of a severe and fatal respiratory disease among horses along with a human case fatality[1,2]. Whereas, in 1998-1999 in Malaysia and Singapore an outbreak of encephalitis in people and respiratory disease in pigs was due to NiV[3,4]. These viruses are capable of causing a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with fatality rates ranging from 40-100%[5,6]. As a result of this high lethality and lack of approved vaccines or antivirals, HeV and NiV are classified as Biosafety Level-4 (BSL-4) pathogens. NiV and henipaviral diseases are also included in the WHO's Blueprint List of Priority Pathogens[7], and NiV is among the Coalition for Epidemic Preparedness Innovations (CEPI) list of Priority Diseases needing urgent research and countermeasure development[8,9].

To date, HeV has spilled over in Australia on 63 occasions, the most recent in 2022[10]. In total HeV has caused the death or euthanasia of >100 horses, 2 HeV antibody positive and euthanized dogs, and 4 fatalities of 9 human cases[10,11]. The yearly outbreaks of NiV in Bangladesh and India have significant human case fatality rates that can reach up to 100%. Transmission in these outbreaks occur by humans consuming contaminated fruits or date palm sap and person-to-person transmission[12-15]. There have been >620 human cases of NiV infection with over 330 fatalities, 7 of them in 2023[16]. The henipavirus genus now includes nine other reported henipaviruses; the four viral isolates of CedV, Gamak virus, Daeryong virus, and Langya virus (LayV)[17-19] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiang virus, Melian virus, Denwin virus, and Angavokely virus (AngV)[20-23]. Pteropid fruit bats, known as flying foxes, are recognized natural reservoir hosts for NiV, HeV[24,25], CedV[18], GhV and AngV. One of these, CedV, has been shown to be nonpathogenic in several animals susceptible to HeV and NiV disease including mice, guinea pigs and ferrets[18,26] and African green monkeys (AGMs) (Geisbert, T.W. and Broder, C.C., unpublished). The remaining six reported henipaviruses are likely of rodent origins. One of these six, the isolate LayV, is associated with nonfatal febrile illness in humans[19].

Henipaviruses are membrane enveloped, single-stranded, negative sense RNA viruses that share a similar genomic makeup. There are two major membrane anchored glycoproteins involved in the virus entry: the attachment (G) glycoprotein that engages the host cell receptor and the fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F glycoproteins are the major antigenic targets for neutralizing antibodies and the main focus for vaccine and therapeutic antiviral strategies[27,28]. CedV is distinct from the other henipaviruses, in that the phosphoprotein (*P*) gene does not have an mRNA editing site to produce the interferon antagonist proteins V and W[18,29-31]. The nonpathogenic phenotype of CedV is most likely attributed to the lack of these nonstructural proteins. Based on genetic data, all identified henipaviruses, except CedV, can potentially express the V and W proteins. Furthermore, CedV is recognized as a BSL-2 restricted agent[32].

B. Innovation

There are multiple layers of innovation within RP2. **First**, the proposed development of henipavirus subunit vaccine candidates using an innovative microneedle array patch (MNP) technology that may be suitable for the incorporation of other undiscovered pathogenic henipaviruses in the future. **Second**, the construction of a rLayV reverse genetics system will be highly valuable in the henipavirus toolbox to study putative differences in pathogenesis and vaccine design between henipaviruses of bat and rodent origin. **Third**, generation of BSL-2 surrogate platforms that are based on rCedV and VSV will allow for a wide-variety of *in vitro* studies to investigate infection and host responses and to screen in a high throughput manner the neutralization potentials of immune sera and monoclonal antibodies all without the requirement for high containment. **Fourth**, considering that the LayV and AngV F and G proteins are functionally fusion active, the generation of viable rCedV chimeras bearing either LayV or AngV F and G glycoproteins is feasible.

C. Approach

Scientific Premise

The most extensively studied NiV/HeV vaccine is the soluble recombinant G glycoprotein of HeV (HeV-sG)[11,27,33] demonstrating complete protection against lethal challenge with NiV or HeV in multiple animal models[34-40]. It has shown complete protection of AGMs against NiV challenge performed a week post-immunization[41]. HeV-sG subunit is marketed by Zoetis as a horse vaccine since November 2012 and is the first commercialized vaccine against any BSL-4 pathogen. HeV-sG /alum intramuscular formulation is in clinical trials funded by CEPI as an anti-NiV vaccine.

We have been developing Cedar virus (CedV) as a new experimental tool set to study henipavirus host cell interactions, including ephrin receptor use, host cell tropism, and in the development of henipavirus countermeasures. Experiments with CedV can be conducted in a BSL-2 containment setting. The rCedV reverse genetics system[42,43] allowed us to develop a rCedV chimeric platform, where the CedV F and G glycoproteins are replaced with that of either NiV-Bangladesh (NiV-B) or with HeV, thus generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV). We have successfully rescued, grown and characterized the chimeric viruses with and without reporter genes[44]. The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G specific mAbs against these rCedV chimeras highly correlate with the potencies obtained for the same antibodies to neutralize authentic NiV-B and HeV in the plaque reduction neutralization tests (PRNTs). The GFP reporter chimeras were employed to developed a rapid, high-throughput, and quantitative fluorescence reduction neutralization test (FRNT). The antibody potencies to neutralize the reporter chimeras in the FRNT corelates with the respective potencies obtained in the PRNT[44].

rCedV chimeras have their most important application in an authentic henipavirus-based surrogate neutralization assay that is rapid, cost-effective, and can be utilized in BSL-2 containment. We believe that the rCedV reverse genetics system can serve as a plug and play platform for other emerging henipaviruses that may have pandemic potential. To accomplish this, we propose here to develop a variety of rCedV variants with reporter genes that will serve as important BSL-2 containment tools for the other RPs and Cores within this U19 ReVAMPP.

Scientific Rigor: The premise of our proposed studies is based on basic science discoveries and clinical observations. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistics and any required testing for multiple comparisons have been properly performed. rCedV laboratory manipulation guidelines and standard operating procedures under BSL-2 conditions were developed for the laboratory and staff, and all procedures were reviewed and approved by the Uniformed Services University Institutional Biosafety Committee in accordance with NIH guidelines.

Aim 1: Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV subunit vaccines.

Rationale and Preliminary Data. We intend to compare NiV vaccine immunogens (sG_{tet} and sF_{stb}), HeV (HeV-sF_{stb}) and a combination of NiV-sF_{stb}+HeV-sG to the clinical subunit HeV-sG. All immunogens will be delivered by microneedle array patches (MNPs). Immunogenicity will be initially tested in mice. The immune mouse sera will be evaluated *in vitro* for neutralizing potencies to either NiV or HeV. Selected top 2 neutralizing immunogens will be evaluated in ferrets and non-human primates (NHPs), AGMs, challenge studies with either NiV-B[45] or HeV-Redlands isolate from the 2008 outbreak. MNPs are promising resources to achieve systemic effects by transdermal delivery. They are a minimally invasive, painless system, which can bypass the stratum corneum and overcome the potential drawbacks of subcutaneous injections[46]. (See **Core C** application for details).

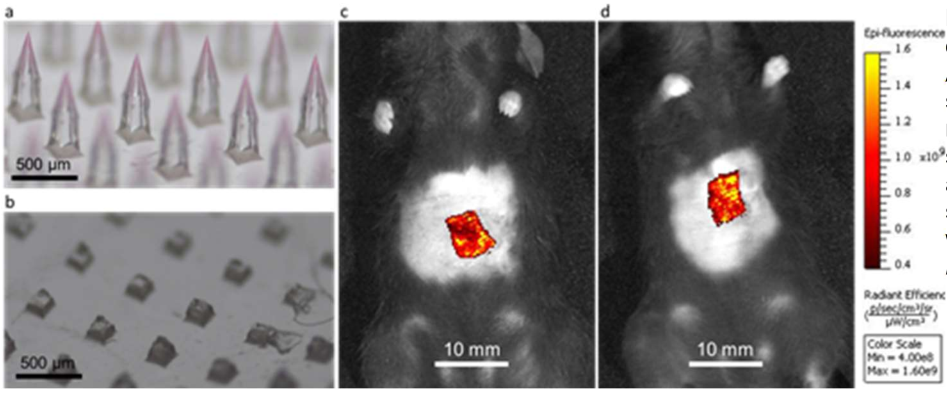


Figure 1: In vivo intracutaneous delivery efficacy of HeV-sG ± PCEP MNPs in mice. An optical stereomicroscopy image of HeV-sG AF555+PCEP CMC/Trehalose microneedles prior to (**a**) and after (**b**) *in vivo* skin application to mice. Effective administration of HeV-sG AF555 **(c)** and HeV-sG AF555+PCEP **(d)** to mouse skin *in vivo* with MNPs, captured by using a fluorescence *in vivo* imaging system (IVIS).

Manufacturing and characterization of HeV-sG ± PCEP MNPs. The technology to manufacture dissolvable MNPs incorporating HeV-sG antigen with or without polydi(sodium carboxylatoethylphenoxy) phosphazene (PCEP) as an adjuvant has been developed by **Core C**. During the development phase HeV-sG was labeled with fluorescent Alexa Fluor 555 dye, HeV-sG AF555, to visualize its penetration into skin. Spin-casting of carboxymethyl cellulose and trehalose was used to create MNPs incorporating HeV-sG AF555±PCEP into obelisk-shaped microneedles (**Figure 1a**). These HeV-sG AF555±PCEP MNPs were applied to the abdomen skin of C57BL/6 mice for 15 min and then removed. HeV-sG AF555±PCEP MNPs were imaged before and after *in vivo* skin applications by using an *in vivo* live animal fluorescent imaging system (IVIS Lumina XR). The acquired images were then post-processed using Living Image software (PerkinElmer). **Figure 1** shows a summary of the intracutaneous delivery capacity of HeV-sG±PCEP MNPs. Images of the HeV-sG AF555±PCEP MNPs before (**Figure 1a**) and after (**Figure 1b**) the patch application, confirm a nearly complete dissolution of the microneedles into the skin demonstrating that the vaccine was effectively deposited into the skin (**Figure 1c**: HeV-sG AF555 and **Figure 1d**: HeV-sG AF555+PCEP).

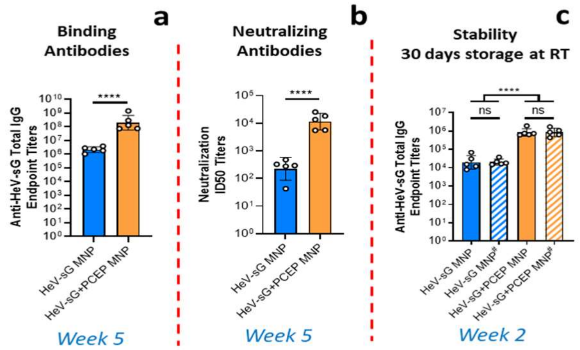


Figure 2: Efficacy of MNPs to induce humoral responses and initial MNP stability data. Mice were immunized using the prime/boost schedule. MNPs were applied to the abdominal skin of anesthetized C57BL/6 mice for 15 min, and then removed on D0 (prime) and D21 (boost). Endpoint binding titers **(a)** were determined by ELISA. Neutralizing ID_{50} titers **(b)** were obtained using the FRNT assay. Stability of the MNPs **(c)** measured as a decrease in the d14 endpoint binding titers when a 30-day-old MNP is used for immunization compared to the titers obtained with a fresh MNP.

Next, HeV-sG vaccine MNPs were tested to induce immune responses in mice. Groups of 5 mice were immunized on D0 (prime) and D21 (boost) with either non-adjuvanted or adjuvanted HeV-sG MNP. Pooled sera from D35 were tested by ELISA for total binding antibodies (endpoint binding titers), **Figure 2a**, and to neutralize henipavirus by using the recently developed FRNT reporter assay[44]. These preliminary data show that MNPs induce significantly higher immune responses. Other tests indicate the efficacy of the MNP is comparable or higher than that of the clinical HeV-sG with Alhydrogel vaccine formulation (see **Core C: Figure 4a**).

To test the stability, mice were immunized with fresh (group 1) and 30-day-old MNP (group 2) by using a single dose immunization. Mice were bled on D14 post immunization and the total IgGs were measured by endpoint antibody binding titers ELISA. **Figure 2c** shows no statistically significant differences in the antibody binding titers induced by the fresh and the 30-day-old MNPs.

Experimental Design.

Aim 1.1: Manufacturing of the MNPs. GLP-like HeV-sG is available in stock, and manufactured by Catalent Pharma Solutions, LLC. Catalent has developed the HeV-sG GLP manufacturing process for Profectus BioSciences, Inc. who led the HeV-sG subunit vaccine preclinical studies. A GLP-like lot manufactured by using the developed process was utilized in the rabbit GLP toxicology study with HeV-sG/Alum formulation, which is currently in clinical studies funded by CEPI. The remaining 3 immunogens will be prepared and delivered to us by **Genovac GmbH**, Freiburg, Germany. **Core C** will prepare fresh MNPs with each of NiV-sG_{tet}, NiV-sF_{stb}, HeV-sF_{stb}, HeV-sG+NiV-sF_{stb} and HeV-sG, all adjuvanted with PCEP. The technology for fabricating MNPs pioneered by Bediz et al.,[47] was developed and is described in detail in the **Core C** application. Based on the experience with fabricating HeV-sG MNP, we expect the technology to be plug and play applicable to the remaining 3 immunogens, also to the combo immunogen with minimal or no adjustments.

Aim 1.2: Mouse immunogenicity study. Six groups of 5 female C57BL/6 mice will be used in the mouse immunogenicity study, which will be executed by **Core C**. Group 1 will be immunized with NiV-sG_{tet}+PCEP formulation MNPs, group 2 – with NiV-sF_{stb}+PCEP, group 3 – with HeV-sF_{stb}+PCEP, group 4 – with HeV-sG+PCEP, group 5 – with combo HeV-sG+NiV-sFstb+PCEP, and group 6 will be naïve mice to read the background naïve mice immunity. Schematics of the immunization and bleeding schedule is given in **Figure 3**. The sera will be tested for binding to the respective immunogens and for cross-binding with the other 3 immunogens by ELISA. IgG1 and IgG2c titers will be recorded. The sera will also be tested at **USUHS** for neutralization of each of HeV and NiV-B by using the respective reporter rCedV chimeras – rCedV-HeV-GFP and rCedV-NiV-B-GFP in the FRNT assay[44]. Cellular immune responses will also be evaluated at the **University of Pittsburg** as described in the **Core C** application (within Core C Aim 2).

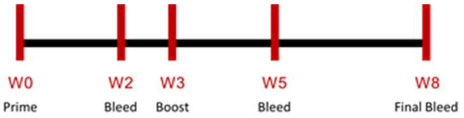


Figure 3. Immunization and bleeding schedule for the mouse immunogenicity study.

Aim 1.3: Efficacy in ferrets. The 2 immunogens with the best immune responses on W2 and W5 combined, *Imun1* and *Imun2*, from the mouse study, will be tested in ferret challenge protection studies executed at **UTMB**. Four vaccine groups and one control group of 6 ferrets each will be challenged with lethal doses of either NiV-B or HeV Redlands, 5000 $TCID_{50}$, in year 2 of the project. Groups 1 and 3 will be immunized with *Imun1*+PCEP MNPs, groups 2 and 4 – with *Imun2*+PCEP and group 5 will be naïve nonvaccinated ferrets. Groups 1 and 2 will be immunized on D0 and D21 (prime/boost schedule), while groups 3 and 4 will be immunized once, on D0. Immunization, challenge and blood and swab sampling schedules are diagramed in **Figure 4**. All protected animals and survived controls will be euthanized on day 21 post-infection (end of study) and tested for cultivatable virus and viral genome in the lung and brain tissue in addition to the blood samples and swabs.

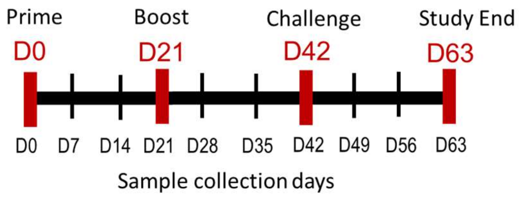


Figure 4. Expected schedule to immunize ferrets and AGMs in the planned efficacy studies. Blood and swabs, oral and rectal, samples will be taken weekly, on days 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63. Single dose groups do not receive boost immunization on D21.

Aim 1.4: Efficacy in AGM. This challenge study will be performed in year 3 of the project at **UTMB**. The immunogen that provides the best cross-protection in the ferret studies will be tested to protect AGMs against either NiV or HeV. Fresh MNPs will be fabricated for this study. Two groups of 3 monkeys per challenge virus, total 4 groups of 3 AGMs each, will be vaccinated by applying the MNP (groups 1 and 2 will be immunized on D0 and D21 while groups 3 and 4 will be immunized only on D0, boost will be skipped). One control per virus will be naïve nonvaccinated. Time schedule will mimick the ferret study schedule in **Figure 4**. Groups 1 and 3 will be challenged with NiV-B, groups 2 and 4 will be challenged with HeV Redlands.

Aim 1.5: Stability Studies will be initiated starting with the mouse immunogenicity studies and finishing at the AGM challenge studies end. Each quarter, a group of 5 mice will be single dose immunized and the SNT titers will be evaluated 2 weeks post immunization by using the FRNT assay[44]. We assume that the MNA patches are stable if the average SNT titers have fallen less than 33%, i.e., from relative 100 the SNT titer stays above 67. Additional stability testing will be initiated with the freshly manufactured MNP for the AGM challenge study.

Bio-sample collection and RNA test. Swabs (oral and rectal), blood and tissue samples (lung, brain) will be tested for viral RNA by TaqMan PCR performed by using the primer sets and probes[35].

Virus isolation will only be attempted from specimens positive for NiV or HeV genome by PCR. Virus titration will be performed by plaque assay on Vero cells. The limit for detection will be 25 pfu/ml.

Expected results, potential pitfalls and alternatives. We expect to obtain sterile protection in AGMs. There is a minimal risk of not finding any protecting immunogen given the complete protection provided by a single dose HeV-sG subunit vaccine[41]. The foreseen problems are already reflected in the experimental design. The team is sufficiently experienced to handle and resolve any possible unforeseen issues and pitfalls.

<u>Aim 2:</u> Rescue Langya virus and construct LayV and AngV glycoprotein bearing reporter virus tools.

Rationale and Preliminary Data: Negative stain electron microscopy (EM) was performed on LayV F and G glycoproteins. The 2D (**Figure 5a**) and 3D classifications (**Figure 5b**) of LayV F and G revealed distinct trimer and tetramer assembly, respectively. In addition, using LayV sF and LayV sG mAbs that we generated at USUHS, we performed ELISAs and demonstrated that there was a dose dependent increase in LayV sF (**Figure 5c**) and LayV sG (**Figure 5d**) binding. This data forms the premise for rescuing a rCedV bearing LayV F and G glycoproteins. We will design and construct a full-length recombinant LayV that encodes a green fluorescent protein (GFP). The LayV F and G coding sequences will be based on the LayV isolate SDQD_H1801 (GenBank: OM101125.1). The GenBank accession numbers for LayV F and LayV G are UUV47205.1 and UUV47206.1, respectively. The authentic, replication competent henipavirus platform, rCedV is an innovative approach that can be utilized at BSL-2. We will design, construct and rescue a new rCedV chimera that encodes the envelope glycoproteins (fusion (F) and attachment (G) glycoproteins) of LayV, i.e., a rCedV-LayV reporter virus encoding GFP. We will characterize it using cell-based infection tropism and growth kinetic assays.

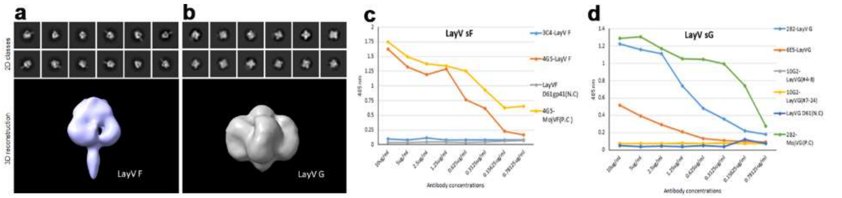


Figure 5. Negative stain EM analysis of LayV envelope glycoproteins. 2D and 3D classifications of LayV F trimers (**a**) and LayV G tetramers (**b**). ELISA indicating cross-reactivity between Mojiang virus (MojV) F mAb (**c**, yellow) with LayV sF and MojV G mAb (**d**, green) with LayV sG.

We utilized an established quantitative fusion assay based on a dual-split-luciferase reporter assay[42,43,48,49] to determine functionality of Angavokely virus (AngV) envelope glycoproteins (F and G glycoproteins). In this assay, content mixing between a virus-infected effector cell or a cell that expresses the F and G glycoproteins and a target cell expressing or not the known henipavirus receptors allows the two halves of Renilla luciferase to functionally interact and produce a luciferase signal that can be measured[43] (**Figure 6a**).

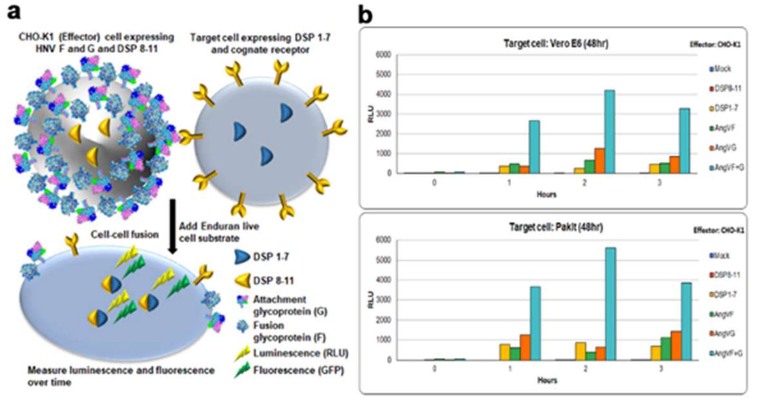


Figure 6. AngV F and G cell-cell fusion using a dual split luciferase. A schematic representation of the dual split luciferase cell-cell fusion assay (**a**). A quantitative dual split luciferase cell-cell fusion assay with AngV F and G plasmid constructs (**b**).

High levels of fusion were observed in Vero E6 cells and the bat kidney cell line, PaKiT, indicating that the AngV glycoproteins are functional and can mediate fusion in susceptible cells (**Figure 6b**). Based on this data, the rescue of a rCedV-AngV reporter virus encoding a GFP and a luciferase protein (Luc) is feasible. We will characterize these viruses using cell-based infection tropism and growth kinetics assays. In other projects in our lab, we have previously used reverse genetics to generate several types of replication competent VSV-GFP reporter viruses including those encoding Australian bat lyssavirus (ABLV) G glycoproteins (VSV-ABLV G-GFP)[50,51], as well as employed reverse genetics to rescue authentic ABLV and various reporter gene-encoding viruses[52,53]. Using a similar strategy, we will also construct a recombinant replication competent VSV encoding the AngV F and G glycoproteins. We will generate reporter versions encoding either GFP or Luc.

Experimental Design.

Aim 2.1: Design and construct a recombinant Langya virus reporter virus. Figure 7 diagrams each of the proposed virus constructs that will be generated. The genome design, length and position of the green fluorescent protein gene are shown, and the experimental plans are described below. LayV helper plasmids for the N, P, and L genes will be synthesized. To construct the rLayV-GFP antigenome plasmid, large gene fragments of LayV will be synthesized and sequentially cloned into pOLTV5$_{opt}$ (**Figure 7a**). Unique restriction sites will be chosen to facilitate ligation of the LayV DNA fragments and to ensure adherence to the genomic “rule of six”. All cloning will be performed with *E.coli* Stbl2 cells. The fully sequenced plasmids will be transferred to **Dr. Thomas Geisbert at UTMB**. Virus rescue will be conducted in collaboration with **Core E** at UTMB. In addition, rLayV will be examined in vivo by **Core E**.

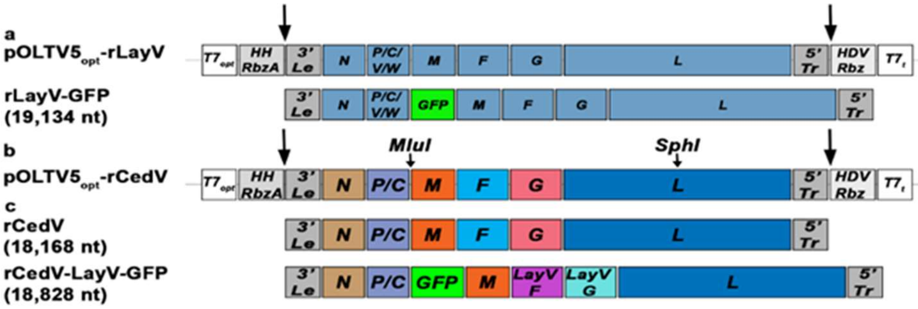


Figure 7. Schematic representation of recombinant reporter viruses. The pOLTV5$_{opt}$-rLayV plasmid (**a** top). The arrows indicate regions of self-cleavage. The rLayV-GFP genome (**b** bottom). The pOLTV5$_{opt}$-rCedV plasmid (**c**). The long arrows indicate regions of self-cleavage. Shown are the unique restriction sites MluI and SphI that will be used to construct rCedV-LayV-GFP. (**c**) The rCedV-LayV-GFP genome.

Aim 2.2: Construct, rescue and characterize a recombinant Cedar reporter virus encoding Langya virus envelope glycoproteins.

Generation of rCedV encoding LayV envelope glycoproteins. rCedV-LayV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the LayV F and G coding sequences. The CedV non-coding intergenic regions are retained to adhere to the genomic “rule of six”. Unique restriction sites, will facilitate the insertion of the LayV F and G gene cassette into the genome clone (pOLTV5$_{opt}$-rCedV) (**Figure 7b**) generating rCedV-LayV-GFP (**Figure 7c**). All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-LayV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (henipavirus CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. When maximal syncytia and GFP is observed (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[42,43,54].

In vitro characterization of rCedV-LayV-GFP. The growth kinetics of rCedV-LayV-GFP compared to rCedV or rCedV-NiV/HeV chimeras will be assessed by infecting Vero cells with MOIs of 0.01, 0.1 and 1 and progeny virus recovered in 24hr intervals over a 72hr period and titered. In parallel experiments, infected cells will also be imaged for fluorescence and syncytia (henipavirus CPE). **Figure 8** illustrates the syncytia (indicated by the yellow arrows) formed in Vero E6 cells infected with either rCedV-NiV-B-GFP or rCedV-HeV-GFP chimeras. The rCedV-LayV-GFP neutralization assays will be conducted by FRNT. We have also already generated several murine mAbs to LayV F and G (3C4-LY and 4G5-LY to LayV F; and 2B2-LY and 6E5-LY to LayV F) and these will be used in neutralization assays. In addition, we will develop rabbit sera to LayV N, P and L proteins.

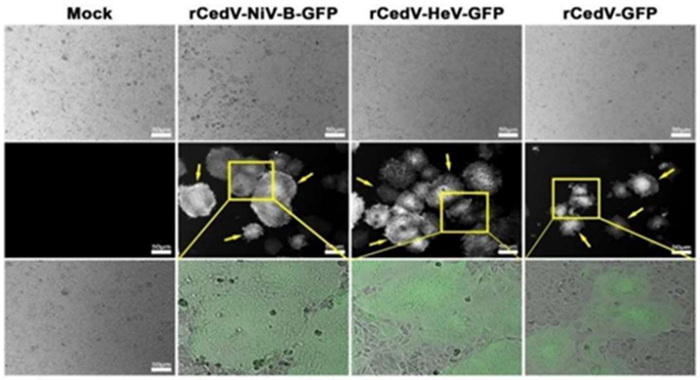


Figure 8. Syncytia induced by rCedV expressing NiV-B or HeV envelope glycoproteins. Vero E6 cells were uninfected (Mock) or infected with either rCedV-NiV-B-GFP, rCedV-HeV-GFP, or rCedV-GFP. All images were taken 24hrs post infection. The respective zoomed-in fluorescence images (3rd row) are regions from the yellow boxes. Arrows indicate giant multinucleated cells (syncytia). Representative images from three independent experiments are shown. Scale bar, 50 µm.

Aim 2.3: Construct, rescue and characterize recombinant reporter viruses encoding Angavokely virus envelope glycoproteins.

Generation of rCedV encoding AngV envelope glycoproteins. Figure 9 diagrams each of the proposed virus constructs that will be prepared. The genome designs, lengths and position of the *GFP* and *Luc* reporter genes are also shown, and the methodology and experimental plans are described below. rCedV-AngV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the AngV F and G coding sequences. The AngV F and G coding sequences will be based on the GenBank: ON613535.1 sequence. The GenBank accession numbers for AngV F and AngV G are UVG43988.1 and UVG43989.1, respectively. The CedV non-coding intergenic regions are retained to adhere to the genomic "rule of six". Unique restriction sites, will facilitate the insertion of the AngV F and G gene cassette into the rCedV antigenome plasmid (**Figure 9a**) generating rCedV-AngV-GFP (**Figure 9b**). The rCedV-AngV-Luc clone will be produced by swapping the reporter gene coding sequences. All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-AngV-GFP or pOLTV5$_{opt}$-rCedV-AngV-Luc antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (henipavirus CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. When maximal syncytia and GFP is observed (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[42,43,54].

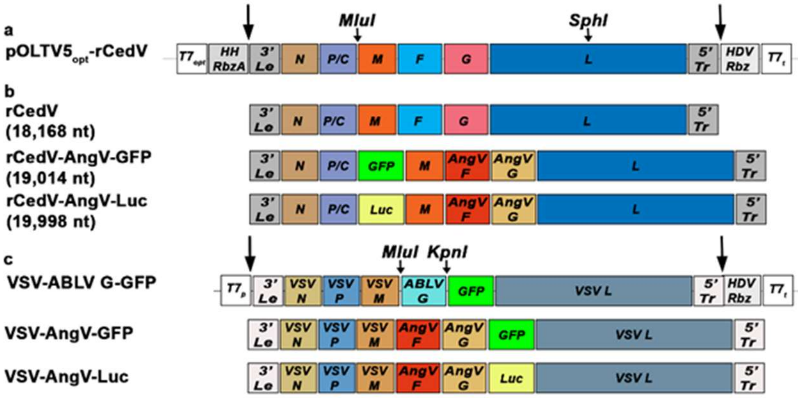


Figure 9. Schematic representation of the AngV chimeric reporter viruses. The pOLTV5$_{opt}$-rCedV plasmid (**a**). Shown are the unique restriction sites MluI and SphI that will be used to construct rCedV-AngV-GFP and rCedV-AngV-Luc. The rCedV-AngV-GFP and rCedV-AngV-Luc genome (**b**). Shown are the unique restriction sites MluI and KpnI that will be used to construct VSV-AngV-GFP and VSV-AngV-Luc (**c**). The long arrows indicate regions of self-cleavage.

Generation of a VSV encoding AngV envelope glycoproteins. We will also construct reporter gene encoding versions of a replication competent VSV encoding the AngV F and G glycoproteins (GFP and Luc) (**Figure 9c**).

A large gene cassette comprising VSV untranslated intergenic regions flanking the AngV F and G coding sequences will be synthesized. Unique restriction enzyme sites, will facilitate the insertion of the AngV F and G gene cassette into the VSV backbone plasmid to generate VSV-AngV-GFP (**Figure 9c**). The VSV-AngV-Luc clone will be produced by swapping the reporter gene coding sequences. Cloning procedures are performed in *E. coli* Stbl2 cells and all plasmid clones verified by sequencing. Rescue of VSV-AngV-GFP and VSV-AngV-Luc, will be performed in BSR-T7/5 cells, in 12-well plates, transfected with helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-AngV-GFP or VSV-AngV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus is performed using HEK293T cells (6-well plates) by inoculation with the supernatant from step 1. Typically, when GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above and the virus stocks stored at -80°C. Larger viral stocks are prepared in T-150 flasks in HEK293T cells, and harvested virus supernatants are purified by buffered sucrose cushion centrifugation and virus pellets resuspended in 10% sucrose/TNE buffer, and small volume single use aliquots stored at -80°C. All virus stocks are deep sequenced. Virus stocks (GFP) are titered (infectious units/ml (IU/ml)) by serial dilution on HEK293T cells in 96-well black walled plates. At 24hrs post-infection plates are fixed with 4% formaldehyde, washed 3x with diH$_2$0 and imaged (fluorescence) with a CTL S6 analyzer. Luciferase encoding VSV stocks are titered by plaque assay[55].

Characterization of the recombinant reporter viruses in cell-based infection assays: The replication kinetics of the rCedV-based and VSV-based AngV F/G encoding viruses will be assayed by Vero cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in several 24hr intervals and titered. rCedV-AngV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Similarly, VSV-AngV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G[51]. The rCedV-AngV-GFP and VSV-AngV-GFP assays will be conducted by FRNTs and rCedV-AngV-Luc and VSV-AngV-Luc in luciferase assays.

Expected results, potential pitfalls and alternatives. We expect that rCedV-LayV-GFP and rCedV-AngV reporter viruses will be readily rescued and viable. In addition, we expect that these chimeric viruses will induce syncytia and replicate to high titers. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. In the event that the rescue of rCedV chimeric viruses are not viable, multiple strategies may be examined. 1) test different ratios of helper plasmids and antigenome plasmid; 2) perform rescue in different cell lines; 3) construct alternate versions of the LayV and/or AngV F and G genes within the antigenome plasmids, such as, fuse the LayV and/or AngV F and G ectodomains with the transmembrane and/or cytoplasmic tail domains of the corresponding CedV F and G elements which may facilitate virus particle formation and membrane fusion activity.

Aim 3: Evaluate HNV-specific antibodies and nanobodies developed by the Center's RPs and Core D.

Rationale and Preliminary Data. We established a surrogate neutralization assay for the pathogenic henipaviruses, NiV and HeV using the rCedV platform[44]. This rCedV chimeric system can serve as surrogate viruses for authentic NiV and HeV in neutralization assays without the requirement for BSL-4 containment. Neutralization values of well-characterized NiV/HeV cross-reactive neutralizing mAbs highly correlated between rCedV-NiV-B-GFP and rCedV-HeV-GFP and authentic NiV-B and HeV at BSL-4 when tested by conventional PRNT[44]. The Pearson correlation analysis between the rCedV chimeras BSL-2 PRNT and the NiV-B or HeV BSL-4 PRNT is shown in **Figure 10a**. We further developed the rCedV-NiV-B-GFP and rCedV-HeV-GFP neutralization assay into a high-throughput and quantitative assay that is based on the reduction of GFP foci, a FRNT[44]. The Pearson correlation analysis between the chimeras PRNT and FRNT is shown in **Figure 10b**. Taken together, these data demonstrate that rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeric viruses are an ideal set of suitable surrogate viruses for authentic NiV-B and HeV for conducting a rapid FRNT-based assay for assessing NiV and HeV antibody neutralization.

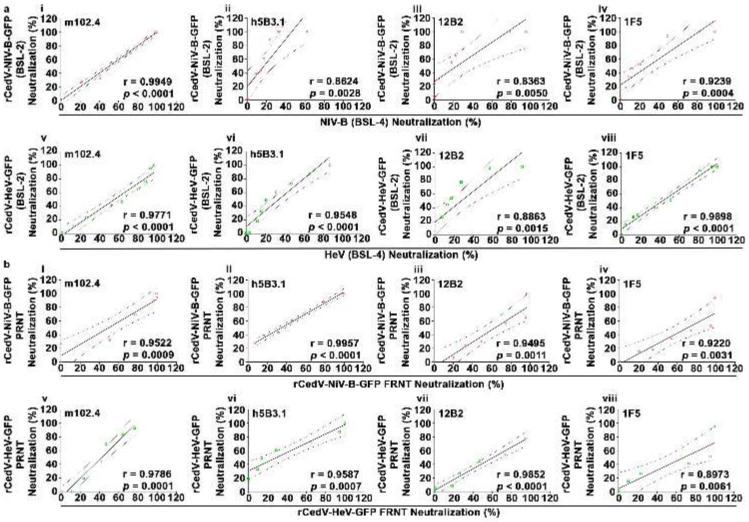


Figure 10. Pearson correlation analysis of rCedV chimeras. Pearson correlation analysis of PRNT neutralization (%) values of rCedV-NiV-B-GFP versus NiV-B (**a**, **i-iv**) and rCedV-HeV-GFP versus HeV (**a**, **v-viii**). Pearson correlation analysis from PRNTs (y-axes) and FRNTs (x-axes) of rCedV-NiV-B-GFP (**b, i-iv**) and rCedV-HeV-GFP (**b, v-viii**). Neutralization values with mAbs m102.4, h5B3.1, 12B2 or 1F5 are shown. The Pearson correlation coefficient 'r,' p-value (two-tailed), linear regression line (solid lines), and 95% confidence intervals (dashed lines).

Experimental Design.
Here, using the newly developed tools (rCedV-LayV-GFP, rCedV-AngV-GFP, VSV-AngV-GFP and VSV-AngV-Luc) we will assess for neutralizing activity, a variety of henipavirus specific mAbs and/or sera from henipavirus immunized mice that will be generated by **Core D**. The FRNT is high throughput and performed in 96-well plates to establish a 7-point dose response. Serially diluted immune sera or mAbs are mixed with either rCedV-NiV-B-GFP or rCedV-HeV-GFP for 2hrs. The virus-mAb or virus-sera mixtures are then added in triplicate to confluent pre-seeded Vero 76 cells in black-walled clear bottom 96-well plates and incubated for an additional 24hrs. Plates are then fixed and imaged and fluorescent foci counted using the CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) will be determined as the mAb concentration or serum dilution at which there was a 50% reduction in fluorescent foci versus untreated control wells. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9.
Expected results, potential pitfalls and alternatives. We do not anticipate any unexpected limitations in assessing the neutralization potential of antibodies and sera generated from other research projects and/or cores as we have successfully demonstrated that the rCedV-GFP chimeric FRNT platform can serve as a surrogate BSL-2 neutralization assay that is high throughput with a fast turnaround time.

Aim 4: Validate PPZ-MNP platform with another paramyxovirus-specific antigen in Phase 2.

At completion of Phase I we will have i) a procedure to fabricate PPZ adjuvanted MNP using sG, sF or a combination of both viral glycoproteins; ii) generated rLayV with an associated animal model in collaboration with **Core E** with reporter virus construct (rCedV-LayV-GFP); and iii) generated reporter viruses AngV (rCedV-AngV-GFP, VSV-AngV-GFP and VSV-AngV-Luc). Here, in Phase II we propose to test the hypothesis that the PPZ-MNP vaccine platform technology can be adapted and transferred as a 'plug and play' strategy for other paramyxoviruses, namely the divergent henipaviruses LayV and AngV. The major goal rests on the rescue of LayV and establishing an infection/disease model of this most recently discovered pathogenic, zoonotic, HNV. Nevertheless, if rescue of rLayV with an associated animal model is not achieved, we will still be able to test the the MNP approach as proof-of-concept to the extent of generating robust immune responses in animals and testing neutralizing antibody responses using both LayV and AngV reporter virus systems.

PPZ-MNP manufacture. The fabrication of the MNPs by **Core C** will be prepared for both the F and G viral glycoproteins: LayV-sG, LayV-sF, AngV-sG and AngV-sF depending on the data and outcomes, and vaccine down-selection in Phase I we may also include a combination of sG and sF. Vaccine antigens will have been prepared by within **Core D**. It will remain important to evaluate both the attachment and fusion glycoprotein immunogens for LayV and AngV in Phase II. Although the preponderance of data to date for the pathogenic prototype HNVs (NiV/HeV) indicates that the attachment glycoprotein (G) alone can serve as an effective vaccine immunogen, this is presently unknown for these two divergent henipaviruses, so it remains prudent to test both sF and sG in this regard.

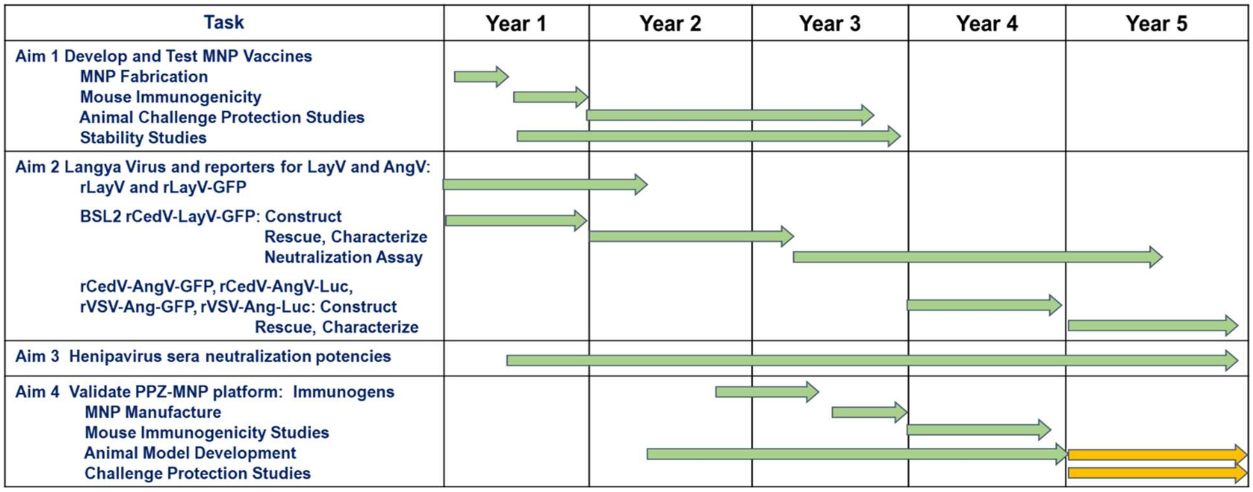
Immunogenicity. Groups of 5 mice will be utilized for each of the chosen immunogens and one control nonimmunized group will serve to measure the naïve mice background immunity. Sera will be drawn as scheduled in **Figure 3**. ELISA titers of sera to vaccine immunogens will be evaluated by **Core C** and sera will be evaluated for virus neutralization with newly developed reporter viruses. Cellular immune responses will be assessed as described in **Aim 1**.

Animal challenge studies. The experimental design for these studies will be adjusted once the animal model has been developed. The basic design will follow those for ferrets and AGMs described in **Aim 1**. The immunogen will be chosen based on the results from the neutralization tests described above.

Expected results, potential pitfalls and alternatives.

We fully anticipate that LayV and AngV recombinant glycoprotein based MNPs will be possible because we have already produced native-like sG and sF ectodomains of each of these viruses. The immunizing mice with the MNPs using the recombinant LayV and AngV recombinant glycoprotein immunogens are likely to be uncomplicated, we will be able to rapidly assess immune mouse sera using newly generated reporter viruses. The new data will also determine whether the sG or sF is the immunogen of choice for eliciting the higher virus neutralization titer. In the absence of rescue of authentic recombinant LayV (rLayV) and establishing an animal model capable of measuring virus infection, immune responses, and/or pathogenesis, we expect at a minimum the establishment of an ability to measure virus infection in cell-based assays and its inhibition, using either the generated reporter viruses or a cell-cell fusion surrogate assay. The results obtained from the mouse immunogenicity studies, together with the virus neutralization assays will provide a strong foundation to support the advancement of a potential anti-LayV and/or anti-AngV vaccine MNPs.

Project Milestones and Timeline:



References

1 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33 (1995).

2 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

3 Chua, K. B. Nipah virus outbreak in Malaysia. *J Clin Virol* **26**, 265-275 (2003).

4 Chua, K. B. *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**, 1257-1259 (1999). https://doi.org:10.1016/S0140-6736(99)04299-3 [doi]

5 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res* **100**, 8-13 (2013). https://doi.org:10.1016/j.antiviral.2013.06.012

6 Geisbert, T. W., Feldmann, H. & Broder, C. C. in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 153-177 (Springer, Berlin, Heidelberg, 2012).

7 Mehand, M. S., Al-Shorbaji, F., Millett, P. & Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral Res* **159**, 63-67 (2018).

8 *Priority Diseases 2021*, <https://cepi.net/research_dev/priority-diseases/> (2021).

9 Brende, B. *et al.* CEPI-a new global R&D organisation for epidemic preparedness and response. *Lancet* **389**, 233-235 (2017). https://doi.org:10.1016/s0140-6736(17)30131-9

10 Anonymous. Hendra virus - Australia (02): (QL) horse. Report No. 20220710.8704331, (International Society for Infectious Diseases, 2022).

11 Broder, C. C., Weir, D. L. & Reid, P. A. Hendra virus and Nipah virus animal vaccines. *Vaccine* **34**, 3525-3534 (2016). https://doi.org:10.1016/j.vaccine.2016.03.075

12 Homaira, N. *et al.* Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect* **138**, 1630-1636 (2010). https://doi.org:10.1017/S0950268810000695 [doi]

13 Homaira, N. *et al.* Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One* **5**, e13570 (2010). https://doi.org:10.1371/journal.pone.0013570 [doi]

14 Luby, S. P. *et al.* Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis* **15**, 1229-1235 (2009).

15 Rahman, M. A. *et al.* Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector borne and zoonotic diseases (Larchmont, N.Y.)* **12**, 65-72 (2012). https://doi.org:10.1089/vbz.2011.0656

16 Anonymous. Nipah virus - Bangladesh (05). Report No. 20230208.8708217, (International Society for Infectious Diseases, 2023).

17 Lee, S. H. *et al.* Discovery and Genetic Characterization of Novel Paramyxoviruses Related to the Genus Henipavirus in Crocidura Species in the Republic of Korea. *Viruses* **13** (2021). https://doi.org:10.3390/v13102020

18 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836 (2012). https://doi.org:10.1371/journal.ppat.1002836

19 Zhang, X. A. *et al.* A Zoonotic Henipavirus in Febrile Patients in China. *N Engl J Med* **387**, 470-472 (2022). https://doi.org:10.1056/NEJMc2202705

20 Drexler, J. F. *et al.* Henipavirus RNA in African bats. *PLoS One* **4**, e6367 (2009). https://doi.org:10.1371/journal.pone.0006367 [doi]

21 Madera, S. *et al.* Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J Virol* **96**, e0092122 (2022). https://doi.org:10.1128/jvi.00921-22

22 Vanmechelen, B. *et al.* The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol* **8**, veac061 (2022).

23 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066 (2014). https://doi.org:10.3201/eid2006.131022

24 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses and public health* **60**, 69-83 (2013). https://doi.org:10.1111/j.1863-2378.2012.01501.x

25 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951 (2011). https://doi.org:10.4269/ajtmh.2011.10-0567 [doi]

26 Schountz, T. *et al.* Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **11** (2019). https://doi.org:10.3390/v11030291

27 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473 (2020). https://doi.org:10.1146/annurev-virology-021920-113833

28 Broder, C. C. *et al.* in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 197-223 (Springer, Berlin, Heidelberg, 2012).

29 Basler, C. F. in *Curr Top Microbiol Immunol* Vol. 359 (eds B. Lee & P. Rota) 123-152 (Springer, Berlin, Heidelberg, 2012).

30 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral Res* **124**, 69-76 (2015). https://doi.org:10.1016/j.antiviral.2015.09.017

31 Marsh, G. A. & Wang, L. F. Hendra and Nipah viruses: why are they so deadly? *Curr Opin Virol* **2**, 242-247 (2012). https://doi.org:10.1016/j.coviro.2012.03.006

32 NIH, C. a. (ed U.S. Department of Health and Human Services) 248-250 (US Government Printing Office, Washington, 2020).

33 Bossart, K. N. *et al.* Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol* **79**, 6690-6702 (2005).

34 Bossart, K. N. *et al.* A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Science translational medicine* **4**, 146ra107 (2012). https://doi.org:10.1126/scitranslmed.3004241

35 McEachern, J. A. *et al.* A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* **26**, 3842-3852 (2008). https://doi.org:10.1016/j.vaccine.2008.05.016

36 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379 (2014). https://doi.org:10.3201/eid2003.131159

37 Mire, C. E. *et al.* A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol* **88**, 4624-4631 (2014). https://doi.org:10.1128/jvi.00005-14

38 Mungall, B. A. *et al.* Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol* **80**, 12293-12302 (2006).

39 Pallister, J. *et al.* A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine* **29**, 5623-5630 (2011). https://doi.org:10.1016/j.vaccine.2011.06.015

40 Pallister, J. A. *et al.* Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J* **10**, 237 (2013). https://doi.org:10.1186/1743-422x-10-237

41 Geisbert, T. W. *et al.* A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. *NPJ Vaccines* **6**, 23 (2021).

42 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084 (2021). https://doi.org:10.1016/j.antiviral.2021.105084

43 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56 (2018). https://doi.org:10.1186/s12985-018-0964-0

44 Amaya, M. *et al.* A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses* **15** (2023). https://doi.org:10.3390/v15051077

45 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916 (2016). https://doi.org:10.1038/srep30916

46 Guillot, A. J. *et al.* Microneedle-Based Delivery: An Overview of Current Applications and Trends. *Pharmaceutics* **12** (2020). https://doi.org:10.3390/pharmaceutics12060569

47 Bediz, B. *et al.* Dissolvable microneedle arrays for intradermal delivery of biologics: fabrication and application. *Pharm Res* **31**, 117-135 (2014). https://doi.org:10.1007/s11095-013-1137-x

48 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715 (2019). https://doi.org:10.1073/pnas.1911773116

49 Navaratnarajah, C. K., Rosemarie, Q. & Cattaneo, R. A Structurally Unresolved Head Segment of Defined Length Favors Proper Measles Virus Hemagglutinin Tetramerization and Efficient Membrane Fusion Triggering. *J Virol* **90**, 68-75 (2016). https://doi.org:10.1128/JVI.02253-15

50 Weir, D. L., Laing, E. D., Smith, I. L., Wang, L. F. & Broder, C. C. Host cell virus entry mediated by Australian bat lyssavirus G envelope glycoprotein occurs through a clathrin-mediated endocytic pathway that requires actin and Rab5. *Virol J* **11**, 40 (2014). https://doi.org:10.1186/1743-422X-11-40

51 Weir, D. L., Smith, I. L., Bossart, K. N., Wang, L. F. & Broder, C. C. Host cell tropism mediated by Australian bat lyssavirus envelope glycoproteins. *Virology* **444**, 21-30 (2013). https://doi.org:10.1016/j.virol.2013.06.016

52 Laing, E. D. *et al.* Enhanced Autophagy Contributes to Reduced Viral Infection in Black Flying Fox Cells. *Viruses* **11** (2019). https://doi.org:10.3390/v11030260

53 Mastraccio, K. E. *et al.* Establishment of a longitudinal pre-clinical model of lyssavirus infection. *J Virol Methods* **281**, 113882 (2020). https://doi.org:10.1016/j.jviromet.2020.113882

54 Weingartl, H. M. *et al.* Recombinant nipah virus vaccines protect pigs against challenge. *J Virol* **80**, 7929-7938 (2006). https://doi.org:10.1128/JVI.00263-06

55 Abdelmageed, A. A. & Ferran, M. C. The Propagation, Quantification, and Storage of Vesicular Stomatitis Virus. *Curr Protoc Microbiol* **58**, e110 (2020). https://doi.org:10.1002/cpmc.110

TITLE: Vaccines and antibodies to henipavirus
Funding announcement: RFA-AI-23-020
Funding Title: Research and Development of Vaccines and Monoclonal Antibodies for Pandemic
Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picronaviridae
PRINCIPAL INVESTIGATOR: Christopher Broder, Ph.D., Site Principal Investigator
Moushimi Amaya, Ph.D and Antony Dimitrov, Ph.D., Co-investigators
Prime PI: Thomas Geisbert, Ph.D., UTMB
Period of Performance: March 1, 2024 - February 28, 2029

Project Summary/Abstract

Pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. Among these are the two emerged zoonotic paramyxoviruses: the ‘prototype’ henipaviruses (HNVs); Nipah virus (NiV) and Hendra virus (HeV), discovered in the mid to late 1990s. The prototype HNVs have a uniquely broad tropism capable of infecting 18 species across 6 orders of mammals using the conserved ephrin-B2 and -B3 ligands as entry receptors. NiV and HeV infection often results in a systemic and fatal respiratory and/or neurological disease in multiple mammalian species including humans. NiV and henipaviral disease are also included in the WHO List of Priority Pathogens, and they are significant biothreats to humans and livestock in South and South East Asia and Australia. HNVs have two membrane glycoproteins: the attachment glycoprotein (G) and the fusion glycoprotein (F) that are the major targets of neutralizing antibodies and the focus of all vaccine and antiviral monoclonal antibody (mAb) strategies. The most extensively studied HNV vaccine is a soluble recombinant form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including nonhuman primates (NHPs). With no licensed vaccines or treatments available for human use, this project will use the prototype HNVs as a model to develop, test, and translate a novel vaccination platform (a thermostable, needle-free, adaptable, and widely deployable polyphosphazene (PPZ) adjuvanted dissolvable microneedle patch (MNP)). Preliminary data demonstrates potent immunogenicity of a PPZ-MNP-HeV-sG vaccine in mice, inducing robust antigen-specific HNV cross-neutralizing antibody and cellular immune responses. The MNP vaccines will be used to evaluate HeV-sG and compare stabilized soluble forms of HNV G and F (sG_{tet} and sF_{stb}) and combinations in challenge studies. A reverse genetics system has been developed to generate recombinant, nonpathogenic Cedar virus (rCedV) and rCedV chimeric virus tools by swapping the G and F glycoproteins with those of NiV/HeV as a rapid, surrogate, BSL-2 high-throughput neutralization platform to be used to rapidly evaluate HNV vaccine responses. Also, divergent and pathogenic zoonotic HNVs have been discovered including the related rodent borne Mòjiāng virus and Langya virus (LayV)), and the divergent bat-borne Angavokely virus (AngV). This project will develop and test the rapid, adaptable and readily deployable MNP vaccine platform together with new virological tools to facilitate the translational nature of this pandemic preparedness approach. Specifically, we will: 1) Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV vaccines; 2) Construct new reporter virus tools; 3) Evaluate vaccine-induced immune responses and HNV-specific mAbs and nbs; and 4) Validate the PPZ-MNP platform with another paramyxovirus antigen.

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Molecular biology tools. Oligonucleotides and gene fragments for PCR and cloning will be purchased and verified by internal quality control at Twist Biosciences, Integrated DNA Technologies or Genscript. All clonal products will be validated by whole plasmid sequencing. All new expression constructs are verified by (1) restriction digest and electrophoretic separation of fragments to ensure fragments of the predicted size are generated and (2) sequencing to ensure the correct sequence is present with no spurious mutations.

Cells. All cells were originally obtained from ATCC or collaborators and maintained by the PI. They are tested monthly for mycoplasma contamination by two complementary methods (qPCR analysis and Lonza MycoAlert biochemical luminescence-based assay). Cells used will be used from the listed initial passage number for a maximum of 50 passages, and then discarded for a fresh vial from the working freezer stock to prevent any long-term selection of altered cell populations. To avoid cross-contamination between different cell lines, only one cell line is used at a time in a biosafety cabinet. Cell lines are routinely validated using either flow cytometry and/or functional tests.

Recombinant Cedar viruses and recombinant vesicular stomatitis viruses (VSV). Viruses are validated through genome sequences or infectivity/functional tests. SOPs, as detailed documents, for work with either recombinant Cedar virus, recombinant Cedar virus chimeras (encoding either Hendra virus or Nipah virus F and G glycoproteins), and recombinant VSVs have been established in the Broder/Amaya laboratory and all laboratory staff are required to be familiar with this documentation. Rescued recombinant viruses are validated through genome sequencing and compared to the original cloned full length viral genomes. Use of recombinant Cedar virus recombinant Cedar virus chimeras has been approved at USU.

Antibodies. Antibodies are tested for specificity as follows: (1) For antibodies used repeatedly in the PI's lab, the staining/blotting performance of new antibody lots are compared against previous lots; (2) For new antibodies, banding/staining patterns are compared against published data; (3) Whenever possible, new antibodies are also validated using objective controls, such as cells known to lack the protein of interest (e.g., knockout cells) or cells engineered to over-express an epitope-tagged form of the molecule (i.e., staining by the new antibody is compared side-by-side with staining by the epitope-tag antibody).

Commercial chemical reagents. Such reagents are validated by one or both of the following: (1) performance comparison to previous lots of the same reagent; (2) use in an established assay that incorporates one or more validated performance controls.

Rigor and Reproducibility

The Broder laboratory employs quality systems to ensure rigor, reproducibility, and robust and unbiased results in all experiments. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility.

Budget Justification - RP 2 - Broder

The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. (HJF) in partnership with the Uniformed Services University of the Health Sciences (USUHS) will manage this proposal, if awarded.

Key Personnel:

Christopher Broder, Ph.D., (Principal Investigator, 1.2 calendar months, 10% effort), is a Professor of Microbiology and Immunology at USUHS and will serve as PI on the proposed project. He is a leading expert in henipavirus biology and genetics, as well as development of glycoprotein-based vaccines and monoclonal antibody-based therapeutics against these viruses; many tested in collaboration with Dr. Thomas Geisbert (Project Lead on this application). Dr. Broder has more than 15 years of collaborative research projects with Dr. Geisbert focused on henipaviruses and together have published on a variety of henipavirus countermeasures including the use of recombinant CedVs. Dr. Broder's group recently developed the reverse genetics platform for designing and rescue of recombinant Cedar henipaviruses (rCedV) that can be utilized at BSL-2, some of which have been successfully used in drug library HTS assays and one report on the successful use of Cedar virus in antiviral drug discovery for henipaviruses including Nipah (NiV), Hendra (HeV) and CedV has been published in collaboration with Dr. Geisbert. Dr. Broder's group generated and characterized the recombinant Cedar chimeric viruses that were used to generate the preliminary data for this application. He will oversee all aspects of the recombinant Cedar chimeric virus construction and characterization. In addition, he and Dr. Amaya will collaborate on the design of all experiments and the preparation of progress reports and manuscripts. Dr. Broder is a federal employee and no salary support is requested.

Moushimi Amaya, Ph.D., (Co-investigator, 2.0 calendar months, 16.67% effort). Dr. Amaya is an experienced biochemist and molecular virologist with expertise in a variety of areas including gene cloning and expression and mutagenesis specifically with viruses and viral culture and propagation, and viral protein purification and analysis. She has considerable experience in henipavirus reverse genetics systems. Using the rCedV platform, Dr. Amaya designed, generated and characterized replication competent chimeric viruses as non-reporter and reporter versions that express the envelope glycoproteins of either NiV-Bangladesh (NiV-B) (rCedV-NiV-B) or HeV (rCedV-HeV) instead of those of CedV. In a collaborative effort, Drs. Amaya, Broder and Geisbert demonstrated that the chimeras can serve as suitable surrogate viruses for authentic NiV and HeV viruses. Dr. Amaya developed and characterized the fluorescent reporter chimeric viruses in a high throughput fluorescence reduction neutralization test (FRNT) to analyze neutralization potencies of sera from henipavirus immunized animals and monoclonal antibodies. She will design, oversee and conduct the research plans related to the generation and characterization of the rCedV chimeric viruses and VSV reporter viruses outlined in this proposal. She will develop, perform quality assurance and test the new chimeric viruses for use in henipavirus neutralization assays. Dr. Amaya is a federal employee and no salary support is requested.

Antony S. Dimitrov, Ph.D., (Co-investigator, 6.0 calendar months, 50% effort). In addition to Dr. Dimitrov's duty to the day-to-day operations of this ReVAMPP and the Admin Core, he will oversee and conduct the research plans related to the production and characterization of all rCedV chimeric viruses and VSV reporter viruses; including generation, quality control, and in vitro cell-based studies. Dr. Dimitrov is highly familiar with all scientific and translational development activities of the proposed ReVAMPP. He was a former member of Broder laboratory and played major roles in the development and biochemical characterization of the HeV-sG subunit glycoprotein (JVI 2005), the discovery of the NiV and HeV entry receptor ephrin B2 (PNAS 2005) and in the development, characterization and efficacy testing of the first neutralizing human monoclonal antibody to NiV and HeV, the latter studies conducted with Profectus Biosciences in collaboration with Dr. Geisbert. When at Profectus Bio, Dr. Dimitrov led their program in the pre-clinical development of HeV-sG as a subunit vaccine and the human antibody, m102.4, for treatment of henipavirus infections. Dr. Dimitrov is an employee of the Henry M. Jackson Foundation and salary support is requested.

Other Personnel

TBD (Research Assistant, 12.0 calendar months, 100% effort). This individual will provide technical laboratory support for this project. In addition, this individual will also be responsible for ordering supplies, organizing shipments, and other laboratory support as instructed by the senior staff. As an employee of the Henry M. Jackson Foundation salary support is requested for this individual.

Lianying Yan (Linda) (Manager/Lead Research Associate 3, 6 calendar months, 50% effort) has been in Dr. Broder's laboratory since 2003 and is well-versed in all aspects of the research in his laboratory. Ms. Yan is a highly trained molecular biologist skilled in expression construct design, screening techniques, and stable cell line development for protein expression using immunoprecipitation and Western blot techniques. Ms. Yan is highly experienced in designing soluble and secreted viral glycoprotein constructs and establishing stable cell-lines to express and produce, purify and characterize the proteins using protocols that include Bio-Plex bead coupling and assay development. She is also expert in confocal and fluorescent microscopy techniques and has been co-author of several manuscripts. Ms. Yan is an employee of the Henry M. Jackson Foundation and salary support is requested.

Madison Thammavong (Research Assistant I, 4 calendar months, 33% effort). Mr. Thammavong will assist Ms. Yan in the production of proteins. He will be trained as a back-up support with various laboratory responsibilities. Mr. Thammavong is an employee of the Henry M. Jackson Foundation and salary support is requested.

Kimberly Boxley, (4.0 calendar months, 33% effort), serves as the Center Financial and Post-Award Administrative Manager. Ms. Boxley, is currently a Program Manager III with the Henry M. Jackson Foundation and her office is located adjacent to Dr. Broder within the Department of Microbiology, USU. Ms. Boxley has been working in grants management for over 15 years and successfully completed the Grants Management Certificate Program (GMCP) from Management Concepts in both the federal and pass-thru tracks. She is enrolled in the Program Management Program (PMP) at the Program Management Institute. She currently manages a department portfolio of approximately $32M in direct funded research awarded during the last 3 years. Her day to day grants management includes budget analysis, employee supervision, training of project managers, forecasting burn rates and compliance issues. Ms. Boxley is an employee of the Henry M. Jackson Foundation and salary support is requested.

Supplies

The costs for materials and supplies are based on historical and current supply needs for these types of studies and on current costs for these supplies. The research teams will be engaged with numerous recombinant gene cloning strategies to produce new expression plasmids and recombinant viral stocks. Several large genomic segments will need to be synthesized and the final clones of all these viral genomes assembled and cloned into expression plasmids.

There will be five different chimeric virus stocks that will need to be prepared, qualified, stored and characterized: three rCedV chimeric viruses and two VSV reporter virus stocks. Piloting and rescuing these new viruses will require significant amounts of materials for all the plasmid preparations and transfection reagents and the multiple anticipated variations in plasmid ratios to achieve virus rescues. We estimate $2000 per construct for gene synthesis and sequencing and $4000 per construct for reagents. Once rescued and cloned each virus genome (the viral stock) will have to be deep sequenced. In addition, the proposed studies will require a significant amount of both cell line and virus culture as well consumables, disposables and reagents (enzyme substrate for the luciferase reporter assays) for the serum neutralization tests with rCedV chimeric reporter viruses. The reporter viruses will be designed and rescued within the first 2 years. The budget for supplies includes molecular biology reagents as well as bacterial culture media and supplements, although reagents and chemicals will be needed to run the assays with the newly created reporter chimeric viruses.

Categories of supplies:

Cell and virus culture media, sera and reagents
Molecular biology reagents; enzymes, primers, plasmid purification kits, ligation kits, gene synthesis, whole plasmid sequencing, virus genome sequencing
Chemicals,
Consumables; flasks, plates, pipettes, gloves, tips

Steady-Glo® Luciferase Assay System for in vitro luciferase assays
CTL S6 analyzer (Fluorescent neutralization tests) and Promega GloMax (luciferase based assays)
Other miscellaneous expenses

Indirect Costs:

HJF indirect cost (IDC) is calculated based on the value-added cost base overhead rates. The fringe rate used is 29.42 % for Tier 1 employees and 7.29% for Tier 2 employees (all employees in this proposal are Tier 1).

The HJF indirect cost is calculated based on the value-added cost base overhead rates. The IDC rate is 36.74% USU Onsite Overhead. Additionally, 16.70% Companywide G&A and applied on the total direct cost less subaward plus the USU Onsite Overhead. For proposals including subawards, an additional 1.93% is applied on total external subaward costs.

The above fringe benefits and indirect cost rates for FY2023 were approved by the U.S. Army Medical Research Acquisition Activity on September 1, 2022.

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role:	Site Principal Investigator										
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	86,601.00	25,478.00	112,079.00
Project Role:	Co-investigator										
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role:	Co-investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 112,079.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant (Thammavong)	4.00	0.00	0.00	15,585.00	4,585.00	20,170.00
1	Research Associate III (Yan)	6.00	0.00	0.00	41,560.00	12,227.00	53,787.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	31,669.00	9,317.00	40,986.00
1	Research Assistant (TBD)	12.00	0.00	0.00	42,000.00	12,356.00	54,356.00

4 **Total Number Other Personnel** **Total Other Personnel** 169,299.00

Total Salary, Wages and Fringe Benefits (A+B) 281,378.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)
Steriguard Biosafety cabinet thermo scientific: 48in, 1300Series	6,450.00
CTL Analyzer	79,000.00

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	85,450.00

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	55,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	55,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	421,828.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	421,828.00	154,980.00
FY23 HJF Companywide G&A	16.70	576,808.00	96,327.00

Total Indirect Costs 251,307.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	673,135.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	673,135.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 03/01/2025 **End Date:** 02/28/2026

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role: Site Principal Investigator											
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	89,978.00	26,472.00	116,450.00
Project Role: Co-investigator											
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role: Co-investigator											

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 116,450.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant(Thammavong)	4.00	0.00	0.00	16,193.00	4,764.00	20,957.00
1	Research Associate III (Yan)	6.00	0.00	0.00	43,181.00	12,704.00	55,885.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	32,904.00	9,680.00	42,584.00
1	Research Assistant (TBD)	12.00	0.00	0.00	43,638.00	12,838.00	56,476.00

4 **Total Number Other Personnel** **Total Other Personnel** 175,902.00

Total Salary, Wages and Fringe Benefits (A+B) 292,352.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: ☐ Add Attachment ☐ Delete Attachment ☐ View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	55,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
10.		
11.		
12.		
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15.		
16.		
17.		
	Total Other Direct Costs	55,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	347,352.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	347,352.00	127,617.00
FY23 HJF Companywide G&A	16.70	474,969.00	79,320.00

Total Indirect Costs 206,937.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number): USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	554,289.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	554,289.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 03/01/2026 **End Date:** 02/28/2027

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role:	Site Principal Investigator										
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	93,488.00	27,504.00	120,992.00
Project Role:	Co-investigator										
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role:	Co-investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 120,992.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant(Thammavong)	4.00	0.00	0.00	16,824.00	4,950.00	21,774.00
1	Research Associate III (Yan)	6.00	0.00	0.00	44,865.00	13,199.00	58,064.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	34,187.00	10,058.00	44,245.00
1	Research Assistant (TBD)	12.00	0.00	0.00	45,340.00	13,339.00	58,679.00

4 **Total Number Other Personnel** **Total Other Personnel** 182,762.00

Total Salary, Wages and Fringe Benefits (A+B) 303,754.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	55,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	55,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	358,754.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	358,754.00	131,806.00
FY23 HJF Companywide G&A	16.70	490,560.00	81,924.00
		Total Indirect Costs	213,730.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	572,484.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	572,484.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 03/01/2027 **End Date:** 02/29/2028

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role: Site Principal Investigator											
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	97,134.00	28,577.00	125,711.00
Project Role: Co-investigator											
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role: Co-investigator											

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 125,711.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant (Thammavong)	4.00	0.00	0.00	17,480.00	5,143.00	22,623.00
1	Research Associate III (Yan)	6.00	0.00	0.00	46,615.00	13,714.00	60,329.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	35,521.00	10,450.00	45,971.00
1	Research Assistant (TBD)	12.00	0.00	0.00	47,108.00	13,859.00	60,967.00

4 **Total Number Other Personnel**

Total Other Personnel 189,890.00

Total Salary, Wages and Fringe Benefits (A+B) 315,601.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	35,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
10.		
11.		
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13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	35,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	350,601.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	350,601.00	128,811.00
FY23 HJF Companywide G&A	16.70	479,411.00	80,062.00
		Total Indirect Costs	208,873.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number): USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	559,474.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	559,474.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 5

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 5** **Start Date:** 03/01/2028 **End Date:** 02/28/2029

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role:	Site Principal Investigator										
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	100,922.00	29,691.00	130,613.00
Project Role:	Co-investigator										
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role:	Co-investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 130,613.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Research Assistant(Thammavong)	4.00	0.00	0.00	18,162.00	5,343.00	23,505.00
1	Research Associate III (Yan)	6.00	0.00	0.00	48,433.00	14,248.00	62,681.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	36,906.00	10,858.00	47,764.00
1	Research Assistant (TBD)	12.00	0.00	0.00	48,945.00	14,399.00	63,344.00

5 **Total Number Other Personnel** **Total Other Personnel** 197,294.00

Total Salary, Wages and Fringe Benefits (A+B) 327,907.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	35,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	35,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	362,907.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	362,907.00	133,333.00
FY23 HJF Companywide G&A	16.70	496,242.00	82,872.00
		Total Indirect Costs	216,205.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	579,112.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	579,112.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		605,845.00
Section B, Other Personnel		915,147.00
Total Number Other Personnel	21	
Total Salary, Wages and Fringe Benefits (A+B)		1,520,992.00
Section C, Equipment		85,450.00
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		235,000.00
1. Materials and Supplies	235,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	0.00	
9. Other 2		
10. Other 3		
11. Other 4		
12. Other 5		
13. Other 6		
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		

Section G, Direct Costs (A thru F)	1,841,442.00
Section H, Indirect Costs	1,097,052.00
Section I, Total Direct and Indirect Costs (G + H)	2,938,494.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	2,938,494.00

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Equipment

The laboratories are located in the Department of Microbiology and Immunology at USU.

The following equipment is supplied in the laboratory:

- Epifluorescence microscopy system (Fully-automated Zeiss AxioObserver epifluorescence microscope)
- BioRad BioPlex 200 HTF multiplexing system
- Luminex MagPIX multiplexing system
- BioRad iQ5 real-time PCR system
- Lonza Nucleofector 4D Shuttle – core unit and X-unit
- Coulter Z1-Dual cell counter
- centrifuges: Eppendorf 5425 microfuges (4); Beckman Allegra 25R; Thermo-Fisher Jouan GR4i; various rotors
- inverted microscopes for tissue culture (3)
- gel electrophoresis equipment for protein (7) and DNA (12) gels
- semi-dry protein transfer apparatus (4)
- Perkin-Elmer and Eppendorf thermal cyclers (5)
- ELISA plate readers (2)
- balances (6)
- pH meter (2)
- refrigerators
- freezers (-20 C, -80 C)
- platform orbital shakers (5)
- sonicator with cup horn and microtip
- bacterial shakers (6)
- heating blocks (16)
- waterbaths (14)
- biosafety cabinets (6)
- CO_2-tissue culture incubators (11)
- Thermo-Fisher Locator 4 liquid nitrogen storage tank with electronic monitoring (6)
- GE-ATKA low pressure chromatography systems, including UV detectors and fraction collectors (2)
- CTL S6 Universal Analyzer

The following equipment is supplied as common support equipment within the department:

- Fuji LAS-4010 chemiluminescence/fluorescence CCD camera imaging system
- IVIS in vivo imaging system (equipped to detect luminescence and fluorescence)
- 2D gel electrophoresis system (protein)
- Alpha-Innotech gel documentation system (EtBr-stained gels)
- MilliQ ultra-pure water system
- Nanodrop spectrophotometer
- Luminometer
- Fluorescence plate readers (2)

The University Biomedical Instrumentation Center, USUHS provides other resources and is also located on- site and provides a wide variety of services, including:

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Translational Imaging Facility

- Siemens Inveon SPECT/PET/CT Scanner
- Bruker In-Vivo Xtreme II: supports bioluminescence, X-ray, and direct radioisotopic imaging
- Bruker Biospec 70/20 USR MRI
- Image Guided Therapy 7T MR guided Focused Ultrasound System for neurological applications

Flow Cytometry

- Becton-Dickinson (10- and 13-parameter) LSRII FACS analyzers (2)
- 15-parameter FACSAria II FACS sorter, including a 541nm laser upgrade for sorting of RFP-expressing cells
- Amnis Image Stream X Mark II imaging flow cytometer with IDEAS statistical image analysis software
- Bio-Rad Bio-Plex 200 multi-analyte bioassay detection system
- Offline data analysis stations (3) with software packages including: FlowJo, WinList, and ModFit

Microscopy

- Zeiss 710NLO confocal system with 405/458/488/514/561/633 conventional lasers and a Coherent Ultra2 Ti-Sapphire laser for multiphoton excitation, continuously tunable over the range of 690 to 1080 nm
- Zeiss 700 confocal system with 405/458/488/514/561/633 laser lines
- Zeiss Elyra PS.1 Superresolution microscope for superresolution structured illumination microscopy (SR-SIM), photoactivated localization microscopy (PALM), and direct stochastic optical reconstruction microscopy (dSTORM). System is fully equipped, including the 3D-PALM/dSTORM option.
- Becker-Hickl 2-detector FLIM system (for FRET – attached to Zeiss 710NLO)
- Zeiss AxioExaminer.Z1 upright confocal system with Coherent Chameleon multiphoton laser for in vivo imaging and electrophysiology
- Zeiss AxioImager.M2 upright microscope and MicroBrightField Stereo Investigator software (stereology analysis).
- Leica AF6000 system, including an inverted microscope equipped with a fully motorized 3-axis stage plus atmosphere and temperature control
- Wide-field fluorescence microscopes (3)
- Transmission electron microscope (Philips CM100 transmission EM)
- Ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment)
- Offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize; Metamorph.

Genomics

- Fuji FLA-5000/LAS-3000 chemiluminescence/fluorescence CCD/laser scanning imaging systems
- ABI 9800 Fast Thermal Cycler
- ABI 7500 Real Time PCR System
- ABI 3500xl Genetic Analyzer
- ABI 394 DNA/RNA Synthesizer (2)
- ABI 3900 DNA Synthesizer
- Eppendorf epMotion 5075 VAC Liquid handling robot
- Thermo Scientific Evolution 300 UV/Vis Spectrophotometer
- Systec MediaPrep & Plate Pourer

Proteomics

- Agilent 1100 HPLC (2)
- AB SCIEX Voyager DSTR MALDI-TOF mass spectrometer

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

- AB SCIEX Q-TOF tandem mass spectrometer
- Genetix QArray2 Micro array printer

Histopathology

- Fee-for-service histology and immunohistochemistry
- Nanozoomer digital slide scanner

Structural Biology

- X-ray crystallography facility with a Rigaku HighFlux HomeLab X-ray diffraction system, including:
    - MicroMax-007 HF, a microfocus rotating anode generator
    - R-AXIS Imaging Plate detector
    - X-stream 2000 cryogenic system
    - AB Sciex 4000 QTRAP LC/MS/MS System

Facilities and Other Resources

Overall Scientific Environment: Uniformed Services University (USU) is the medical school at which approximately half of the physicians in the Armed Services receive their graduate training. Research at USU is supported primarily by extramural grants, as in other medical schools. Dr. Broder (PI) is a tenured Professor and Chair of the Department of Microbiology and Immunology, which includes 12 full-time Faculty members. The overall focus of the Department is mechanisms of infectious diseases and the host response/immunology. Faculty interests and active research programs at USU are diverse, with many nationally- and internationally-known investigators. Dr. Broder, the PI, has had and is currently involved in active collaborations within the University, in areas of viral immunology and vaccine and antiviral therapeutics and animal model development. USU is also physically located directly across from the main NIH campus in Bethesda. The overall broad scientific environment at both USU and the NIH is highly conducive to productive collaborations. The PI often uses these resources to his advantage, both for his research objectives and interests and in assisting in adjunct faculty appointments within the Emerging Infectious Diseases graduate program for both on and off-campus scientists interested in participating in graduate education and graduate student training. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional. In addition, multi-lab Immunology journal club, Immunology data club, Virology journal club, and virology data club exist. Approximately 10 groups regularly participate in the immunology journal/data club and 5 groups regularly participate in the virology journal/data club.

Dr. Broder has been well-supported by the Department of Microbiology and Immunology and the USU Office of Research. Although USU is a modest-sized medical school, the technical resources are outstanding, rivaling many very large institutions (see below). The modest size of the school is an advantage, as key pieces of equipment (e.g., Bruker In Vivo Xtreme II; Siemens Inveon SPECT/PET/CT Scanner) are not overbooked and therefore readily available to the laboratory personnel. In addition to University funds, we have successfully used the NIH S10 mechanism to purchase advanced and very expensive equipment, including the Zeiss 710NLO system and the FACSAria 15-parameter cell sorter. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional, as is illustrated by the most recent USU purchase of cutting-edge equipment: the Zeiss Elyra PS.1 super-resolution microscope.

Laboratory: Dr. Broder’s laboratories are divided into 3 rooms totaling 1,440 sq. ft. Both investigators possess routine equipment for microbiology, molecular biology, and biochemistry including incubators, centrifuges, inverted microscopes, gel electrophoresis equipment for protein and DNA gels, thermal cyclers, balances, pH meters, refrigerators, freezers, platform orbital shakers, sonicator, UV transilluminator, shakers, heating blocks, water baths, free-standing biosafety cabinets, and CO_2-tissue culture incubators. Additionally, Dr. Broder’s lab is equipped with an epifluorescence microscopy system (a Zeiss AxioObserver inverted fluorescence microscope, upgraded in 2015), a BioRad iQ5 real-time PCR system, and a Lonza Nucleofector 4D Shuttle. Dr. Broder’s lab also includes two complete GE-ATKA low pressure chromatography systems, with integrated UV detectors, fraction collectors, and pump systems, and gradient fractionator apparatus. Dr.
Broder’s lab performs virus based assays that incorporate primarily 2 techniques: plaque assays and neutralization assays. Both of these techniques determine viral concentration and/or viral quantity and require manual counting of plaques/clear areas (plaque assay) or manually counting GFP foci using a fluorescent microscope (neutralization assays). Since a large number of iterations with multiple viruses are performed routinely, Dr. Broder acquired a CTL S6 analyzer, which is an automated plate reader that can read up to 4 fluorescent colors, captures 25 megapixel images and is able to recognize over 11,000 spots per well in a 96-well plate format. The analyzer can efficiently handle a large number of 96-well plates required for assay characterization and development thus allowing for a high-throughput workflow with the use of minimal reagents. Operator bias is also diminished when counting plaques/GFP foci from processed images allowing for consistent, objective and statistically reliable results. Washed formalin fixed 96-well plates are first loaded into the analyzer and the software takes a high quality image of each well. Using the Basic count feature in the software package the user establishes parameters, which is used to enumerate GFP foci in each well of a single 96-well plate or from batch processing multiple plates. The counts can then be exported as an Excel file, which can be used to generate neutralization profiles for example. Conventional plaque assays and virus neutralization assays can take between 5- 7 days to complete, whereas the protocol established with the CTL

S6 analyzer has reduced the time to 3 days. Postdocs, students, and technicians have desks and computers in the laboratory.

Animal: Animals are maintained in University facilities under the supervision of a full-time veterinarian. USUHS maintains a modern AAALC Accredited, Central Animal Facility of about 50,000 square feet. It, and the University's animal care and use program, is managed by the Center for Laboratory Animal Medicine, which is directed by a veterinarian who is an ACLAM Diplomat and staffed with three other veterinarians, a graduate animal husbandry man, and about 30 technicians. The University is able to provide appropriate care for a wide variety of laboratory animal species, from invertebrates to lower vertebrates, to higher vertebrates including non-human primates and domestic livestock, as well as the more commonly used species such as rodents and rabbits. The facility also includes a number of properly equipped ABSL-2 rooms.

Computer: Windows-based computers as well as Apple Mac all within 4 years of life cycle. They are available for routine use by postdoctoral fellows, graduate students, and technicians located in the laboratories. The University maintains site licenses for other image and data analysis software, including Zeiss Axiovision, VisioPharm, and OriginPro.

Office: Dr. Broder has an office separate from but proximal to the laboratories. Trainees and technicians have desks in the laboratory. The department employs one senior program manager, administrative officer, administrative specialist and a microbiologist. Other personnel can be provided as necessary to support the program.

Clinical: N/A

Other: The Biostatistics Consulting Center (BCC), a service of the Department of Preventive Medicine and Biometrics, provides statistical consulting to USUHS scientific investigators. We routinely consult with Cara Olsen, Research Assistant Professor (the full-time Biostatistics Consultant of the BCC), regarding proper design of experiments for statistical testing and for statistical analysis of the resulting data.

The USU Translational Imaging Facility (TIF) houses state-of-the art equipment for live animal imaging, including a Siemens Inveon SPECT/PET/CT Scanner, a Bruker Biospec 70/20 USR Magnetic Resonance Imaging system, and a Bruker In-Vivo Xtreme II bioluminescence and X-ray imaging sytem.

The USU Biomedical Instrumentation Center (BIC) houses core equipment for use by investigators throughout the University. Instrumentation is available either free or on a fee-for-service basis, depending on which instruments have annual service contracts (which are paid largely through per-hour use fees). The BIC Flow Cytometry Core includes two Becton-Dickinson (10- and 13-parameter) LSRII FACS analyzers, one 15-parameter FACSAria FACS sorter, and one Amnis Image Stream X Mark II imaging flow cytometer, as well as off-line analysis workstations.

The BIC Imaging Core houses three confocal microscopes, including a Zeiss 700 inverted system with 405/458/488/514/561/633 laser excitation; a Zess 710NLO inverted system with 405/458/488/514/561/633 conventional lasers and a Coherent Ultra2 Ti-Sapphire laser for multiphoton excitation, continuously tunable over the range of 690 to 1080 nm; and a Zeiss AxioExaminer-Z1 upright microscope equipped with a direct-coupled Coherent Chameleon tunable infrared laser for ex vivo and in vivo mutliphoton imaging projects. A Becker-Hickl two-detector FLIM system (for FRET analyses) is connected to the inverted Zeiss 710NLO system. Recently, the BIC has also acquired a Zeiss Elyra PS.1 super-resolution microscope, which is capable of 4-parameter SR-SIM (super-resolution structured illumination) imaging, 3-parameter PALM (Photoactivation localization microscopy) and dSTORM (direct stochastic optical reconstruction microscopy), as well as 3D-PALM/dSTORM. The BIC also houses a Leica AF6000 system, consisting of an inverted microscope equipped with a fully motorized 3-axis stage plus atmosphere and temperature control, allowing extended term (days) live cell analyses. Additionally, there is a stereology system consisting of a Zeiss AxioImager.M2 upright microscope connected to MicroBrightField's Stereo Investigator software package. The facility also includes several additional wide-field fluorescence microscopes, and three offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize; Metamorph Basic. The Imaging Core also includes a transmission electron microscope (Philips CM100 transmission EM) and an ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment).

The BIC Genomics core includes an ABI 3900 DNA synthesizer, an ABI3500xl Genetic Analyzer (for sequencing), a RochLightCycler 480 for real-time PCR, and Systec Mediaprep and Plate Pourer instrument. There is also an integrated Fuji FLA-5000/LAS-3000 imaging system for many applications that involve fluorescence and chemiluminescence imaging of gels and blots. The BIC Proteomics Core includes two Agilent 1100 HPLCs, an AB SCIEX Voyager DSTR MALDI-TOF mass spectrometer, and an AB SCIEX Q-TOF tandem mass spectrometer.

The BIC Structural Biology Core includes a Rigaku HighFlux HomeLab X-ray diffraction system, with a MicroMax-007 HF microfocus rotating anode generator, an R-AXIS Imaging Plate detector, and an X-stream 2000 cryogenic system. Other available BIC instruments and services include histopathology and PET/CT instrumentation for small animal research.

A full-time statistician is available at the University for consultation on data analysis and to help with statistical analysis.

The University also has an equipment repair service, central duplicating service, audiovisual service, and microcomputer support service. The University Learning Resource Center is a high quality medical and scientific library with additional microcomputers and support. A wide variety of scientific journals are available in print and via remote computer access. A central autoclave/glassware washroom serves the Department of Microbiology and Immunology and is maintained through extramural grant support.

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Professor and Chair

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May 16, 2023

Thomas W. Geisbert, PhD
Professor, Microbiology and Immunology
University of Texas Medical Branch
Galveston National Laboratory
301 University Blvd.
Galveston, TX 77550-0610

Dear Tom,

We are writing to indicate our full support for your Center grant submission: "**Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)**" in response to RFA-AI-23-020: Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP). The Research Projects and Scientific Cores you have assembled for this effort will have superior and synergistic plan for the Center's research objectives, and will significantly advance the rapid development of vaccines and antibody-based countermeasures against several very important viral pathogens within the *bunyavirales* and paramyxoviridae.

Our lab's have been working together on emerging viruses and host cell interactions, with particular focus on vaccines and antibody-based countermeasures for henipaviruses for more than 15 years, and together our groups have made a number of significant contributions to this field.

Together, we have developed and tested several new Nipah/Hendra virus vaccine platforms, discovered some new antiviral drug leads, and have made great progress in mAb based pre- and post-exposure treatments against Nipah and Hendra virus infection. More recently the new authentic henipavirus tools using the nonpathogenic Cedar henipavirus (CedV) for use at BSL-2 we developed in collaboration with you, will be very useful in rapidly accessing vaccine induced Nipah/Hendra neutralizing antibody responses and mAbs that the Center will be testing and developing. For Center programs like this, having collaborative and multidisciplinary teams is critical and the partners UTMB and USU have in place will augment this Center's success in achieving its objectives. This ReVAMMP proposal is critically important, and I, Drs. Amaya and Dimitrov, and our team at USU look forward to this opportunity to work towards the development of new and effective antivirals together with the world class team you and your colleagues have pulled together!

Sincerely,

(b) (6)

Christopher C. Broder, Ph.D.
Professor and Chair Staff
Microbiology and Immunology

(b) (6)

Moushimi Amaya, Ph.D.
Res. Assistant Professor

(b) (6)

Antony S Dimitrov, Ph.D.
Senior Staff Scientist
Henry Jackson Foundation

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Resource Sharing Plan (to include sharing research data, model organisms and data)

We routinely present data derived from our studies at scientific meetings such as the American Society for Virology (ASV), American Society for Microbiology, Keystone Symposia among others, and when components are completed, invention disclosures filed, and following consultation with our appropriate technology transfer offices, we publish and disclose our findings. We have always made our reagents and data available to other investigators, and when appropriate, complete MTAs in a timely fashion. If research findings we deem sensitive are requested by outside parties, prior to publication mutual non-disclosure agreements will be put in place. In addition, we will have a free exchange of findings and data with all our consultants and collaborators. We will adhere to all institutional, state and federal resource sharing guidelines and agree to share knowledge, research materials, and any other resources necessary and relevant to the particular focus of the grant, recognizing that final authorization will be cleared with all appropriate organizational parties. We will make all data gathered available quickly and in an easy-to-interpret format, adhering to confidentiality regulations, provided that this does not conflict with our local requirements.

Research resources, research tools, research procedures and protocols, and novel materials such as recombinant viruses like VSV and rCedV based reporters for LayV and other emerging viruses that may arise out of the funded studies will be made available to others in the private and public sector as soon as appropriate agreements covering such transfer can be executed, or in the case of project data, shall be published as soon as possible or otherwise shared with other researchers pursuant to NIH policies regarding data sharing.

The USU and HJF Joint Office of Technology Transfer (JOTT) will coordinate all technology transfer activities for data and resources generated by USU and UTMB and Novartis. The JOTT will adhere to all applicable policies, principles, guidelines and procurement rules in making unique research resources readily available for research purposes to qualified individuals and entities, and in ensuring that there is no more than a short–term restriction on publication or public dissemination of data in order for the JOTT to evaluate and file for patent protection on any subject invention that may arise from the funded study. Dissemination of unpublished data that is considered to be proprietary or confidential shall occur pursuant to a suitable confidential disclosure agreement prepared by the JOTT as required. Material transfer agreements, license agreements, and Cooperative Research and Development Agreements (CRADAs), as appropriate, will be used to transfer material property. Material transfer agreements will be established to share resources among the research community for noncommercial research use.

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Select Agents

No select agents are used in this research.

We will generate and characterize recombinant forms of Cedar virus and Vesicular Stomatitis Virus. These viruses are categorized as BSL-2. Drs. Broder and Amaya have approved biosafety protocols for in vitro research with Cedar virus. All members of the Broder lab have received specific training in laboratory safety and the proper use and disposal of BSL-2 organisms. Dr. Broder has BSL-2 certified laboratories where work with these agents will be conducted.

SPECIFIC AIMS

Pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. Among these are the two emerged zoonotic paramyxoviruses: the ‘prototype’ henipaviruses (HNVs); Nipah virus (NiV) and Hendra virus (HeV), discovered in the mid to late 1990s. The prototype HNVs have a uniquely broad tropism capable of infecting 18 species across 6 orders of mammals using the conserved ephrin-B2 and -B3 ligands as entry receptors, and infection often results in a systemic and fatal respiratory and/or neurological disease in multiple mammalian species including humans. NiV and henipaviral disease are also included in the WHO List of Priority Pathogens, and are significant threats to humans and livestock in South and South East Asia and Australia. HNVs have two membrane glycoproteins: the attachment (G) and the fusion (F) glycoproteins that are the major targets of neutralizing antibodies and focus of all vaccine and antiviral monoclonal antibody (mAb) strategies. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including nonhuman primates (NHPs). With no licensed vaccines or treatments available for human use, **Research Project 2 (RP2)** will address this critical need using the prototype HNVs as a model to develop, test, and translate a novel vaccination platform. We have recently tested a thermostable, needle-free, adaptable, and widely deployable polyphosphazene (PPZ) adjuvanted dissolvable microneedle patch (MNP)-based HeV-sG vaccine as a new tool for pandemic preparedness against HNVs. Our preliminary data demonstrate potent immunogenicity of the PPZ-MNP-HeV-sG vaccine in mice, inducing robust antigen-specific HNV cross-neutralizing antibody and cellular immune responses. This new approach may afford a new, rapid, adaptable and readily deployable vaccination strategy. We will use MNP vaccines and evaluate HeV-sG and new stabilized soluble forms of HNV G and F glycoproteins **(**sG_{tet} and sF_{stb}) in immunogenicity and challenge studies. We have also extensively studied the nonpathogenic HNV, Cedar virus (CedV); develop a reverse genetics system, generated recombinant CedV (rCedV) and rCedV chimeric virus tools by swapping the G and F glycoproteins with those of NiV/HeV as a rapid, surrogate, BSL-2 high-throughput neutralization platform. Recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not use ephrins as entry receptors. Also, another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. Here, we also propose to adapt the MNP vaccine platform to LayV, rescue rLayV in collaboration with **Core E,** and develop additional LayV and AngV reporter virus tools, and we will translate and test the MNP vaccine **plug-and-play** approach, and evaluate new mAbs and nanobodies generated by **Core D** and **RP4** and **RP5** to the prototype HNVs and new divergent and/or potential zoonotic HNVs. Specifically, we will:

Aim 1: Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV subunit vaccines.

We will test and compare NiV-sG_{tet}, NiV-sF_{stb}, HeV-sF_{stb} and HeV-sG as vaccine immunogens using PPZ-MNPs. Murine studies will evaluate their immunogenicity and antiviral humoral responses and homologous and heterologous neutralizing antibody inducing potentials. Best-in-class vaccines will then be evaluated in NiV-B and HeV challenge studies in ferrets, and then selected vaccines advanced to evaluation in NHPs.

Aim 2: Rescue Langya virus and construct LayV and AngV glycoprotein bearing reporter virus tools.

We will construct reverse genetics tools and rescue rLayV and reporter-gene encoding rLayV. Virus rescue will be conducted in collaboration with **Core E** at UTMB. Alternate reporter virus tools will also be generated using rCedV and vesicular stomatitis virus (VSV) platforms encoding HNV F/G glycoproteins. Rescued viruses will be characterized in cell-based assays. rLayV virus will be examined in vivo (new animal models) **by Core E**.

Aim 3: Evaluate HNV-specific antibodies and nanobodies developed by the Center’s RPs and Core D.

We will utilize our unique HNV tools and high-throughput virus neutralization platforms to evaluate PPZ-MNP vaccine-induced immune sera and the virus specific mAbs and nanobodies developed by Center. These experiments will help facilitate down-selection of vaccine antigens and prioritize best-in-class antiviral antibodies.

Aim 4: Validate PPZ-MNP platform with another paramyxovirus-specific antigen in Phase 2.

We will demonstrate the broad applicability of the PPZ-MNP subunit-based vaccine platform for the development of safe, skin-targeted, and effective vaccines using alternate recombinant paramyxovirus glycoprotein antigens.

These proposed studies to develop and apply the rapid, adaptable and readily deployable MNP vaccination platform is innovative and unique, and the development of the new virological tools to help facilitate the translational nature of this approach and evaluate new antiviral antibody-based countermeasures will foster pandemic preparedness tool-set to combat future emerging paramyxovirus threats.

TITLE: Cedar henipavirus animal model

Funding announcement: RFA-AI-23-020

Funding Title: Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picronaviridae

PRINCIPAL INVESTIGATOR: Brian Schaefer, Ph.D., Site Principal Investigator

Prime PI: Thomas Geisbert, Ph.D., UTMB

Period of Performance: March 1, 2024 - February 28, 2029

Abstract

Henipaviruses are single-stranded, negative-sense enveloped RNA viruses of the paramyxovirus family. Two henipaviruses, Nipah virus (NiV) and Hendra virus (HeV), cause a systemic and often fatal respiratory and/or encephalitic disease in humans and ten other mammalian species. Importantly, NiV and HeV are significant biothreats to humans and economically important livestock in Australia and Southeast Asia. There are currently no vaccines or therapeutics approved for human use. Notably, development of countermeasures for NiV and HeV is hampered by the fact that these viruses require BSL-4 containment, meaning that very few research groups worldwide have access to the required biocontainment facilities to perform preclinical studies with these important human viral pathogens. To address this problem, we are developing a BSL-2 animal model that is based on Cedar virus (CedV), which is a non-pathogenic henipavirus. Specifically, we are employing recombinant Cedar viruses (rCedVs) in which the NiV and HeV fusion (F) and receptor-binding glycoprotein (G) are expressed in the rCedV genome, replacing CedV F and G. Additionally, we have also incorporated our recently developed *in vivo* bioluminescence methodology to longitudinally trace the dynamics and anatomical progression of rCedV-luciferase (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host innate immune response in mice, we have demonstrated sustained replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Importantly, whereas rCedV-luc does not establish stable expression in the brain, both of the chimeric viruses do. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in specific strains of mice. The rCedV-luc platform is thus an authentic henipavirus system that can be used to study henipavirus *in vivo* biology safely and expediently under BSL-2 containment. Our overall hypothesis is that rCedV-luc infection of mice lacking specific innate immune responses represents a BSL-2 platform for the study of henipavirus biology and pathogenesis, as well as for development and testing of anti-viral countermeasures. We will address this hypothesis through three Specific Aims: Aim 1: To optimize the use of immunodeficient mice and rCedV-NiV-luc/rCedV-HeV-luc chimeras as a robust BSL2 model of pathogenic henipavirus disease; Aim 2: To determine the mechanism of rCedV-NiV neurovirulence; Aim 3: To define the efficacy and mechanisms of mAb-based therapeutics for CNS-resident henipavirus infections. Aims involve synergistic collaborations with several other research projects and cores in this U19 program. Successful completion of these Aims will establish the rCedV-luc mouse model as a robust BSL-2 platform for the exploration of henipavirus pathogenesis and countermeasures.

USUHS

Site Principal Investigator: Brian Schaefer, Ph.D.

Authentication of Key Biological and Chemical Resources

The reagents that are used in the Schaefer lab are routinely authenticated as follows:

Expression constructs: All new expression constructs are verified by (1) restriction digest and electrophoretic separation of fragments to ensure fragments of the predicted size are generated and (2) sequencing to ensure the correct sequence is present with no spurious mutations.

Antibodies: Antibodies are tested for specificity as follows: (1) For antibodies used repeatedly, the staining/blotting performance of new antibody lots are compared against previous lots; (2) For new antibodies, banding/staining patterns are compared against published data; (3) Whenever possible, new antibodies are also validated using objective controls, such as cells known to lack the protein of interest (e.g., knockout cells) or cells engineered to over-express an epitope-tagged form of the molecule (i.e., staining by the new antibody is compared side-by-side with staining by the epitope-tag antibody).

Cell lines: Cell lines are routinely validated using flow cytometry for key cell surface markers and/or functional tests. Cell lines transfected for virus production are validated via measuring titers of virus in the resultant supernatants.

Viruses: Viruses are validated through a combination of genome sequencing, infectivity and functional testing. Rescued recombinant viruses are validated through genome sequencing, with comparison to the original cloned full length viral genomes.

Mice: Every new litter of knockout animals is PCR screened for both the wild-type and inactivated allele, with validated wild-type and knockout control DNAs included in every screen. For transgenic animals, presence of the transgene is PCR verified in every litter, with validated controls included in the screen. Additional screens include periodic western blotting and/or FACS analysis of tissues/cells from knockout animals, to validate that PCR-identified knockouts indeed fail to express the protein product of the inactivated gene.

Commercial chemical reagents: Such reagents are validated by one or both of the following: (1) performance comparison to previous lots of the same reagent; (2) use in an established assay that incorporates one or more validated performance controls.

Budget Justification – RP3 – Schaefer

The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. (HJF) in partnership with the Uniformed Services University of the Health Sciences (USUHS) will manage this proposal, if awarded.

Key Personnel:

Brian. C. Schaefer, Ph.D. (Site Principal Investigator, 1.2 calendar months, 10% effort) is a Professor of Microbiology and Immunology at USUHS. His expertise is in cellular immunology, animal models of immune responses in the central nervous system and applying advanced imaging methods to biological questions. He and Dr. Broder have been collaborating for nearly a decade on creating animal models and investigating therapeutic interventions for neurotropic viral infections. The preliminary data in this application resulted from this collaboration, through an NIH-funded U19 grant (with aims that do not overlap the aims of this proposal). Dr. Schaefer will direct all animal studies in this project, managing and advising staff and overseeing data collection and data analysis. Dr. Schaefer will manage the overall experimental direction, the writing and editing of manuscripts for publication and the preparation of progress reports and other administrative functions related to this project, with assistance from the Senior Staff Scientist. (Dr. Schaefer is a full-time Federal employee. Hence, no salary is requested for this project).

Other Personnel

†**To be named** (Scientist II, 9 calendar months, 75% effort). A Scientist with experience in animal models and transcriptomics analysis will be recruited for this project. This Scientist will supervise animal research studies, coordinate, conduct and supervise data analyses for transcriptomics studies. The scientist will also coordinate overall data analysis and will participate with Dr. Schaefer in preparation of manuscripts for funding and in preparing progress reports and performing other administrative tasks related to the conduct of this project. The Scientist II will be employed by the Henry M. Jackson Foundation, and salary support is requested on this project.

†**To be named** (Postdoctoral fellow, 12 calendar months, 100% effort). A Postdoctoral fellow with experience in animal research and virology will be recruited for this project. The Postdoctoral fellow will perform approximately half of the animal experiments proposed for this study. The Postdoctoral fellow will be employed by the Henry M. Jackson Foundation, and salary support is requested on this project.

†**Celeste Huaman** (Graduate Student, 12 calendar months,100% effort) will perform approximately half of the proposed in vivo experiments described in this proposal. Ms. Huaman is a senior graduate student in Dr. Schaefer's lab, who has been working collaboratively with members of the Broder lab on this project. Notably, Ms. Huaman generated the majority of preliminary data in this proposal, and has extensive experience in all procedures and analysis techniques required for the execution of the proposed experiments. Ms. Huaman worked in Dr. Schaefer's lab as a Research Assitant II for 5 years before joining the USU Emerging Infectious Diseases graduate program, and she acquired a very deep technical skill set during that time. Ms. Huaman completed the CEEZAD BSL-3 training course at the Biosecurity Research Institute of Kansas State University in 2021, and she has trained most current laboratory staff in proper BSL-2 technique. Ms. Huaman is currently employed by the Henry M. Jackson Foundation, and salary support is requested for her on this project.

†**Caitlyn Clouse** (Research Assistant II, 9 calendar months, 75% effort) will provide general technical support, performing routine tissue culture, ordering reagents, and maintaining databases, as required for the project. Ms. Clouse will also perform genotyping of animals breeding at Charles River Lab and she will maintain detailed records of animal genotypes. She will also assist the postdoctoral fellow and Ms. Huaman with all animal experiments. Ms. Clouse completed the CEEZAD BSL-3 training course at the Biosecurity Research Institute of Kansas llent technical support, including assistance to Ms. Huaman in gathering the preliminary data for this project. Ms. Clouse is an employee of the Henry M. Jackson Foundation and we are requesting salary support for her on this project.

[†] **Camille Alba** (Research Associate, The American Genome Center (TAGC), 1.85 calendar months, 15.4% effort) will be responsible for leading the collaborative performance task for this study. She will oversee performance of library preparation, library quality assessment, library clustering and whole genome sequencing of the samples across the entire project. She will report Illumina NovaSeq platform performance metrics to the collaborating staff scientist and oversee data transfer batches. Ms. Alba is employed as a Henry Jackson Foundation employee therefore salary support is requested.

[†] A merit increase has been added to years 2-5 of the personnel budget to account for salary increases for Henry M. Jackson Foundation personnel.

Equipment:

An ultralow (-80 C) freezer, ThermoFisher Scientific Model TSX60086A ($12,123), is needed for storage of the many RNA samples, viral stocks and frozen tissues (for sectioning) and slides with frozen sections. Dr. Schaefer's current -80 freezers are filled to capacity.

Travel: (Year 1)

Funds are requested to attend two domestic scientific conferences or one international conference. This travel is for data presentation, exchange of ideas with colleagues, and for keeping up-to-date regarding the latest research findings relevant to the proposed project. Meeting attendance and data presentation is a key component of postdoctoral and graduate student training.

Materials and Supplies: $48,000 - year 1-2; $43,200 - year 3; $33,000 - year 4; $20,000 - year 5

For the laboratory to successfully complete the experiments, the project will need to procure the following reagents and supplies:

General Laboratory Supplies

Serological pipettes, microscope slides and coverslips, pipet tips, microcentrifuge tubes, sterile conical tubes, ultracentrifuge tubes, syringes, needles, gloves, respirators, gowns, buffers, flasks, plates, etc.

Materials

Trizol reagents, RNAse-free DNAse, RNAse-free water and buffers, Reverse Transcriptase, Taq Polymerase, qPCR master mix, RNAse-free consumables for tissue homogenization, magnetic beads for cell sorting, tissue culture media, fetal bovine serum, antibiotics, etc

Real-time PCR probes

DNA oligonucleotide

Labeled antibodies for histology and flow cytometry

Animal costs (procurement and husbandry): $34,728 - year 1; $ 48,046 - year 2-5

Animal Breeding at Charles River Labs

The breeding of experimental mice at USU is contracted to Charles River Labs (CRL). All aspects of animal care and breeding are managed by CRL, while genotyping of progeny is performed by Dr. Schaefer's group, using biosamples collected and shipped by CRL. We maintain a colony of B6-albino Ifnar1-/- mice and B6-albion STAT1-/- mice in our breeding colony at CRL. For this project, we expect to maintain 12 breeding cages and 12 progeny cages to supply the animals needed for the proposed experiments. ($1.80/cage/day x 24 cages x 365 days = $15,768 + Transportation charges $1,500 = $20,768).

Animal Husbandry at USU

Animal facility (DLAR) mouse per diems:
USU DLAR FY24 per diem for biohazardous mice is $4.78/cage/day. We expect to have an average of 8 experimental cages housed in the facility (8 cages x 365 days x $4.78/cage/day = $13,957.60/yr)

Translational Imaging Core (BRIC): $23,400 year 1; $39,000 year 2 - 5
BRIC – IVIS Spectrum CT bioluminescence imaging (9 hr/week x 52 weeks/yr x $50/hr = $23,400/yr)

Biomedical Instrumentation Core (BIC): $10,608 year 1 - 5
BIC – flow cytometry (4 hr/week x 52 weeks/yr x $36/hr = $7,488/year).
BIC – confocal microscopy (2 hr/week x 52 weeks/yr x $30/hr =$3,120/year)

The American Genome Center (TAGC): $52,680 year 1 - 5

Transcriptomics studies performed at the USU American Genome Center

Total RNAseq on brain slices from 32 animals/year x $300 per animal

Supplies for Visium Spatial Gene Expression:

Visium CytAssist Spatial Gene Expression for FFPE, Mouse Transcriptome 6.5mm slides (quantity of 16 per year)

Visium CytAssist Tissue Slide Cassettes

Dual INdex Kit TS set A

S1 Novaseq Flow cells (for spatial transcriptomics sequencing), 2 flow cells/year

Supplies for Single-cell RNAseq:

Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1

Chromium Next GEM Automated Chip G Single Cell Kit

S1 Novaseq Flow cells (for single-cell RNAseq), 1 flow cell/year

Publication costs: $5,000 - year 2
We estimate 2 manuscripts per year for an average cost of $2,500 per publication. This cost estimate is based on our average fee paid per open access journal publication for the last 3 years.

Indirect Costs:

HJF indirect cost (IDC) is calculated based on the value-added cost base overhead rates. The fringe rate used is 29.42 % for Tier 1 employees and 7.29% for Tier 2 employees (all employees in this proposal are Tier 1).

The HJF indirect cost is calculated based on the value-added cost base overhead rates. The IDC rate is 36.74% USU Onsite Overhead. Additionally, 16.70% Companywide G&A and applied on the total direct cost less subaward plus the USU Onsite Overhead. For proposals including subawards, an additional 1.93% is applied on total external subaward costs.

The above fringe benefits and indirect cost rates for FY2023 were approved by the U.S. Army Medical Research Acquisition Activity on September 1, 2022.

USUHS

Site Principal Investigator: Brian Schaefer, Ph.D.

EQUIPMENT

Schaefer Laboratory

Epifluorescence microscopy system (Fully-automated Zeiss AxioObserver epifluorescence microscope)
BioRad CFX Connect real-time PCR system
Lonza Nucleofector 4D Shuttle – core unit and X-unit
Coulter Z1-Dual cell counter
DeNovix CellDrop-FL cell counter with fluorescence
centrifuges: Eppendorf 5425 microfuges (3); Beckman Allegra 25R; Thermo-Fisher Jouan GR4i; various rotors NestAdvance Bullet Blender bead mills (2)
Miltenyi QuadroMACS separator (2)
inverted microscopes for tissue culture (2)
gel electrophoresis equipment for protein (3) and DNA (5) gels
semi-dry protein transfer apparatus (2)
Bio-Rad thermal cyclers (2)
balances (4)
pH meter
refrigerators
freezers (-20 C, -80 C)
platform orbital shakers (5)
sonicator with cup horn and microtip
bacterial shaker
heating blocks (6)
waterbaths (8)
biosafety cabinets (2)
CO_2-tissue culture incubators (3)
Thermo-Fisher Locator 4 liquid nitrogen storage tank with electronic monitoring

Departmental

GE Healthcare Life Sciences Amersham Imager 680 (for imaging of protein and DNA gels and blots)
Alpha-Innotech gel documentation system (EtBr-stained gels)
2D gel electrophoresis system (protein)
MilliQ ultra-pure water system
Nanodrop spectrophotometer
Luminometer
Fluorescence plate readers (2)

USU CORE FACILITIES (fee for service or free)

Biomedical Research Imaging Core (BRIC)

IVIS® Spectrum CT preclinical *in vivo* imaging system (2D and 3D bioluminescence and CT imaging)
Siemens Inveon SPECT/PET/CT Scanner
Bruker Biospec 70/20 USR MRI
Image Guided Therapy 7T MR guided Focused Ultrasound System for neurological applications

The American Genome Center (TAGC)

Illumina NovaSeq 6000 systems (7)
Illumina HiSeq X systems (6)
Illumina NextSeq 500 system
10x Genomics Visium CyAssist transcriptomic probe transfer system
10x Genomics Chromium Connect automated single-cell NGS library workstation
10x Genomics Chromium Controller massively parallel sample partitioning platform
Agilent Fragment Analyzer automated parallel capillary electrophoresis systems (2)
Bio-Rad Experion LabChip Platform automated electrophoresis system
PRIMER Data Science Core - direct connection to a high-speed network switch for data storage on a petabyte-scale Isilon SAN

Biomedical Instrumentation Center (BIC)

Flow Cytometry
Cytek Aurora 5-laser, 64-channel Spectral Analyzer
Becton-Dickinson 5-laser, 30-parameter FACSymphony A5
Becton-Dickinson 4-laser, 15-parameter LSRII FACS analyzer
BD FACSAria Fusion 4-laser, 18-parameter flow sorter
BD FACSAria IIIu 4-laser, 17-parameter flow sorter
Amnis Image Stream X Mark II imaging flow cytometer with IDEAS statistical image analysis software
Bio-Rad Bio-Plex 200 multi-analyte bioassay detection system
Agilant Seahorse XPe96 Analyzer
Offline data analysis stations (3) with software packages including: FlowJo, WinList, and ModFit

Microscopy
Zeiss 980 Confocal with Airyscan2
Zeiss 700 confocal system with 405/458/488/514/561/633 laser lines
Zeiss 7 MP Multi-photon Confocal Electrophysiology System
Zeiss Elyra PS.1 Superresolution microscope for superresolution structured illumination microscopy (SR-SIM), photoactivated localization microscopy (PALM), and direct stochastic optical reconstruction microscopy (dSTORM). System is fully equipped, including the 3D-PALM/dSTORM option.
Zeiss Lightsheet Z.1
Zeiss Axioscan Z1 Slide Scanner
Zeiss AxioImager M2 upright microscope and MicroBrightField Stereo Investigator software (stereology analysis).
Leica AF6000 system, including an inverted microscope equipped with a fully motorized 3-axis stage plus atmosphere and temperature control
Transmission electron microscope (JEOL JEM-1011)
Ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment)
Grid stainer (Leica EM AC20)
Wide-field fluorescence microscopes (3)
Offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize; Metamorph.

Genomics
3500 XL Applied Biosystems Genetic Analyzer
Gene Forge 3900 and ABI 3900 and DNA Synthesizer
U-3000 Thermo-Scientific HPLC system
Agilent 1200 HPLC
QX200 Droplet Digital PCR System (ddPCR)
Roche LightCycler 480 Real-Time PCR System
Agilent Capillary Electrophoresis Fragment Analyzer

Structural Biology/Proteomics
AB Sciex 4000 QTRAP LC/MS/MS System
Agilent 1200 HPLC
Wyatt DynaPro NanoStar Dynamic Light Scattering
GE Healthcare Biacore 3000 Surface Plasmon Resonance
Art Robbins Instruments Phoenix Crystallization Robot
JANSi UVEX Fluorescence Microscope

Histopathology
Leica Autostainer XL
Leica CV 5030 Robotic Coverslipper
Sakura Tissue-Tek VIP tissue Processor
Leica CM 3050 S Cryostat
Microm HM 325 Microtome

Facilities and Other Resources

Overall Scientific Environment: Uniformed Services University (USU) is the medical school at which approximately half of the physicians in the Armed Services receive their graduate training. Research at USU is supported primarily by extramural grants, as in other medical schools. Dr. Schaefer is a tenured Professor in the Department of Microbiology and Immunology, which includes 12 full-time Faculty members. The overall focus of the Department is mechanisms of infectious diseases and the host response/immunology. Faculty interests and active research programs at USU are diverse, with many nationally- and internationally-known investigators, including the Department of Microbiology and Immunology chair, Dr. Christopher Broder, who is an internationally-known virologist (and centrally involved in this proposal).

USU is directly across the street from the main NIH campus and close to Johns Hopkins, the University of Maryland and several other outstanding institutions, facilitating opportunities to establish productive collaborations with world-class investigators. For example, Dr. Schaefer has had a multi-PI U01 grant with Dr. Wolfgang Losert (Dept of Physics, University of Maryland) as the co-PI and Dr. Hari Shroff (NIH/NIBIB) as a collaborator. Dr. Schaefer is also primary PI on a multi-PI R01 from NIAID with Dr. YouWen He at Duke University Medical Center, and Dr. Thomas Conrads (Women's Health Integrated Research Center). Dr. Schaefer and his staff have also collaborated with the Advanced Imaging Center (AIC) at the Howard Hughes Medical Institute (HHMI) for some of their imaging-based research. Overall, the broader scientific environment at USU and the greater Bethesda/Washington D.C. metropolitan region is highly conducive to productive collaborations. Dr. Schaefer has been using these resources to his advantage, and has been very active in establishing collaborative relationships to facilitate his research objectives. Notably, Dr. Schaefer and Dr. Broder have been collaborating for several years on lyssavirus- and henipavirus-related project, and they have a very strong working relationship.

Members of the Schaefer lab participate in a weekly multi-lab Immunology journal club, Immunology data club, virology journal club, and virology data club. Approximately 10 groups regularly participate in the immunology journal/data club and 5 groups regularly participate in the virology journal/data club. Also, members of the Schaefer lab participate in a bi-weekly data presentation/discussion group which meets monthly.

Dr. Schaefer has been well-supported by the Department of Microbiology and Immunology and the USU Office of Research. Although USU is a modest-sized medical school, the technical resources are outstanding, rivaling many very large institutions (see below). The modest size of the school is an advantage, as key pieces of equipment (e.g., The IVIS® Spectrum CT preclinical in vivo imaging system; Cytek Aurora spectral flow cytometer), and 10x Genomics Visium Spatial Transcriptomics instruments are not overbooked and therefore readily available to laboratory personnel. Dr. Schaefer is also Chair of the USU Biomedical Instrumentation Center (BIC), and thus plays a central role in directing equipment purchases, ensuring that needed technologies are available for the research of the PI and his USU colleagues. In addition to University funds, we have successfully used the NIH S10 mechanism to purchase core facility equipment. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional, as is illustrated by recent USU purchases of cutting-edge equipment including a Perkin-Elmer IVIS® Spectrum CT preclinical in vivo imaging system, a Zeiss Elyra PS.1 super-resolution microscope, an Agilant Seahorse XPe96 Analyzer, a BD FACSAria Fusion flow sorter, and many other pieces of advanced research equipment. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional.

USU has several Ph.D. programs, including an Emerging Infectious Diseases (EID) program, a Molecular and Cellular Biology (MCB) program and a Neuroscience (NES) program. Dr. Schaefer holds secondary appointments in all three programs and is currently Interim Director of the Emerging Infectious Diseases Graduate Program. Ds. Schaefer has been very active in training graduate students, having trained >10 Ph.D. students. USU recruits strong graduate students, and Dr. Schaefer's students have been very productive, during and beyond their dissertation studies. The availability of talented graduate students is a further resource, promoting productivity and enhancing the overall vibrancy of the scientific environment at USU.

Laboratory: Dr. Schaefer's laboratory area consists of three rooms of 450 sq. ft, 300 sq. ft and 225 sq.ft., and a dedicated microscopy suite of 110 sq. ft. The Schaefer lab contains routine equipment for microbiology, molecular biology, and biochemistry including incubators, centrifuges, inverted microscopes, gel electrophoresis equipment for protein and DNA gels, thermal cyclers, balances, pH meters, refrigerators, freezers, platform orbital shakers, sonicator, UV transilluminator, shakers, heating blocks, waterbaths, free-standing biosafety cabinets, and CO_2-tissue culture incubators. Additionally, Dr. Schaefer's lab is equipped

with an epifluorescence microscopy system (a Zeiss AxioObserver inverted fluorescence microscope), a BioRad CFX Connect real-time PCR system, a Coulter Z1-Dual cell counter, a DeNovix CellDrop-FL cell counter and a Lonza Nucleofector 4D Shuttle. Postdocs, students, and technicians have desks and computers in the laboratory.

Animal: Animals are maintained in University facilities under the supervision of a full-time veterinarian. The USUHS maintains a modern AAALC Accredited, Central Animal Facility of about 50,000 square feet. The facility also includes two preclinical behavior cores (one for mice, one for rats). The facility and the University's animal care and use program is managed by the Department of Laboratory Animal Medicine (DLAR), which is directed by a veterinarian who is an ACLAM Diplomate and staffed with three other veterinarians, a graduate animal husbandryman, and about 30 technicians. The University is able to provide appropriate care for a wide variety of laboratory animal species, from invertebrates and lower vertebrates to higher vertebrates including non-human primates and domestic livestock, as well as more commonly used species such as rodents and rabbits. The facility also includes a number of properly equipped ABSL-2 rooms.

Computer: Windows-based computers (Intel i5/i7 or similar processors and ≥8GB of RAM) are available for routine use by postdoctoral fellows, graduate students, and technicians located in the laboratories of both investigators. Dr. Schaefer's laboratory also has a dedicated workstation for use for analysis of microscopy and in vivo imaging data. Image analysis software in the Dr. Schaefer's lab includes Zeiss Zen, with a digital deconvolution module and numerous tools for quantitative image analysis. The University maintains site licenses for other image and data analysis software, including Zeiss Axiovision, VisioPharm, and OriginPro. Dr. Schaefer also maintains a license for FlowJo software, which can be used on any networked computer in the laboratory. The computers are connected by a central server to each other and to the Internet. A variety of USU-supported software programs are available, including EndNote, Microsoft Office, Adobe Creative Cloud, Geneious Prime and Graph Pad Prism 9.

Office: Drs. Schaefer's office is separate from but proximal to each of his laboratories. Trainees and technicians have desks in the laboratory. Four full-time administrative officers and one part-time administrator are available to provide support within the department.

Clinical: N/A

Other: The Biostatistics Consulting Center (BCC), a service of the Department of Preventive Medicine and Biometrics, provides statistical consulting to USUHS scientific investigators. We routinely meet with BCC consultants regarding proper design of experiments for statistical testing and for statistical analysis of the resulting data.

The USU Biomedical Research Imaging Core (BRIC) houses state-of-the art equipment for pre-clinical studies, including a IVIS® Spectrum CT, a Siemens Inveon SPECT/PET/CT Scanner and a Bruker Biospec 70/20 USR Magnetic Resonance Imaging system. A Scientist and several staff provide technical support and manage the facility.

The USU American Genome Center (TAGC) is one of only four academic genome centers in the United States. It is the Department of Defense operations subsidiary of the Collaborative Health Initiative Research Program (CHIRP) established between the National Heart, Lung, and Blood Institute (NHLBI) and USU. Since inception, TAGC has generated more than 40,000 whole Genome Sequences across 107 research studies sourced from NHLBI, other NIH Institutes, DoD and USU investigators. TAGC is also the sequencing center and genome data analysis center for the APOLLO Cancer Moonshot project, and collaborates for this purpose with the Murtha Cancer Center (MCC) at Walter Reed, the National Cancer Institute, and the Veterans Administration. Through a collaborative relationship with 10x Genomics, USU has early access to newly released platforms such as Visium Spatial Transcriptomics (including improvements to that platform).

The USU TAGC includes a large array of next-generation sequencing instruments including Illumina NovaSeq 6000 systems, Illumina HiSeq X systems, and an Illumina NextSeq 500 system. A 10x Genomics Chromium Connect automated single-cell NGS library workstation is available for automated single cell partitioning, barcoding and NGS library preparation. A 10x Genomics Chromium Controller massively parallel sample partitioning platform is available for barcoding single cell RNA for library preparation and for single cell transcriptome profiling, including CITE-seq workflows. 10x Genomics Visium CyAssist transcriptomic probe transfer system is available to facilitate spatial transcriptomics workflows. Electrophoresis systems include Agilent Fragment Analyzers and a Bio-Rad Experion LabChip Platform. Sequencing platforms are connected to a high-speed network switch for data storage on a petabyte-scale Isilon SAN.

The USU Biomedical Instrumentation Center (BIC) consists of five core equipment facilities for use by investigators throughout the University. Instrumentation is available either free or on a fee-for-service basis, depending on which instruments have annual service contracts (which are paid largely through per-hour use fees). Each core is run by a full-time Director with an advanced science background (most hold a PhD) and a high degree of technical expertise. The majority of cores have at least one additional employee to help manage the core and provide guidance and assistance to users.

The BIC Flow Cytometry Core includes three analyzers (Cytek Aurora 5-laser, 64-channel Spectral Analyzer; Becton-Dickinson 5-laser, 30-parameter FACSymphony A5; Becton-Dickinson 4-laser, 15-parameter LSRII) and two sorters (BD FACSAria Fusion 4-laser, 18-parameter flow sorter; BD FACSAria IIIu 4-laser, 17-parameter flow sorter) and an Amnis Image Stream X Mark II imaging flow cytometer. Other equipment includes a Bio-Rad Bio-Plex 200 multi-analyte detection system and an Agilant Seahorse XPe96 Analyzer. The core has several off-line analysis workstations with FlowJo and other data analysis platforms.

The BIC Imaging Core houses a wide array of microscopes, including a Zeiss Elyra PS.1 super-resolution microscope, which is capable of 4-parameter SR-SIM (super-resolution structured illumination) imaging, 3-parameter PALM (Photoactivation localization microscopy) and dSTORM (direct stochastic optical reconstruction microscopy), as well as 3D-PALM/dSTORM. Other microscopes include aZeiss 980 Confocal with Airyscan2, a Zeiss 700 confocal system, a Zeiss 7 MP Multi-photon Confocal Electrophysiology System, a Zeiss Lightsheet Z.1 and a Zeiss Axioscan Z1 Slide Scanner. The facility also includes several additional wide-field fluorescence microscopes, and three offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize. For electron microscopy needs, the core is equipped with a JEOL JEM-1011 transmission electron microscope, an ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment), and a grid stainer (Leica EM AC20).

The BIC Genomics core includes a 3500 XL Applied Biosystems Genetic Analyzer, a Gene Forge 3900 and an ABI 3900 and DNA Synthesizer, a U-3000 Thermo-Scientific HPLC system, an Agilent 1200 HPLC, a QX200 Droplet Digital PCR System, a Roche LightCycler 480 Real-Time PCR System and an Agilent Capillary Electrophoresis Fragment Analyzer.

The BIC Structural Biology and Proteomics Core includes a GE Healthcare Biacore 3000 Surface Plasmon Resonance instrument, an AB Sciex 4000 QTRAP LC/MS/MS System, an Agilent 1200 HPLC, a Wyatt DynaPro NanoStar Dynamic Light Scattering analyzer, an Art Robbins Instruments Phoenix Crystallization Robot, and a JANSi UVEX Fluorescence Microscope (for crystal inspection).

The BIC Histopathology Core includes a Leica Autostainer XL, a Leica CV 5030 Robotic Coverslipper, a Sakura Tissue-Tek VIP tissue Processor, a Leica CM 3050 S Cryostat, and a Microm HM 325 Microtome.

The University also has an equipment repair service, central duplicating service, audiovisual service, and microcomputer support service. The University Learning Resource Center is a high quality medical and scientific library with additional microcomputers and support. A wide variety of scientific journals are available via remote computer access. A central autoclave/glassware washroom serves the Department of Microbiology and Immunology.

DEPARTMENT OF DEFENSE
UNITED STATES OF AMERICA

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

1972

June 1st, 2023

Dr. Schaefer,

It is with great enthusiasm that I can confirm support for your proposed research study: *Research Project 3 (RP3), Cedar henipavirus animal model*, that is part of the multi-site center application, Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX). This project is a sound representation of your research expertise in cellular, histological and molecular methodologies to understanding the modulation of infectious viral consequences in the mammalian brain and central nervous system, using mice as a model system. This proposal is centered on exciting preliminary data suggesting that infection of STAT1-/- mice with chimeric recombinant Cedar viruses (rCedVs) causes neurovirulence and mortality (whereas immunocompetent animals rapidly control infection). Through your proposed transcriptomics studies, we can facilitate your experimental plan to define gene expression changes across cell types in the brains of infected animals. Moreover, through your use of distinct recombinant viruses and different immunocompromised mouse strains, we can identify those gene expression changes that correlate with neurovirulence and mortality. The proposed unbiased approach to defining pathogenic mechanisms will represent a truly important step in establishing, validating and gaining biological insights from this novel BSL-2 model of henipavirus disease.

We are well poised to collaboratively support the high complexity molecular workflows from the well curated tissue specimens your team will provide. At the laboratories of The American Genome Center (TAGC), we have model system experience in transcriptomic and proteomic profiling for experimental models using whole rodent brain (see PubMed Id (PMID): 21190398, 22291617 and 23118733). Specifically, we will assist your laboratory in bulk transcriptomics, single cell RNA-sequencing and Visium spatial transcriptomics using 10x Genomics workflows. We have current standard operating procedures for your experiments using the 10x Genomics CytAssist platform and tissue compatibility for fixed tissues. We will also provide data analysis through validated pipelines and data storage from our compute infrastructure and storage area network. Importantly, we will build cross-compatible protocols with your laboratory team, including a key liason in your laboratory, who has previously trained in our genome center. Our laboratory and Data Science division computational biology associates will be available for analysis and interpretation of the data, with your leadership.

I look forward to working with you on this exciting project!

Sincerely,

(b) (6)

Clifton Lee Dalgard, PhD
Professor
Department of Anatomy, Physiology & Genetics
Director, Center for Military Precision Health
Director, The American Genome Center
Uniformed Services University of the Health Sciences
USU is "America's Medical School"

RESEARCH STRATEGY

Significance and Premise

The health threats and pandemic potential posed by Nipah virus and Hendra virus. The emergence and reemergence of pathogenic zoonotic viruses are continuous threats to global public health. Two particularly pathogenic examples with clear pandemic potential are the henipaviruses, Nipah virus (NiV) and Hendra virus (HeV). NiV and HeV are bat-borne paramyxoviruses that are distinguished from the many paramyxoviruses that cause human and animal diseases by their uniquely broad host tropism (18 animal species across 6 orders of mammals) and their high degree of lethality[13]. Henipaviruses, like all paramyxoviruses, are membrane enveloped, single-stranded negative-sense RNA viruses, and they possess two major membrane anchored glycoproteins involved in virus entry: One mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F glycoproteins are both the major antigenic targets of neutralizing antibodies and the main focus of vaccine and therapeutic antiviral strategies[14]. NiV and HeV are Biosafety Level-4 (BSL-4; risk group 4) pathogens. Moreover, they are classified as priority agents by US Department of Health and Human Services (HHS) because they could be engineered for mass dissemination, and they possess the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the World Health Organization (WHO) as an epidemic threat needing urgent research and development (R&D) action, and NiV has been included in the WHO R&D Blueprint list of priority pathogens with epidemic potential[15]. To date, there have been >630 cases of human NiV infection with >370 fatalities[10]. Characteristics of NiV enhancing its global pandemic potential include: (i) humans are susceptible to infection; (ii) it is capable of person-to-person transmission; (iii) it is an RNA virus with potential to mutate; and (iv) if a human-adapted NiV strain were to infect communities in South Asia, high population densities and global interconnectedness would rapidly spread the infection[16].

Additional members of the henipavirus family, including a non-pathogenic species, Cedar virus. Nucleic acid-based detection studies have also identified additional henipavirus species for which complete genomic sequences are now available[17, 18], including Langya virus, which has also been reported to cause human morbidity during zoonotic spillovers[19], and Angavokely virus, which may be capable of mammalian pathogenesis, due to likely ability to produce V and W proteins[20]. Another member of the henipavirus family, Cedar virus (CedV), was identified in pteropid bats in 2012[7]. Importantly, although CedV is closely related to HeV and NiV and shares the same bat host species as HeV, CedV is not known to be zoonotic and has been shown to be **non**pathogenic in animals susceptible to HeV and NiV disease including mice, guinea pigs, and ferrets[7, 21, 22]. A notable distinction between CedV and HeV/NiV lies in the phosphoprotein gene (P), which contains the open reading frames for the C, V and W proteins in HeV/NiV. The CedV P gene does not encode the C, V and W proteins, which contribute to HeV/NiV pathogenicity[21, 23-25]. The absence of C, V and W is an important factor underlying the failure of CedV to cause disease in infections of mice, ferrets or guinea pigs[7, 22, 25]. Despite the pathogenic differences and lower level of sequence homologies of CedV G to HeV and NiV G (29% and 30%, respectively), CedV infection, like both HeV and NiV, is supported by the cell surface protein ephrin-B2[7, 26] (as well as additional ephrins[26]) as a functional entry receptor.

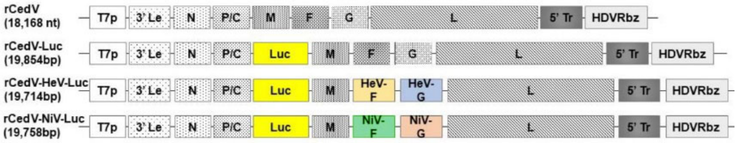


Figure 1. rCedPV variants used in this proposal. Maps are of plasmid-based rCedV antigenomes, all of which have been generated, sequence validated and utilized to produce infectious virus via an in vitro rescue system[27]. The top line shows the genome features of the parental rCedV with only CedV open reading frames (ORFs) and no reporter gene. The second line is rCedV with a firefly luciferase reporter inserted between P and M (rCedV-luc). The third and fourth lines are the rCedV-luc chimeras in which HeV F and G and NiV F and G (respectively) replace CedV F and G. T7p: promoter; HDVRbz: hepatitis D virus ribozyme; Le: leader; Tr: trailer.

CedV as a potential BSL-2 platform for the study of henipavirus biology and preclinical testing of candidate henipavirus therapies. The **Broder group (RP2)** has been developing CedV as a new experimental tool set to study henipavirus host cell interactions, including ephrin receptor use, and host cell tropism. Moreover, they have recently developed a CedV reverse genetics system to rescue recombinant CedV (rCedV)[27], adding a gene for firefly luciferase (rCedV-luc)[28] to enable bioluminescence imaging of viral replication. Additionally, they have created chimeric viruses that express the F and G glycoproteins of NiV or HeV in place of the endogenous CedV F and G (rCedV-NiV-luc and rCedV-HeV-luc; see **Fig. 1**). Importantly, there is **no** evidence that NiV and HeV F and G suppress the immune response or contribute to any viral activity beyond binding and fusion to target cells, nor is there evidence that NiV or HeV F and/or G increase the pathogenicity of rCedV in immunocompetent hosts. Thus, work with rCedVs can be carried out under BSL-2 containment settings[28].

The Schaefer group has optimized use of bioluminescence imaging to semi-quantitatively track lyssavirus replication in individual animals in a longitudinal manner[8, 9]. Working collaboratively, the Schaefer group and **Broder group (RP2)** groups are now developing the rCedV-luc platform as a new technology for the study of henipavirus *in vivo* biology, using mouse models deficient in interferon signaling. *From a translational perspective, rCedVs are an authentic henipavirus platform suitable for developing live-attenuated NiV/HeV vaccines and for preclinical testing of other types of NiV/HeV-directed countermeasures (e.g., mAb and nanobody therapy; novel small molecule antivirals).*

In this proposal, we will build on important preliminary data, showing that (i) rCedVs replicate and infect multiple organs in mouse strains deficient in interferon signaling; (ii) rCedV-NiV-luc and rCedV-HeV-luc rapidly enter and efficiently replicate in the brains of Ifnar1-/- mice; and (iii) rCedV-NiV-luc infection causes neurological disease signs and mortality in STAT1-/- mice. Our goal is to further develop and optimize this model for the study of henipavirus neuropathogenesis and the development and validation of novel countermeasures, under BSL-2 conditions. A robust rCedV chimera-based model would represent a considerable advance in throughput for preclinical validation of NiV- and HeV-directed therapeutics, in contrast to the current cumbersome and costly BSL-4 containment required for such work with NiV and HeV.

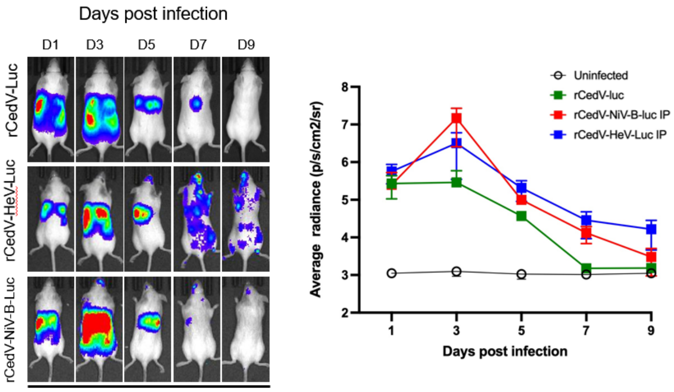


Figure 2. rCedV-luc and rCedV-luc chimeras replicates in Ifnar1-/- albino mice with similar kinetics. B6-albino-Ifnar1-/- mice were infected IP with 10^7 pfu of rCedV-luc, rCedV-HeV-luc or rCedV-NiV-luc. Bioluminescence intensity is reported as average radiance (log scale).

Innovation

Scientific Innovation: The generation of a BSL-2 mouse model of authentic henipavirus infection is novel. Due to the use of the mouse model, there is a potential to leverage powerful genetic tools and a wide array of antibody-based phenotyping reagents to characterize the mechanistic basis of the host-virus interaction, particularly with regard to the antiviral response. A further innovation is use of the STAT1-/- strain in combination with rCedV chimeras to achieve a model of CNS-tropic infection that leads to neurological disease and mortality. We have thus created a tractable *in vivo* system in which henipavirus-directed therapeutics can be evaluated for protection against lethality. Collectively, these innovations should dramatically reduce costs and enhance throughput of translational studies of henipavirus countermeasures, generally making such studies widely accessible to the scientific community.

Technical Innovation: Our use of luciferase-expressing henipaviruses in mice enables longitudinal tracking of infection kinetics and anatomical location[8, 9] of rCedV replication. This innovation was crucial for following and semi-quantitatively measuring the progression of infection with a virus that causes no apparent disease signs (in the Ifnar1-/- strain that we utilized for our initial pilot investigations). Application of robust transcriptomics approaches, including spatial transcriptomics, represents a further technical innovation that may yield a more granular understanding of mechanisms of henipavirus-dependent CNS pathogenesis, thereby potentially suggesting novel targets for countermeasures.

Approach

Scientific Rigor: We will utilize both male and female animals for experiments in this proposal. Animal studies are planned in consultation with the USU Biostatistics Consulting service. These planning phases include power analyses to determine group size and discussion of appropriate statistical methods for the given type of data. Additionally, prior to publication, collected data are analyzed via in-depth discussions with biostatistics consultants to ensure that statistical analyses and any required testing for multiple comparisons have been properly performed. rCedV laboratory manipulation guidelines and standard operating procedures under BSL-2 conditions were developed for the laboratory and staff, and all procedures were reviewed and approved by the Uniformed Services University Institutional Biosafety Committee in accordance with NIH guidelines (see *Select Agents* for further details regarding **biosafety measures** and approvals).

Aim 1: To optimize the use of Stat1-/- mice and rCedV-NiV-luc/rCedV-HeV-luc chimeras as a robust BSL2 model of pathogenic henipavirus disease. Hypothesis: *Infection of Stat1-/- mice with recombinant rCedVs will recapitulate major hallmarks of pathogenic NiV and HeV infections in humans*.

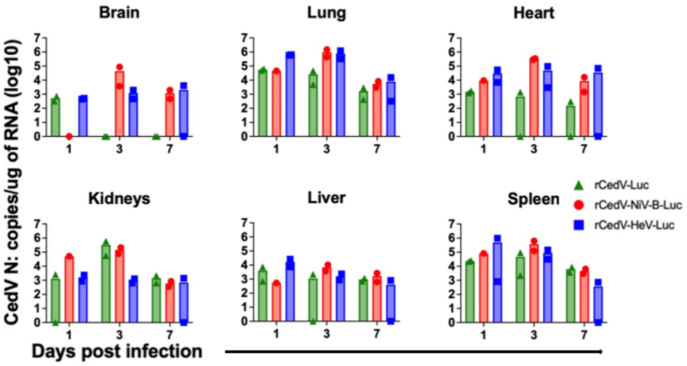


Figure 3. *N*-gene transcription in Ifnar1-/- albino mice infected with rCedV-luc and rCedV-luc chimeras. B6-albino-Ifnar1-/- mice were infected IP with 10^7 pfu of rCedV-luc, rCedV-HeV-luc or rCedV-NiV-luc. Animals were sacrificed and organs were harvested at the indicated time points. qPCR was performed to determine N-gene transcripts/µg total RNA.

Rationale:

The **Broder (RP2)** group at USU has constructed and rescued a series of rCedV chimeras that include the *Photinus pyralis* (firefly) luciferase gene to enable longitudinal *in vivo* bioluminescence imaging of individual animals, analogous to the system we have developed for the study of lyssavirus *in vivo* infection kinetics[8, 9]. In addition to the wild-type rCedV reporter virus (rCedV-Luc)[28], they have also generated two types of rCedV chimeras; one bearing NiV-Bangladesh[29] F and G (rCedV-NiV-luc) and the other bearing HeV F and G (rCedV-HeV-luc). For these reporters, the NiV and HeV F and G replace the Cedar F and G (**Fig. 1**). Notably, using cell-based infection tropism and growth kinetics assays, the luciferase-expressing chimeras behave identically to the rCedVs that do not encode a reporter gene (data not shown and [28]), indicating that there is no *in vitro* growth inhibitory effect of the added luciferase reporter gene.

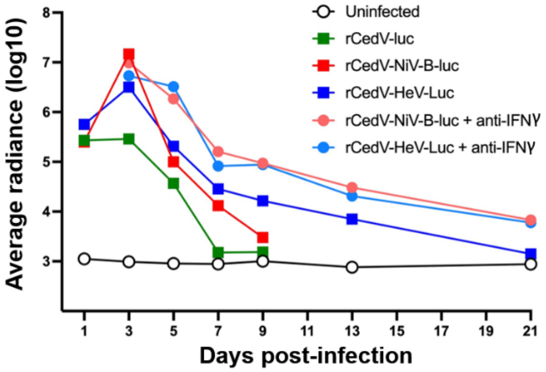


Figure 4. In vivo neutralization of IFNγ inhibits host control of replication of rCedV-luc chimeras. Ifnar1-/- mice were infected with rCedV-luc, rCedV-NiV-luc or rCedV-HeV-luc and untreated or treated every 3-4 days with an IFNγ neutralizing antibody. Whole body bio-luminescence is shown over a 21-day time course.

Pathogenesis by NiV and HeV can largely be attributed to specific viral gene products, C, V and W. Each of these gene products are encoded within the P gene. C is encoded as an alternative reading frame within P, whereas V and W are produced via an RNA editing process, in which non-templated G residues are added at a specific editing site within the nascent P transcript, resulting in a reading frame shift. V and W thus share N-terminal sequence with P, but the C-termini differ from P and from each other[30]. Variants of NiV engineered to lack C, V or W are less pathogenic in a variety of animal models, with V being essential for lethality in ferrets[23, 24, 31, 32]. Although data suggest that these proteins inhibit host immunity via multiple independent mechanisms[33], a very well documented inhibitory effect involves the targeting of the host type-I IFN system. Specifically, data show that the NiV P, V and W protein family can bind to multiple STAT transcription factors[34]. Moreover, these NiV proteins strongly inhibit the STAT1-induced host interferon response via these binding interactions[35, 36]. Although one ferret study has shown that the STAT1 binding site can be eliminated without impacting lethality, NiV (and HeV) have multiple mechanisms of interfering with induction of the interferon response, including antagonism of STAT2 and RIG-I signailing[37, 38]. Collectively, evidence thus indicates that targeting of the host interferon response is a major immune evasion mechanism of HeV and NiV.

CedV does not have the RNA editing site that gives rise to the V and W proteins, and it lacks HeV and NiV mechanisms of antagonizing the host interferon response[21]. Previous studies have shown that wild-type mice are inherently resistant to HeV and NiV infection; however, both HeV and NiV can replicate and cause lethal infection in mice in which the type I interferon receptor is not expressed (Ifnar1-/- mice)[39], further implicating the host interferon response as a major mechanism of limiting HeV and NiV replication and pathogenesis. We thus postulated that rCedV would replicate in Ifnar1-/- mice, but not in WT C57BL/6 mice. As expected, upon intraperitoneal (IP) infection of WT mice with rCedV-luc, no replication could be detected *in vivo*, whereas several days of replication was detected via bioluminescence imaging and qPCR in Ifnar1-/- mice (data not shown, and see **Fig. 2**). Consistent with the non-pathogenic nature of CedV infection in other species[7, 21], there was no morbidity or mortality and no detectable pathogenesis in mice infected with rCedV-luc.

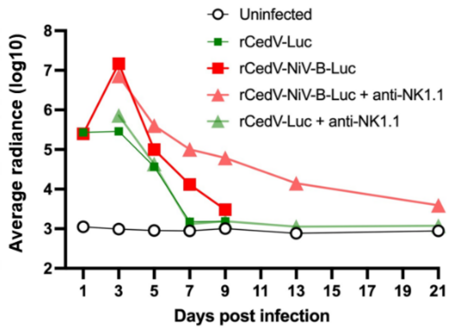


Figure 5. In vivo depletion of NK cells inhibits host control of replication of rCedV-luc chimeras. Ifnar1-/- mice were infected with rCedV-luc or rCedV-NiV-luc and untreated or treated every 3-4 days with an anti NK1.1 antibody to deplete NK cells. Whole body bioluminescence (viral replication) is shown over a 21-day time course.

Because the bioluminescence signal produced within an experimental animal is substantially attenuated by melanin in the skin and fur[40], we crossed Ifnar1-/- mice with B6-albino mice to produce a B6-albino-Ifnar1-/- strain (We maintain this strain in our Charles River Laboratories (CRL) breeding colony). These mice were then infected with rCedV-luc, rCedV-HeV-luc and rCedV-NiV-luc to assess how the chimeric virus replicated in comparison to wild-type over a 9-day infection time course. Interestingly, the chimeric viruses exhibited distinct *in vivo* properties, replicating to a level approximately a log higher than the wild-type rCedV-luc at days 3 and 7 post-infection, and with replication still detectable at day 9. By contrast, rCedV-luc replication was at undetectable levels by day 7 **(Fig. 2**). Viral infection was further assessed on a per-organ basis by qPCR quantification of *N*-gene transcription, demonstrating sustained viral transcription in an array of organs on days 1, 3 and 7. Although viral transcription was generally similar across rCedVs, the notable exception was the brain, where weak *N*-gene transcription was detected for rCedV-luc on day 1 only, whereas both chimeras exhibited *N*-transcription in the brain on days 3 and 7 post-infection (**Fig. 3**). Disease signs were not apparent in any infected animal (data not shown). Thus, rCedVs replicate in B6-albino-Ifnar1-/- mice with an infection kinetic that suggests host immune control of viral replication beginning near day 3 post-infection, with substantially reduced levels of viral replication by day 7 or 9 post-infection (**Fig. 2**). In the brain, rCedV-luc is present only transiently, whereas rCedV chimeras replicate at that site for at least 5 days.

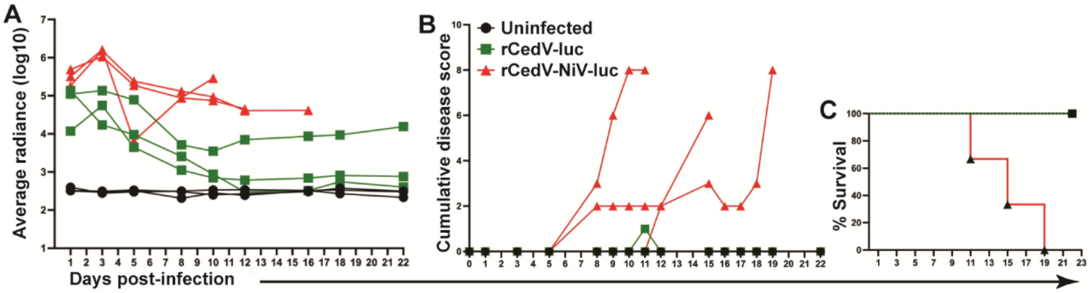


Figure 6. Infections of STAT1-/- mice with rCedV-NiV-luc leads to disease manifestations and death. STAT1-/- mice were uninfected or infected with rCedV-luc or rCedV-NiV-luc and monitored for (**A**) viral replication by bioluminescence; (**B**) disease signs; and (**C**) mortality. Animals infected with rCedV-NiV-luc experienced disease signs and death, whereas animals infected with rCedV-luc survived with no disease signs through this 22-day experiment.

Based on the above data, we hypothesized that more robust *in vivo* replication of rCedVs might be achieved by further impairing the interferon response in the B6-albino-Ifnar1-/- strain. We thus injected rCedV-infected Ifnar1-/- albino mice every 3 - 4 days with the neutralizing anti-IFNγ mAb XMG1.2 (200 µg/injection, starting on day 0). As shown in **Fig. 4**, inhibition of IFNγ activity resulted in increased in vivo levels of rCedV-NiV-luc and rCedV-HeV-luc bioluminescence after day 3 post-infection, as well as a substantially extended *in vivo* replication kinetic, such that both viruses were still replicating at detectable levels at day 21 post-infection. In a similar experiment, we used repeated administration of anti-NK1.1 to deplete NK cells, which are a major source of IFN-γ during viral infection[41]. Depletion of NK cells resulted in an extended kinetic of rCedV-NiV-luc replication, very similar to IFNγ depletion. Interestingly, there was little to no effect on replication of rCedV-luc (**Fig. 5**). Notably, despite the lengthy kinetic of viral replication for chimeric rCedVs, animals treated with either anti-IFNγ or anti-NK1.1 exhibited no disease signs throughout the 21-day course of these infections.

Overall, the above data suggest that type I and II interferons work together to suppress replication of rCedV chimeras. We thus reasoned that type III interferons may also play a role in viral suppression, particularly given that in vitro data have shown that there is a type III interferon response *in vitro* in response to CedV infection, but not HeV infection (presumably due to the suppressive activity of HeV C, V and W genes)[42]. To achieve complete blockade of signaling by types I, II and III interferons, including in the brain (where access of IP-administered neutralizing mAbs is limited by the blood-brain barrier (BBB)), we performed infections of STAT1-/- mice, in which signals from all interferon receptors are highly attenuated. In this initial experiment, we observed that mice infected with rCedV-luc survived for the entire experiment (22 days), with no disease signs and with 2/3 of animals exhibiting virus levels reduced to near the limit of detection by day 12 post-infection. By contrast, animals infected with rCedV-NiV-luc experienced disease signs consistent with neurological dysfunction, with all animals reaching euthanasia criteria between days 11-19 post-infection (**Fig. 6**). As the major differences between these viruses is the ability to sustain infection in the brain (**Fig. 3**), these data suggest that interferon responses play a particularly important role in protecting animals from CNS/encephalitic disease. Thus, our overall interpretation of our preliminary data is: (i) the host interferon response blocks features of viral infection shared across henipaviruses (including CedV) that result in CNS pathogenesis; and (ii) once henipaviruses enter the CNS, pathogenesis will result, if the host interferon response is blocked (e.g., by C, V, W) or not present (e.g., in STAT1-/- mice).

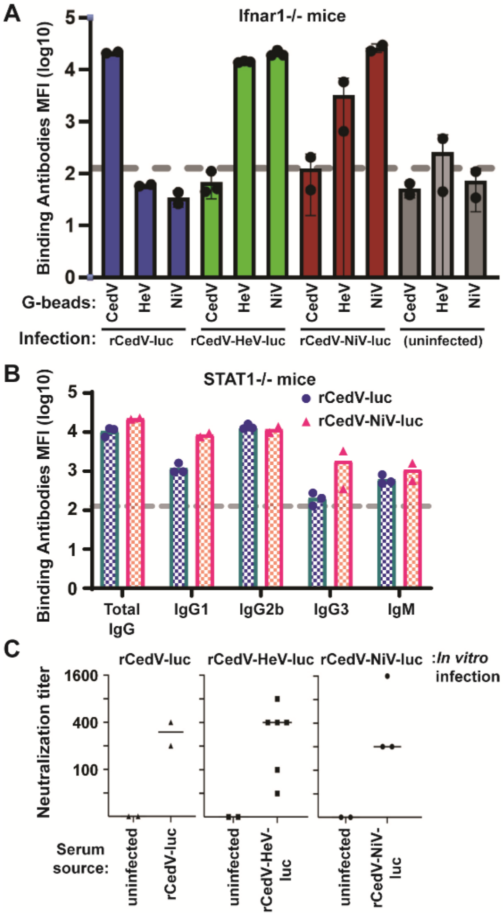


Figure 7. Infections with rCedVs lead to production of G-specific neutralizing antibodies in Ifnar1-/- and STAT1-/- mice. (**A**) Ifnar1-/- mice were uninfected or infected with the indicated rCedVs. G-specific total IgG was detected in sera harvested at the indicated time points using Luminex beads coupled to G ectodomains from CedV, HeV or NiV, as indicated. These specificity controls show the predicted cross-reactivity between anti-HeV and anti-NiV. (**B**) G-specific total IgG, IgG isotypes and IgM were detected in sera of STAT1-/- mice infected as indicated, using methodology of (A). Dashed line is average background. (**C**) *In vitro* neutralization of the indicated viruses with sera from mice infected with the indicated rCedVs. Beads prepared in the **Broder lab (RP2)**; serology run in the **Gunn (Core D, RP1,2) and Laing (RP2) labs**.

In this Aim, we will further optimize the chimeric rCedV-STAT1-/- infection model, to fully define the in vivo kinetics of viral replication, the characteristics of viral pathogenesis and the antiviral humoral response.

Experimental Design

Generation of an albino STAT1-/- strain: Because STAT1-/- mice are not carried in large numbers by the vendor (Jackson Labs) and because the pigmentation (including hyper-melanated birthmarks) of this strain impedes bioluminescence imaging, we will cross STAT1-/- mice to the B6-albino strain. Sequential intercrossings will be performed to produce enough STAT1-/- albino breeding animals to generate age-matched animals in sufficient quantities for our proposed experiments (notably, the F_1 breeding generation has been initiated in our contracted breeding colony at Charles River Labs).

Quantifying in vivo replication kinetics of rCedVs in mouse models. As our findings with the STAT1-/- strain are very recent and limited to the data in **Fig. 6**, further experiments will be conducted using larger groups and *albino*-STAT1-/- animals. We will first perform in vivo titration experiments to determine the lowest amount of virus that leads to 100% mortality. Next, whole animal bioluminescence data will be used to define the kinetics of infection, providing some indication regarding on which days individual organs or groups of organs reach peak infection titers. These studies will be followed up by further bioluminescence tracing studies, in which some individual animals are euthanized at key time points for more detailed analysis of individual organs (liver, lungs, spleen, kidneys, heart, lymph nodes, brain, etc), to quantify transcript levels of rCedVs, comparing rCedV-luc, rCedV-NiV-luc and rCedV-HeV-luc, and providing more granular data regarding the per-organ kinetics of viral replication and transcription. These data will also be compared with infection data from infections of the Ifnar1-/- albino strain (with and without inhibition of IFNγ), to determine the degree to which types II and III interferons contribute to suppression of viral replication across organ systems.

Histological identification of cell types infected and pathological features of infection by rCedVs. We will perform histological analyses, to determine if there is evidence of organ-specific pathology during the course of infection. While brain-specific pathology is of particular interest and will be explored in depth in **Aim 2**, we will perform a thorough analysis of other possible organ-specific pathology in this Aim. To add power to these histological analyses, we are currently developing an anti-Cedar N mAb (via a contract with GenScript), with the goal of producing a highly-sensitive reagent for histological identification of cells infected with rCedVs (note that Cedar N is expressed by all viruses to be used in this proposal). If successfully generated, this reagent will be employed in our protocols using paraformaldehyde-fixed frozen tissues for multiparameter immunofluorescence microscopy, which we perform routinely. The goal of these experiments will be to determine the types of cells infected by rCedVs and how cellular tropism varies between distinct rCedVs (e.g., rCedV-luc vs rCedV-NiV-luc), potentially as a result their incompletely overlapping usage of ephrin entry receptors (xxxRef). A distinct goal of these experiments is to identify subtypes of immune cells that may expand in or infiltrate tissues in areas of viral replication. We will also formalin-fix and paraffin-embed (FFPE) organs for standard H&E staining and histological analysis, using this complementary histological approach to identify features of pathology. Analyses of H&E-stained FFPE sections will either be performed by in-house veterinary pathologists or contracted to Histoserv, Inc. Additionally, we will conduct analogous experiments in albino Ifnar1-/- animals, with and without antibody-mediated inhibition of IFNγ (Figs. xxx), to evaluate whether specific pathological features differ as interferon signaling is combinatorially inhibited (i.e, inhibition of only Type I IFN signaling in the Ifnar1-/- strain; inhibition of Types I+II IFN signaling in Ifnar1-/- mice with anti-IFNγ injection, and inhibition of Types I+II+III IFN signaling in STAT1-/- mice). Our goal is to use the resulting data to form new mechanistic hypotheses regarding the roles of specific interferon types in the virus-host interaction, particularly with regard to *in vivo* control of infection and pathogenesis.

Analyses of humoral responses to rCedVs. To assess the humoral response to rCedV infections, sera will be recovered from animals at various time points post-infection. We will analyze sera for G- and F-specific isotypes, and we will determine neutralization efficiency (**Fig. 7**). In collaboration with the **Gunn group (Core D, RP1,2)**, we will use a systems serology approach to define antibody-dependent mechanisms of host defense against rCedV infection, analogous to what they have published for Ab-dependent host defenses in human and NHP studies (e.g., [43, 44]). Specifically, aliquots of sera will be shipped to the Gunn group for analysis via phagocytosis, antibody-mediated complement deposition (AMCD) and antibody dependent NK cell degranulation (NK-ADCC) assays, in order to determine the degree to which the anti-rCedV Ab response exhibits these Fc-dependent activities. Additionally, sera will be analyzed via Luminex-based approaches for cytokine and chemokine levels, with a particular goal of defining the kinetics of inflammatory cytokine presence (if any). We will also perform Luminex-based analysis of cytokine production in homogenates of any organs that show evidence of virus-induced pathogenesis.

Expected Results

Overall, we expect these data to define infection of STAT1-/- mice with chimeric rCedVs as a highly relevant BSL-2 model for lethal henipavirus disease, in which encephalitis is the major mortality-driving pathological feature, analogous to human disease (xxxRefs). We expect to rigorously demonstrate that as we progressively inhibit the interferon system (type I, then I + II, then I + II + III), replication of rCedVs will increase across organ systems, both in magnitude and duration of infection. Via histological analyses of distinct organs, we expect to identify cell types that are predominant targets for rCedV-luc infection and replication, via combinatorial staining for Cedar N and markers of distinct cell types. We furthermore expect that these cellular targets will only partially overlap the targets of the chimeric rCedVs, due to their incompletely overlapping patterns of ephrin binding (xxxRef). Also, due to its ability to invade the brain, we expect rCedV-HeV-luc to induce mortality with kinetics and frequency indistinguishable from rCedV-NiV-luc.

We expect histological analyses to identify hallmarks of pathogenesis in a subset of organs, particularly in the STAT1-/- model. Our preliminary data (not shown) suggest an enlargement of the liver on days 1 and 3 post-infection, suggestive of a transient hepatitis. Notably, liver involvement in the form of vasculitis has been noted in HeV infections of hamsters[45]. Thus, we expect to observe vasculitis in the liver, and/or leukocyte infiltration. The lungs are also a major henipavirus target, and our qPCR data demonstrate replication of all rCedVs in the lungs of infected animals (**Fig. 3**). As vasculitis/vascular lesions of the lungs have been observed in multiple distinct henipavirus animal models[46], we anticipate similar findings in the STAT1-/- model upon infection with rCedVs. Indeed, given the presence of viral transcripts in many organs (**Fig. 3**), evidence of lymphocyte and macrophage infiltrates is likely across organ systems, particularly in perivascular regions[46].

Preliminary data performed collaboratively between our group and the **Broder, Laing and Gunn groups (RP2)** show robust G-specific IgM and IgG responses are induced in rCedV-infected animals, across all animal genotypes and interferon blockade conditions (**Fig. 7A-B**). Interestingly, our preliminary studies indicate that neutralization titers from sera of infected animals are rather low (**Fig. 7C**). We thus expect that the systems serology assays of the **Gunn group (Core D, RP1,2)** will substantially clarify the potency of various Ab-mediated effector mechanisms against rCedVs in this model system. For example, we may find that although neutralization titers are low, the ability of anti-G (or anti-F) Abs to facilitate NK-ADCC is high.

Pitfalls and Alternative Approaches

Although our pilot study of rCedV-NiV-luc infection of STAT1-/- mice yielded promising results, we may encounter technical hurdles in further development of this model. For example, the frequency of fatality may be less than the 100% rate observed in our pilot study. Alternatively, although disease signs strongly indicate a neurological basis for progression to euthanasia criteria, which is highly consistent with human disease, our histological studies may indicate other effects on major organ systems that are inconsistent with human disease. While many such scenarios can be envisioned that would complicate this model, we believe that the initial data are compelling enough for us to be confident that the STAT1-/- model of henipavirus disease will be of high value for these studies and the overall scientific community. One technical pitfall is that our in-process development of the CedV N monoclonal may fail to yield a reagent of high enough specificity/sensitivity for use in histology. In such a circumstance, we can use the equivalent GFP-expressing variant of each rCedV (the Broder (RP2) group routinely generates both luciferase- and GFP-expressing variants of each new rCedV).

Aim 2: To determine the mechanism of rCedV-NiV neurovirulence in the Stat1-/- mouse model.

Hypothesis: *CNS infection by rCedV chimeras requires functional interaction between G and host ephrin B3; neurovirulence is a property shared by henipaviruses in the absence of host IFN-dependent defenses*.

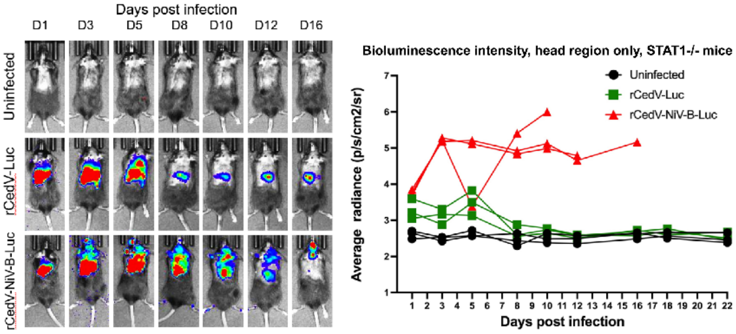


Figure 8. Persistent replication of rCedV-NiV-luc in the brains of STAT1-/- mice precedes death. STAT1-/- mice were uninfected or infected with rCedV-luc or rCedV-NiV-luc. Fur was shaved from the head and back to enable bioluminescence imaging. An ROI was drawn around the head and radiance was quantified (log10 scale). Data show strong replication in the head region of rCedV-NiV-luc, but not rCedV-luc (weak head signal on days 1-5 may reflect bleed of bright signal from chest cavity). See **Fig. 6** for related whole-body luminescence, disease score, and mortality data for these animals.

Rationale

Data in **Figs. 3 and 6** demonstrate that unlike rCedV-luc, both rCedV-NiV-luc and rCedV-HeV-luc are able to establish days-long infections in the brains of Ifnar1-/- and STAT1-/- mice. Because these viruses differ only at their F and G loci, the ability of these viruses to access the CNS must map to either F, G or both cooperatively. Importantly, although CedV G binds to more ephrins than NiV/HeV G, CedV G does **not** bind to ephrin B3, whereas both NiV and HeV G do interact with ephrin B3[26]. Moreover, data suggest that the ability of NiV and HeV (and the inability of CedV) to enter the CNS is due to the fact that brain cells express ephrin B3, but little to no ephrin B2[47]. Thus, our *in vivo* observations with rCedVs lend additional support to the hypothesis that efficient henipavirus entry into the CNS requires interaction between G and ephrin B3.

Notably, for the rCedV chimeras, both viral persistence and pathogenesis appear to be highly influenced by interferon-dependent mechanisms. Specifically, in the case of Ifnar1-/- animals, regardless of IFN-γ blockade or NK cell depletion (**Figs. 2-5**), infection with rCedV-NiV-luc and rCedV-HeV-luc causes neither disease signs nor mortality. However, Ifnar1-/- mice given an mAb that blocks IFNγ or that depletes NK cells exhibited a longer kinetics of viral replication over the 21-day experimental time frame (**Figs. 3-5**), in contrast to the lack of impact of NK cell depletion on the transient rCedV-luc infection of Ifnar1-/- mice (**Fig. 3, 5**). In STAT1-/- animals, which fail to transduce signals from interferon receptors, rCedV-NiV-luc replicated to high levels (**Fig. 6**), including a persistent infection in the head/brain region of these animals (**Fig. 8**), with animals experiencing rapid escalation of disease signs, culminating in death (**Fig. 6**). However, as observed in viral transcription analysis in Ifnar1-/- mouse experiments (**Fig. 3**), rCedV-luc replication in the head/brain region was transient and at a much lower level than rCedV-NiV-luc (**Fig. 8**). Collectively, these data strongly suggest that interferon-dependent signals are required to control henipavirus infection in the brain and to prevent consequent neuropathogenesis and death.

Experimental Design

Defining viral determinants of CNS entry and pathogenesis. To further validate the postulate that CNS entry leads to morbidity and mortality, we will conduct several distinct experiments. Firstly, we will repeat the experiment shown in **Fig. 8** with the addition of rCedV-HeV-luc, a chimera that also stably infects the brain

(**Fig. 3**). Secondly, we will perform experiments in which we infect STAT1-/- albino mice with rCedV-NiV-luc and the rCedV-NiV($B3_{mut}$)-luc virus (expressing a NiV G mutant that binds to ephrin B2 but ***not*** ephrin B3, which is being generated under **RP2)**, to test the requirement for ephrin B3 binding for brain entry and subsequent mortality. Also, the **Broder (RP2)** group has generated data showing that the combination of CedV G and HeV F can mediate viral fusion. Thus, we will test if rCedV-(CedV G/HeV F)-luc is capable of brain infection, to provide a further test regarding whether G or F is the crucial henipavirus determinant of CNS entry. Additionally, we will directly inject rCedV-luc and rCedV-NiV-luc into the brains of Ifnar1-/- and STAT1-/- albino mice, in order to evaluate development of disease signs and mortality under conditions in which CNS entry is independent of the ability to cross the BBB.

Each of the above experiments will be conducted with the necessary number of iterations to robustly define elements of CNS pathology in these animals. In some iterations, animals will be euthanized at intermediate time points prior to the expected earliest mortality date. Brains will be harvested, with one hemisphere being used for RNA collection and the other being used for histology (details below). RNA will be analyzed in qPCR experiments, to determine absolute numbers of both N gene transcripts (**Fig. 3**) and rCedV genomes (we have validated genome-specific primer sets for this purpose), to achieve a thorough understanding of the relationship between viral transcriptional activity and viral load over time and across animal genotypes.

Transcriptomics analysis of gene expression in the brain. To define gene expression changes that correlate with henipavirus pathogenesis in the CNS, we will use a complementary set of unbiased transcriptomics approaches. Our central approach will be 10x Genomics Visium transcriptomics, to map gene expression changes to specific regions and cell types within the brains of infected animals. For these experiments, we will compare rCedV-NiV-infected Ifnar1-/- animals treated with anti-IFNγ (which exhibit an extended kinetic of viral replication in the brain, without morbidity or mortality) with rCedV-NiV-infected STAT1-/- mice (which exhibit viral replication in the head/brain region, leading to disease signs and mortality). We will also include a custom-designed RNA probe for rCedV *N*, to enable correlation of host gene expression changes with viral infection. Based on our initial data (**Fig. 6**), we anticipate choosing a time point in the day 7-10 range, during which virus is present in the brains of both genotypes, and STAT1-/- mice exhibit early disease signs, but have not yet reached euthanasia criteria, and do not have severe brain pathology (**Aim 1** experiments will enable more refined determination of this time point). To further validate and reinforce spatial transcriptomics experiments, we will also perform transcriptomics analysis on total RNA isolated from brain sections adjacent to the sections used for spatial transcriptomics, and from equivalent sections from additional mice in the same group. We will also use a third complementary transcriptomics approach to provide confirmation and additional granularity regarding resident and infiltrating leukocyte transcriptional programs. Specifically, we will perform single-cell transcriptomics on leukocytes recovered from brains of additional animals (i.e., of the same groups and time points as assessed by spatial transcriptomics), to provide further granularity and confirmation regarding leukocyte-centric transcriptional changes that correlate with neurovirulence and mortality. Key differences identified by transcriptomics approaches will be further confirmed by qPCR, using total RNA from sections.

Histological and flow cytometry analysis. To complement and reinforce data collected through the above transcriptomics approaches, we will use multiparameter fluorescence labeling a Zeiss Axioscan Z1 slide scanner to define regions of the brain and cell types that are infected by each rCedV, using a CedV anti-N mAb (see Aim 1) and markers of brain-resident cells and peripheral immune cells that may be recruited to the brains of animals with rCedV infections. These studies will be informed both by transcriptomics data, and analysis of H&E-stained sections, described in **Aim 1**. Additionally, we will also perform dedicated experiments for flow cytometry analysis, in which brains are collected and processed to purify resident and infiltrating lymphocytes, with populations and activation states defined by antibody staining for specific cell surface and intracellular markers (e.g., transcription factors). In some experiments, cells will be stimulated and stained for intracellular cytokines to aid in identification of cellular subpopulations (e.g., types of effector T cells). We are already performing analyses of leukocytes in the mouse brain in similar studies of lyssavirus pathogenesis (xxxRef). We will also extend these flow cytometry studies to the peripheral tissues listed above, in order to determine the degree to which phenotypes of resident or infiltrating leukocytes reflect cellular phenotypes in the brain.

Expected Results

In our experiments testing the requirements for brain entry and mortality, we expect that rCedV-HeV-luc will behave analogously to rCedV-NiV-luc, replicating to high levels in the brain and causing mortality of all STAT1-

/- albino animals, as we have already shown that rCedV-HeV-luc efficiently enters and replicates in the brains of Ifnar1-/- mice. As existing data are consistent with the model that G interaction with ephrin B3 is the key determinant for productive replication of rCedVs within the CNS (**Fig. 3** and [26, 47]), we expect that neither rCedV-NiV($B3_{mut}$)-luc nor rCedV-(CedV G/HeV F)-luc will exhibit stable CNS replication and trigger morbidity and mortality in the STAT1-/- strain. Whether stable CNS infection and mortality can be driven by direct injection of rCedV-luc into the brains of STAT1-/- animals is more difficult to predict. Notably, a wide array of anatomical subregions of the brain express high levels of ephrin B3 and little to no ephrin B2[47], perhaps meaning that viruses expressing CedV G may be unable to efficiently infect brain cells, regardless of BBB penetrance. Thus, our expectation is that we will observe an increased level of brain infection upon direct injection of rCedV-luc as compared to IP delivery, but likely at lower levels and perhaps less persistently; and morbidity and mortality may not occur within the 21-day experiment. Across the above experiments, we expect qPCR experiments to be consistent with longitudinal bioluminescence imaging, with viral transcription levels correlating well with bioluminescence intensity, as we have shown in our lyssavirus studies[9].

We expect that the three complementary transcriptomics approaches will yield data that provide a highly granular picture of rCedV-NiV-luc-induced transcriptional changes that correlate with morbidity and mortality in the STAT1-/- model. Given that we will have rCedV-NiV-luc-infected IFNγ-treated IFNAR1-/- mice as controls in these experiments, analysis of the resulting data should identify gene expression changes *that specifically correlate with pathology* and not simply with CNS viral infection. Notably, the mechanism by which HeV and NiV trigger encephalitis and death remains unknown, with neither a direct cytopathic effect or an immune cell-mediated inflammatory response clearly implicated as primary drivers of morbidity and mortality. We anticipate that both mechanisms are important, and that our transcriptomics data will implicate leukocyte-specific inflammatory gene expression programs as more robustly induced in the STAT1-/- model as compared to Ifnar1-/- mice. We also anticipate that our data will provide gene expression data consistent with induction of stress and apoptosis programs in STAT1-/- animals, with spatial transcriptomics data demonstrating that infected neurons are the focal points of these indicators of cytopathic effects.

In addition, we expect histological and flow cytometry approaches to confirm key pathogenesis-associated changes in cellular phenotypes identified through our combined transcriptomics approaches. Moreover, we expect histological results to provide data that can help to discriminate between potential mechanisms of viral entry into the brain, particularly transcytosis of infected leukocytes, vs. infection of microvascular endothelia cells during viremia. Staining with anti-N plus markers of specific cell types at early post-infection time points should enable identification of initial cell types in the brain that are infected with rCedVs. Later time point data should suggesting which point(s) of early entry ultimately seed infection of the brain parenchyma. We also expect that brain pathology will be observed only with those viruses that cause morbidity and that such pathology will share many similarities with what has been observed in human post-mortem tissues and in animal models of HeV and NiV infection[46, 48].

Pitfalls and Alternative Approaches

While it is possible that our predictions regarding the Ephrin B3-centric role for viral entry are incorrect, experiments have been designed in such a way that data should indicate which viral features are crucial (e.g., F rather than G, or a determinant of G outside of the B3-interaction region). The basis of henipavirus neurovirulence has remained poorly understood for many reasons, including the expense of BSL-4 research and the relatively small number of *in vivo* experiments that have been performed. While it is unclear whether our rCedV-mouse model will recapitulate all crucial aspects of henipavirus neuropathogenesis in humans, there should be commonalities. The use of three separate unbiased transcriptomics approaches will provide multiple independent measures of gene expression changes, leading to new mechanism-based hypotheses regarding henipavirus neuropathogenesis. These data and hypotheses will be of high value to investigators across the henipavirus field, who can perform experiments in diverse models to further test these models.

Aim 3: To define the efficacy and mechanisms of mAb-based therapeutics for CNS-resident henipavirus infections. Hypothesis: *G- and F-directed mAb-based therapeutics will demonstrate Fc-dependent efficacy against CNS-resident henipavirus.*

Rationale

Monoclonal antibodies have been utilized effectively against several disease-causing viruses in humans (e.g., RSV, SARS-CoV-2[49]). Indeed, m102.4, an anti-HeV/NiV G mAb, developed by the **Broder group**, has been employed in humans under circumstances of compassionate use for those exposed to HeV and NiV[2, 50], with all of those treated experiencing no symptoms of henipavirus disease. While virus neutralization has historically been viewed at the key mechanism of action of such mAbs, accumulating data are suggesting varying degrees of contribution of the antibody Fc domain in mediating therapeutic benefits[51, 52].

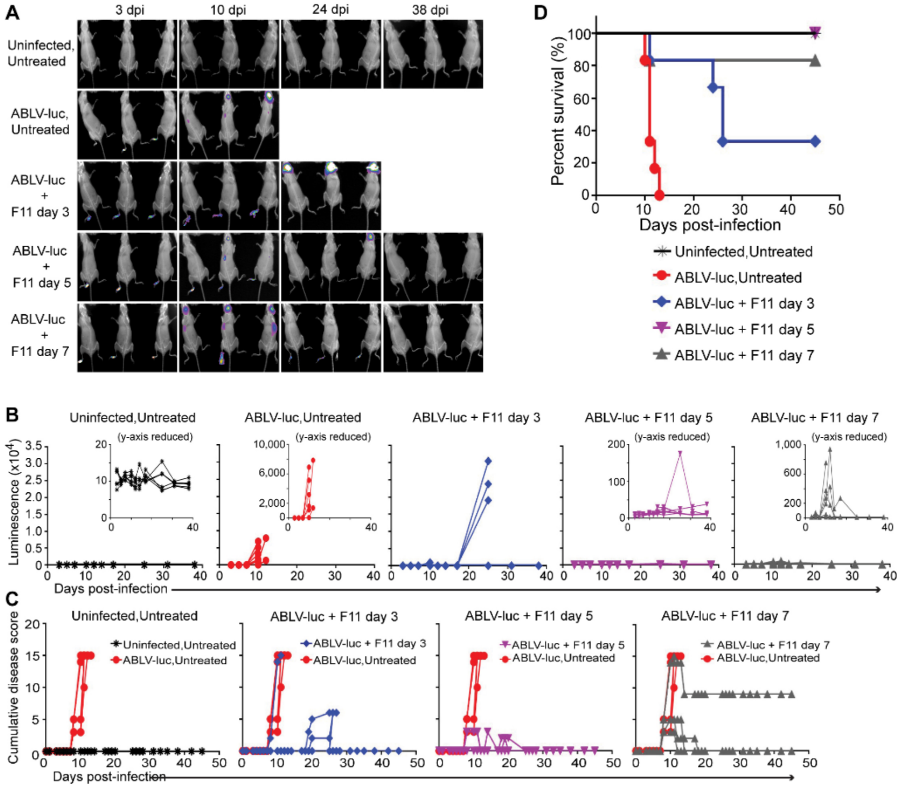


Figure xxx. Single-dose F11 mAb therapy reverses disease signs and prevents mortality in animals with CNS-resident lyssavirus infection. A. ABLV-luc bioluminescence (pseudocolor intensity) overlaid on X-ray images of mice at the indicated days post-infection. Treatments are indicated in the left column. mAb F11 was administered as a single dose of 10 mg/kg on the indicated day. **B.** Mean luminescence intensity (MLI) values from the head/brain region of the indicated infection and treatment groups. Inset y-axis scales are reduced (and **not** × 10^4), to enable clear visualization of lower luminescence values. **C.** Pairwise comparison of cumulative disease scores of different infection and treatment groups. **D.** Kaplan-Meier survival analysis of same groups of mice analyzed in panels A-C..

Importantly, in a distinct Schaefer lab-Broder lab collaborative project focused on development of novel lyssavirus-directed therapeutics, we have made the surprising finding that a single dose of a neutralizing human anti-lyssavirus G mAb, F11[53], can protect mice from a lethal lyssavirus infection, even when administered (via the IP route) after virus is already replicating robustly in the CNS (xxxRef or Fig?). Specifically, day 3, 5 and 7 treatments with mAb F11 had a dramatic effect on reducing virus burden in both the spine and brain during the acute phase of infection (through ~day 14), versus infected untreated controls (**Fig. xxxA-B** and data not shown). Additionally, although most infected, F11-treated animals experienced weight loss and other disease manifestations, F11 therapy completely reversed disease signs in the majority of these animals (some of the day 7 animals had permanent partial paralysis in the inoculated limb, but all animals recovered their lost weight) (**Fig. xxxC** and data not shown). Most remarkably, *100% of the day 5 and >80% of the day 7-treated animals survived* (**Fig. xxxD**). *Indeed, we have monitored day 5 and day 7 F11-treated animals for more than 4 months, and there has been no re-emergence of disease signs in any animal*. Recovery occurred despite the fact that the virus progressed to and replicated within the brain, as evidenced by bioluminescence imaging (**Fig. xxxA-B**). Thus, our data strongly suggest that single-dose human anti-ABLV mAb therapy provides long-term control of viral infection, even when therapy is administered after CNS entry. We also found that F11 therapy was effective against the lethal mouse-adapted rabies strain, CVS-11 (data not shown). Importantly, we have shown that the mechanism of efficacy requires participation of the adaptive immune response, particularly CD4 T cells, with F11 therapy leading to a CD4-dominant T cell infiltration in the brain (in contrast to the balanced CD4/CD8 ratio in the brains of untreated animals) (xxxRef). Moreover, mutation of the Fc region to impede FcR binding leads to dramatically-reduced F11 therapeutic efficacy (xxxRef). Collectively, these data demonstrate that an IP-administered mAb can have therapeutic efficacy against a *brain-resident* virus in a manner that requires CD4 T cells and a functional Fc region.

In this aim, we will investigate whether similar therapeutic efficacy can be achieved via IP administration of henipavirus-directed mAbs, following CNS invasion by chimeric rCedVs. We will also test therapeutic efficacy of nanobodies, including chimeric nanobodies engineered for transport across the BBB.

Experimental Design

IP-administration of mAbs and nanobody reagents as a therapy for CNS infections with rCedVs. Our preliminary data (**Fig. 8**) show that rCedV-NiV-luc reaches high expression in the brain of infected STAT1-/- animals by day 3, with initial disease signs apparent by day 8 and animals beginning to succumb to disease on day 11 (**Fig. 6**). For modeling of human henipavirus disease, we consider it most important to identify therapeutics that demonstrate efficacy in neutralizing virus that is already replicating in the nervous system, with the most broadly useful therapeutics being those that can protect from death after disease signs are apparent. We will thus investigate the day 3-10 post-infection period for administration of therapeutic antibodies, with initial studies investigating therapeutic efficacy on (a) day 3 (early post-CNS entry) and (b) the first day animals show disease signs. Time points for later experiments will be determined based on the results from these initial time points (e.g., if therapy is effective on the first day post-appearance of disease signs, later post-disease sign time points will be tested). As we already have stocks of neutralizing mAbs m102.4 (highly potent human anti-HeV G which cross-neutralizes NiV G[54]) and 1F5 (mouse anti-HeV/NiV F[55]), we will use these candidate therapeutics for our initial investigations.

STAT1-/- albino mice will be infected with rCedV-NiV-luc or rCedV-HeV-luc and treated with m102.4 or 1F5 (with a control group given a non-henipavirus specific mAb of the same isotype). Initial experiments will employ a single 10 mg/kg dose at d3 or d8 post-infection. Viral replication will be monitored by bioluminescence imaging, and cumulative disease scores and mortality will be recorded for each animal over the 21-day experimental time frame. Successful therapeutics will be those that both limit viral replication in the brain and reduce disease manifestations and mortality. In follow-on experiments, the antibody administration protocol may be adjusted, depending on strength of the therapeutic effect, e.g., the mAb could be delivered in a larger dose or in multiple doses over several days. In collaboration with **RP5**, we will also perform therapeutic efficacy experiments as described above with newly developed nanobody reagents that perform well during *in vitro* testing. Of particular interest are chimeric nanobodies that have been engineered to cross the BBB. Such reagents have the potential to both enhance the adaptive immune response and directly neutralize virus in the CNS, following transport across the BBB.

Mechanistic studies of therapeutic antibody efficacy. For any mAb or nanobody found to be therapeutically effective, we will perform additional experiments to investigate the mechanism. To define the importance of Fc function, we will generate a LALA-PG mutant (xxxRef) of the therapeutic mAb, to impair FcR binding and complement activation. Infected mice will be given a control mAb, the WT therapeutic mAb or the LALA-PG mutant. Analysis of outcomes will be as described above. To define gene expression and cellular changes that correlate with therapeutic efficacy, we will use the transcriptomics, histological and flow cytometry approaches of **Aim 2** as independent avenues to analyze the impact of therapies on animal brains. In this way, we can identify the experimental paradigms that lead to the highest therapeutic efficacy. Such data will also contribute to the **Aim 2** goal of defining cellar and transcriptional responses that correlate with pathogenesis.

Expected Results

Given our results with mAb F11 in the lyssavirus system (Fig xxx and xxxRef), we expect to demonstrate significant therapeutic efficacy via IP administration of m102.4 and 1F5 in the d3 – d10 window during which virus is replicating in the brain. Indeed, we have demonstrated >80% efficacy with administration of mAb F11 on d7 (we have not yet attempted later time points). Animals typically first exhibit disease signs on d8 – d10 and generally reach euthanasia criteria 3 – 4 days later. Although mice administered F11 on d7 still exhibit disease signs (Fig xxx and xxxRef), these signs reverse and most animals survive. Thus, the mAb-induced therapeutic effect on CNS disease is relatively rapid, resulting in survival of animals that would otherwise succumb to infection within 4 – 7 days. In our initial experiment with STAT1-/- mice (**Fig. 6**), animals infected with rCedV-NiV-luc reached euthanasia criteria 3 – 7 days after first showing disease signs. Thus, if there is a similar therapeutic effect of mAbs during rCedV-chimera infection of STAT1-/- mice, it is reasonable to expect that mortality rates will drop in response to therapeutic mAbs, possibly even after disease signs are apparent.

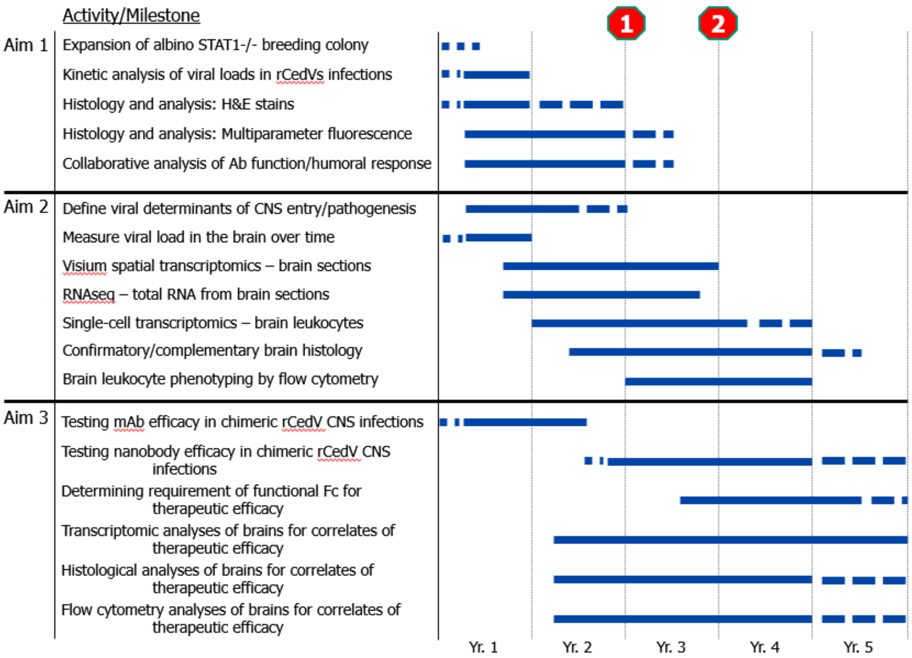
Because the nanobodies produced in **RP5** will include the IgG1 Fc domain, we also anticipate that these reagents will exhibit therapeutic efficacy. Although it is difficult to predict which therapeutics will perform best, we expect chimeric nanobodies engineered for transport across the BBB may demonstrate enhanced efficacy. These therapeutics could potentially both directly neutralize virus in the brain, and facilitate an effective CD4 T cell antiviral response in the brains of infected animals, as we have observed in our lyssavirus work (xxxRef). If BBB-crossing nanobodies prove effective, we predict that multiple doses would increase efficacy, as this approach should lead to higher concentrations of neutralizing nanobodies in the brains of infected animals. For all effective therapeutic antibody reagents, we predict that the LALA-PG mutation will impair functionality, as an intact Fc region appears critical for promoting an effective CD4 T cell response in the brain (xxxRef).

By applying transcriptomics, histological and flow cytometry approaches to the analysis of brains of animals that experience therapeutic benefit from antibody therapy, we expect to identify therapy-driven changes in gene transcription, cellularity and/or cellular phenotype in the brains of treated animals. Such data could suggest mechanisms by which mAb therapy prevents virus-induced death, as well as providing correlates of therapeutic efficacy for development of other types of therapeutics (e.g., small molecule drugs). As discussed in **Aim 2**, the mechanism by which NiV and HeV induce encephalitic death is still undetermined (xxxRef). Thus, the mechanistic studies in this Aim could synergize with **Aim 2** findings to yield novel insights into mechanisms of henipavirus neuropathogenesis, thereby suggesting key target pathways for development of future small molecule therapeutics.

Pitfalls and Alternative Approaches

Based on our surprising findings regarding the potency of IP-administered therapeutics in the lyssavirus system (Fig xxx and xxxRef), we anticipate a similar mechanism and efficacy against chimeric rCedVs with IP-administered neutralizing antibodies. However, it is possible that the mechanism of mAb enhancement of the CNS-directed CD4 T cells response is specific to the lyssavirus model, and will not be generalized to the henipavirus model. If our early data suggest such an outcome, we will focus our efforts on the nanobodies of **RP5**, engineered to cross the BBB and directly neutralize virus in the brain. Another possibility is that IP administered mAbs are effective at early times post-CNS entry of rCedVs, but ineffective once disease signs appear. With such an outcome, we focus our efforts on (i) identification of the most effective mAb/nanobody reagent, and (ii) optimization of the dosing amount and schedule to achieve efficacy as late as possible post-CNS invasion.

Project Milestones and Timelines



Gannt chart outlining project milestones (left). Solid lines represent periods of likely activity to achieve milestone; dashed lines indicate periods of possible early initiation or extended completion of activities. Go/no-go decision points are indicated by *red octagons*.

Go/no-go#1: **Cessation of pathogenesis-associated goals** if neuropathogenesis of rCedV-chimera infections of albino-STAT1-/- mice clearly has no overlapping features with known pathology in currently accepted animal models or human post-mortem cases. Note that therapeutics can still be evaluated on the basis of reduction of viral loads in the brain.

Go/no-go#2: **Cessation of Aim 3 studies** if there is no sign of CNS therapeutic efficacy from any tested mAb or nanobody (*note:* decision will be delayed if not all major planned nanobody designs have been generated by **RP5** AND tested by **RP3**, by the end of year 3).

References

1. Mathieu, C. and B. Horvat, *Henipavirus pathogenesis and antiviral approaches.* Expert Rev Anti Infect Ther, 2015. **13**(3): p. 343-54.
2. Broder, C.C., K. Xu, D.B. Nikolov, Z. Zhu, D.S. Dimitrov, D. Middleton, J. Pallister, T.W. Geisbert, K.N. Bossart, and L.F. Wang, *A treatment for and vaccine against the deadly Hendra and Nipah viruses.* Antiviral Res, 2013. **100**(1): p. 8-13.
3. Chua, K.B., S.K. Lam, C.T. Tan, P.S. Hooi, K.J. Goh, N.K. Chew, K.S. Tan, A. Kamarulzaman, and K.T. Wong, *High mortality in Nipah encephalitis is associated with presence of virus in cerebrospinal fluid.* Ann Neurol, 2000. **48**(5): p. 802-5.
4. Geisbert, T.W., H. Feldmann, and C.C. Broder, *Animal challenge models of henipavirus infection and pathogenesis.* Curr Top Microbiol Immunol, 2012. **359**: p. 153-77.
5. Bishop, K.A. and C.C. Broder, *Hendra and Nipah: Lethal Zoonotic Paramyxoviruses*, in *Emerging Infections*, W.M. Scheld, S.M. Hammer, and J.M. Hughes, Editors. 2008, American Society for Microbiology: Washington, D.C. p. 155-187.
6. Eaton, B.T., C.C. Broder, D. Middleton, and L.F. Wang, *Hendra and Nipah viruses: different and dangerous.* Nat Rev Microbiol, 2006. **4**(1): p. 23-35.
7. Marsh, G.A., C. de Jong, J.A. Barr, M. Tachedjian, C. Smith, D. Middleton, M. Yu, S. Todd, A.J. Foord, V. Haring, J. Payne, R. Robinson, I. Broz, G. Crameri, H.E. Field, and L.F. Wang, *Cedar virus: a novel Henipavirus isolated from Australian bats.* PLoS pathogens, 2012. **8**(8): p. e1002836.
8. Mastraccio, K.E., C. Huaman, E.D. Laing, C.C. Broder, and B.C. Schaefer, *Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging.* Methods Mol Biol, 2022. **2524**: p. 369-394.
9. Mastraccio, K.E., C. Huaman, D. Warrilow, G.A. Smith, S.B. Craig, D.L. Weir, E.D. Laing, I.L. Smith, C.C. Broder, and B.C. Schaefer, *Establishment of a longitudinal pre-clinical model of lyssavirus infection.* J Virol Methods, 2020. **281**: p. 113882.
10. Skowron, K., J. Bauza-Kaszewska, K. Grudlewska-Buda, N. Wiktorczyk-Kapischke, M. Zacharski, Z. Bernaciak, and E. Gospodarek-Komkowska, *Nipah Virus-Another Threat From the World of Zoonotic Viruses.* Front Microbiol, 2021. **12**: p. 811157.
11. Uday, U., L.J. Tadi, Z. Islam, P. Mohanan, S. Ghazanfar, M.S. Babar, and S. Ismail, *Zika, Nipah and Kala-azar: Emerging lethal infectious diseases amid COVID-19 as an escalating public health threat in South India.* Ann Med Surg (Lond), 2022. **79**: p. 103972.
12. Arunkumar, G., R. Chandni, D.T. Mourya, S.K. Singh, R. Sadanandan, P. Sudan, B. Bhargava, P. Nipah Investigators, and G. Health Study, *Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018.* J Infect Dis, 2019. **219**(12): p. 1867-1878.
13. Luby, S.P. and C.C. Broder, *Paramyxoviruses: Henipaviruses*, in *Viral Infections of Humans, Epidemiology and Control*, R.A. Kaslow, L.R. Stanberry, and J.W. Le Duc, Editors. 2014, Springer Science+Business Media: New York. p. 519-536.
14. Broder, C.C., T.W. Geisbert, K. Xu, D.B. Nikolov, L.F. Wang, D. Middleton, J. Pallister, and K.N. Bossart, *Immunization strategies against henipaviruses.* Curr Top Microbiol Immunol, 2012. **359**: p. 197-223.
15. Sweileh, W.M., *Global research trends of World Health Organization's top eight emerging pathogens.* Global Health, 2017. **13**(1): p. 9.
16. Luby, S.P., *The pandemic potential of Nipah virus.* Antiviral Res, 2013. **100**(1): p. 38-43.
17. Drexler, J.F., V.M. Corman, M.A. Muller, G.D. Maganga, P. Vallo, T. Binger, F. Gloza-Rausch, V.M. Cottontail, A. Rasche, S. Yordanov, A. Seebens, M. Knornschild, S. Oppong, Y. Adu Sarkodie, C. Pongombo, A.N. Lukashev, J. Schmidt-Chanasit, A. Stocker, A.J. Carneiro, S. Erbar, A. Maisner, F. Fronhoffs, R. Buettner, E.K. Kalko, T. Kruppa, C.R. Franke, R. Kallies, E.R. Yandoko, G. Herrler, C. Reusken, A. Hassanin, D.H. Kruger, S. Matthee, R.G. Ulrich, E.M. Leroy, and C. Drosten, *Bats host major mammalian paramyxoviruses.* Nat Commun, 2012. **3**: p. 796.
18. Wu, Z., L. Yang, F. Yang, X. Ren, J. Jiang, J. Dong, L. Sun, Y. Zhu, H. Zhou, and Q. Jin, *Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012.* Emerg Infect Dis, 2014. **20**(6): p. 1064-6.
19. Zhang, X.A., H. Li, F.C. Jiang, F. Zhu, Y.F. Zhang, J.J. Chen, C.W. Tan, D.E. Anderson, H. Fan, L.Y. Dong, C. Li, P.H. Zhang, Y. Li, H. Ding, L.Q. Fang, L.F. Wang, and W. Liu, *A Zoonotic Henipavirus in Febrile Patients in China.* N Engl J Med, 2022. **387**(5): p. 470-472.
20. Madera, S., A. Kistler, H.C. Ranaivoson, V. Ahyong, A. Andrianiaina, S. Andry, V. Raharinosy, T.H. Randriambolamanantsoa, N.A.F. Ravelomanantsoa, C.M. Tato, J.L. DeRisi, H.C. Aguilar, V. Lacoste, P.

Dussart, J.M. Heraud, and C.E. Brook, *Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar.* J Virol, 2022. **96**(18): p. e0092122.

21. Lieu, K.G., G.A. Marsh, L.F. Wang, and H.J. Netter, *The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2.* Antiviral Res, 2015. **124**: p. 69-76.
22. Schountz, T., C. Campbell, K. Wagner, J. Rovnak, C. Martellaro, B.L. DeBuysscher, H. Feldmann, and J. Prescott, *Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters.* Viruses, 2019. **11**(3).
23. Satterfield, B.A., R.W. Cross, K.A. Fenton, K.N. Agans, C.F. Basler, T.W. Geisbert, and C.E. Mire, *The immunomodulating V and W proteins of Nipah virus determine disease course.* Nat Commun, 2015. **6**: p. 7483.
24. Yoneda, M., V. Guillaume, H. Sato, K. Fujita, M.C. Georges-Courbot, F. Ikeda, M. Omi, Y. Muto-Terao, T.F. Wild, and C. Kai, *The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals.* PLoS One, 2010. **5**(9): p. e12709.
25. Lawrence, P. and B. Escudero-Perez, *Henipavirus Immune Evasion and Pathogenesis Mechanisms: Lessons Learnt from Natural Infection and Animal Models.* Viruses, 2022. **14**(5).
26. Laing, E.D., C.K. Navaratnarajah, S. Cheliout Da Silva, S.R. Petzing, Y. Xu, S.L. Sterling, G.A. Marsh, L.F. Wang, M. Amaya, D.B. Nikolov, R. Cattaneo, C.C. Broder, and K. Xu, *Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus.* Proc Natl Acad Sci U S A, 2019. **116**(41): p. 20707-20715.
27. Laing, E.D., M. Amaya, C.K. Navaratnarajah, Y.R. Feng, R. Cattaneo, L.F. Wang, and C.C. Broder, *Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species.* Virol J, 2018. **15**(1): p. 56.
28. Amaya, M., H. Cheng, V. Borisevich, C.K. Navaratnarajah, R. Cattaneo, L. Cooper, T.W. Moore, I.N. Gaisina, T.W. Geisbert, L. Rong, and C.C. Broder, *A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery.* Antiviral Res, 2021. **193**: p. 105084.
29. Harcourt, B.H., L. Lowe, A. Tamin, X. Liu, B. Bankamp, N. Bowden, P.E. Rollin, J.A. Comer, T.G. Ksiazek, M.J. Hossain, E.S. Gurley, R.F. Breiman, W.J. Bellini, and P.A. Rota, *Genetic characterization of Nipah virus, Bangladesh, 2004.* Emerg Infect Dis, 2005. **11**(10): p. 1594-7.
30. Lo, M.K., B.H. Harcourt, B.A. Mungall, A. Tamin, M.E. Peeples, W.J. Bellini, and P.A. Rota, *Determination of the henipavirus phosphoprotein gene mRNA editing frequencies and detection of the C, V and W proteins of Nipah virus in virus-infected cells.* J Gen Virol, 2009. **90**(Pt 2): p. 398-404.
31. Mathieu, C., V. Guillaume, V.A. Volchkova, C. Pohl, F. Jacquot, R.Y. Looi, K.T. Wong, C. Legras-Lachuer, V.E. Volchkov, J. Lachuer, and B. Horvat, *Nonstructural Nipah virus C protein regulates both the early host proinflammatory response and viral virulence.* J Virol, 2012. **86**(19): p. 10766-75.
32. Satterfield, B.A., V. Borisevich, S.L. Foster, S.E. Rodriguez, R.W. Cross, K.A. Fenton, K.N. Agans, C.F. Basler, T.W. Geisbert, and C.E. Mire, *Antagonism of STAT1 by Nipah virus P gene products modulates disease course but not lethal outcome in the ferret model.* Sci Rep, 2019. **9**(1): p. 16710.
33. Lo, M.K., M.E. Peeples, W.J. Bellini, S.T. Nichol, P.A. Rota, and C.F. Spiropoulou, *Distinct and overlapping roles of Nipah virus P gene products in modulating the human endothelial cell antiviral response.* PLoS One, 2012. **7**(10): p. e47790.
34. Keiffer, T.R., M.J. Ciancanelli, M.R. Edwards, and C.F. Basler, *Interactions of the Nipah Virus P, V, and W Proteins across the STAT Family of Transcription Factors.* mSphere, 2020. **5**(6).
35. Becker, N. and A. Maisner, *Nipah Virus Impairs Autocrine IFN Signaling by Sequestering STAT1 and STAT2 into Inclusion Bodies.* Viruses, 2023. **15**(2).
36. Talbot-Cooper, C., T. Pantelejevs, J.P. Shannon, C.R. Cherry, M.T. Au, M. Hyvonen, H.D. Hickman, and G.L. Smith, *Poxviruses and paramyxoviruses use a conserved mechanism of STAT1 antagonism to inhibit interferon signaling.* Cell Host Microbe, 2022. **30**(3): p. 357-372 e11.
37. Childs, K., R. Randall, and S. Goodbourn, *Paramyxovirus V proteins interact with the RNA Helicase LGP2 to inhibit RIG-I-dependent interferon induction.* J Virol, 2012. **86**(7): p. 3411-21.
38. Rodriguez, J.J., J.P. Parisien, and C.M. Horvath, *Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation.* J Virol, 2002. **76**(22): p. 11476-83.
39. Dhondt, K.P., C. Mathieu, M. Chalons, J.M. Reynaud, A. Vallve, H. Raoul, and B. Horvat, *Type I interferon signaling protects mice from lethal henipavirus infection.* J Infect Dis, 2013. **207**(1): p. 142-51.

40. Sadikot, R.T. and T.S. Blackwell, *Bioluminescence: imaging modality for in vitro and in vivo gene expression.* Methods Mol Biol, 2008. **477**: p. 383-94.
41. Piersma, S.J. and I. Brizic, *Natural killer cell effector functions in antiviral defense.* FEBS J, 2022. **289**(14): p. 3982-3999.
42. Chen, M., M. Tachedjian, G.A. Marsh, J. Cui, and L.F. Wang, *Distinct Cell Transcriptomic Landscapes Upon Henipavirus Infections.* Front Microbiol, 2020. **11**: p. 986.
43. Gunn, B.M., V. Roy, M.M. Karim, J.N. Hartnett, T.J. Suscovich, A. Goba, M. Momoh, J.D. Sandi, L. Kanneh, K.G. Andersen, J.G. Shaffer, J.S. Schieffelin, R.F. Garry, D.S. Grant, and G. Alter, *Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses.* J Infect Dis, 2020. **221**(1): p. 156-161.
44. Gunn, B.M., R.P. McNamara, L. Wood, S. Taylor, A. Devadhasan, W. Guo, J. Das, A. Nilsson, A. Shurtleff, S. Dubey, M. Eichberg, T.J. Suscovich, E.O. Saphire, D. Lauffenburger, B.A. Coller, J.K. Simon, and G. Alter, *Antibodies against the Ebola virus soluble glycoprotein are associated with long-term vaccine-mediated protection of non-human primates.* Cell Rep, 2023. **42**(4): p. 112402.
45. Guillaume, V., K.T. Wong, R.Y. Looi, M.C. Georges-Courbot, L. Barrot, R. Buckland, T.F. Wild, and B. Horvat, *Acute Hendra virus infection: Analysis of the pathogenesis and passive antibody protection in the hamster model.* Virology, 2009. **387**(2): p. 459-65.
46. Williamson, M.M. and F.J. Torres-Velez, *Henipavirus: a review of laboratory animal pathology.* Vet Pathol, 2010. **47**(5): p. 871-80.
47. Lee, B., C.C. Broder, and L. Wang, *Henipaviruses: Hendra and Nipah Viruses*, in *Fields Virology: Emerging Viruses*, P.M. Howley, D.M. Knipe, and S.P. Whelan, Editors. 2019, Wolters Kluwer. p. 559-595.
48. Wong, K.T. and K.C. Ong, *Pathology of acute henipavirus infection in humans and animals.* Patholog Res Int, 2011. **2011**: p. 567248.
49. Pantaleo, G., B. Correia, C. Fenwick, V.S. Joo, and L. Perez, *Antibodies to combat viral infections: development strategies and progress.* Nat Rev Drug Discov, 2022. **21**(9): p. 676-696.
50. Johnson, K., M. Vu, and A.N. Freiberg, *Recent advances in combating Nipah virus.* Fac Rev, 2021. **10**: p. 74.
51. Chan, K.R., E.Z. Ong, D.Z. Mok, and E.E. Ooi, *Fc receptors and their influence on efficacy of therapeutic antibodies for treatment of viral diseases.* Expert Rev Anti Infect Ther, 2015. **13**(11): p. 1351-60.
52. Keeler, S.P. and J.M. Fox, *Requirement of Fc-Fc Gamma Receptor Interaction for Antibody-Based Protection against Emerging Virus Infections.* Viruses, 2021. **13**(6).
53. Weir, D.L., S.A. Coggins, B.K. Vu, J. Coertse, L. Yan, I.L. Smith, E.D. Laing, W. Markotter, C.C. Broder, and B.C. Schaefer, *Isolation and Characterization of Cross-Reactive Human Monoclonal Antibodies That Potently Neutralize Australian Bat Lyssavirus Variants and Other Phylogroup 1 Lyssaviruses.* Viruses, 2021. **13**(3).
54. Zhu, Z., K.N. Bossart, K.A. Bishop, G. Crameri, A.S. Dimitrov, J.A. McEachern, Y. Feng, D. Middleton, L.F. Wang, C.C. Broder, and D.S. Dimitrov, *Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody.* J Infect Dis, 2008. **197**(6): p. 846-53.
55. Dang, H.V., R.W. Cross, V. Borisevich, Z.A. Bornholdt, B.R. West, Y.P. Chan, C.E. Mire, S.C. Da Silva, A.S. Dimitrov, L. Yan, M. Amaya, C.K. Navaratnarajah, L. Zeitlin, T.W. Geisbert, C.C. Broder, and D. Veesler, *Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins.* Nat Struct Mol Biol, 2021. **28**(5): p. 426-434.

Resource Sharing Plan

No model organisms will be generated from this research. Strains of mice to be used in this proposal are directly available from Jackson labs, or will be derived via breeding of these commercial strains. As these strains are readily available to the scientific community, no further action on our part is required.

All genomic data resulting from this project will be deposited in NCBI GEO (Gene Expression Omnibus) prior to or concurrent with publication.

In general, we will adhere to NIH policies regarding sharing of resources and data (https://sharing.nih.gov/). Our lab has demonstrated its commitment to sharing by providing published reagents upon request for the last 20+ years. Regarding any novel materials and resources that may be generated in this proposal, the general Resource Sharing Plan developed by the Henry M. Jackson Foundation and USUHS is as follows: Unique research resources, research tools, novel materials, including model organisms or modified strains, and related protocols, genetic and phenotypic data (including data from Genome-Wide Association Studies) that may arise out of the funded studies will be made available to others in the private and public sector as soon as appropriate agreements covering such transfer can be executed. In the case of project data, findings shall be published as soon as possible or otherwise shared with other researchers pursuant to NIH policies regarding data sharing. The USUHS-HJF Joint Office of Technology Transfer (JOTT) will manage all technology transfer activities for data and resources generated by researcher of either USUHS or HJF. The JOTT will adhere to all applicable policies, principles, guidelines and procurement rules in making unique research resources readily available for research purposes to qualified individuals and entities, and in ensuring that there is no more than a short–term restriction on publication or public dissemination of data in order for the JOTT to evaluate and file for patent protection on any subject invention that may arise from the funded study. Dissemination of unpublished data that is considered to be proprietary or confidential shall occur pursuant to a suitable confidential disclosure agreement prepared by the JOTT. Patient data shall be de-identified and shared in accordance with USU and HJF policies and applicable laws and regulations. Data associated with Genome-Wide Association Studies (GWAS) will be submitted to the NIH-designated GWAS data repository. Material transfer agreements, license agreements, and Cooperative Research and Development Agreements (CRADAs), as appropriate, will be used to transfer material property. Material transfer agreements will be established to share resources among the research community for noncommercial research use.

USUHS

Site Principal Investigator: Brian Schaefer, Ph.D.

Select Agents

No select agents are used in this research.

We will perform animal studies with recombinant forms of Cedar virus. These viruses are categorized as BSL-2. Dr. Schaefer has an approved BSL-2 biosafety protocol for research with Cedar virus, including in vivo ABSL-2 studies. All members of the Schaefer research group have received specific training in laboratory safety and the proper use and disposal of BSL-2 organisms (and two current laboratory members have certifications for BSL-3). Dr. Schaefer has BSL-2 certified laboratories where work with these agents will be conducted. Animal experiments are conducted in approved ABSL-2 rooms within the USU animal facility, conducted under USU IACUC-approved protocols.

Specific Aims

Henipaviruses are single-stranded, negative-sense enveloped RNA viruses of the paramyxovirus family[1]. Two henipaviruses, Nipah virus (NiV) and Hendra virus (HeV), cause a systemic and often fatal respiratory and/or encephalitic disease in humans and ten other mammalian species[2-4]. Importantly, NiV and HeV are significant biothreats to humans and economically important livestock in Australia and Southeast Asia[5]. There are currently no vaccines or therapeutics approved for human use. Notably, development of countermeasures for NiV and HeV is hampered by the fact that these viruses require BSL-4 containment, meaning that very few research groups worldwide have access to the required biocontainment facilities to perform preclinical studies with these important human viral pathogens[6].

To address this problem, we are developing a BSL-2 animal model that is based on Cedar virus (CedV), which is a non-pathogenic henipavirus[7]. Specifically, we have engineered recombinant Cedar viruses (rCedVs) in which the NiV and HeV fusion (F) and receptor-binding glycoprotein (G) are expressed in the rCedV genome, replacing CedV F and G. Additionally, we have also incorporated our recently developed *in vivo* bioluminescence methodology[8, 9] to longitudinally trace the dynamics and anatomical progression of rCedV-luciferase (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, we have demonstrated sustained replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Importantly, whereas rCedV-luc does not establish stable expression in the brain, both of the chimeric viruses do. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. The rCedV-luc platform is thus an authentic henipavirus system that can be used to study henipavirus *in vivo* biology safely and expediently under BSL-2 containment. Our **overall hypothesis** is that rCedV-luc infection of mice lacking interferon responses represents a BSL-2 platform for the study of henipavirus biology and pathogenesis, as well as for development and testing of anti-viral countermeasures. We will address this hypothesis through three Specific Aims:

Aim 1: To optimize the use of Stat1-/- mice and rCedV-NiV-luc/rCedV-HeV-luc chimeras as a robust BSL2 model of pathogenic henipavirus disease. Hypothesis: *Infection of Stat1-/- mice with recombinant rCedVs will recapitulate major hallmarks of pathogenic NiV and HeV infections in humans*. We will establish a breeding colony of Stat1-/- mice on a B6-albino background. We will infect Stat1-/- mice with rCedV-luc chimeras. We will use flow cytometry, spatial transcriptomics approaches and histological methods to define cell types infected, gene expression changes and immune phenotypes that characterize pathological (Stat1-/- model; rCedV-luc chimeras) vs. non-pathological (Ifnar1-/- model; rCedV-luc) infections.

Commented [BS1]: -Collaboration with Core D (systems serology - Gunn)
-Collab with RP2 (other rCedV chimeras)
-Possible exploration of hamster infection (rCedV-NiV-luc), using much larger dose than what was used for UTMB studies to determine if (1) efficient CNS infection can be achieved and (2) if CNS infection is highly correlated with lethality. Hamsters may represent a rCedV lethal model employing a host that is not immunocompromised. Limitations: few specific analysis tools (mAbs in particular) for the hamster model.

Aim 2: To determine the mechanism of rCedV-NiV neurovirulence in the Stat1-/- mouse model. Hypothesis: *CNS infection by rCedV chimeras requires functional interaction between G and host ephrin B3; neurovirulence is a property shared by henipaviruses in the absence of host IFN-dependent defenses*. We will evaluate whether interaction between NiV/HeV G and host ephrin B3 is required for neurotropism. We will employ direct injection of rCedV into the brains of Stat1-/- mice to determine whether neurological disease is a property shared by brain-resident henipaviruses in the absence of a host interferon response.

Commented [BS2]: -Collab with RP2 (rCedV-NiV ephrin B3 binding mutant)

Aim 3: To define the efficacy and mechanisms of mAb-based therapeutics for CNS-resident henipavirus infections. Hypothesis: *G- and F-directed mAb-based therapeutics will demonstrate Fc-dependent efficacy against CNS-resident henipavirus*. Stat1-/- mice infected with rCedV chimeras will be treated with anti-G and anti-F mAbs and nanobodies. We will employ the approaches of Aim 1 to determine the immunological basis of any observed mAb therapeutic effects. We will also specifically investigate the role of mAb-FcR interactions for therapeutic efficacy.

Commented [BS3]: -Collaboration with RP5, nanobodies
- Collaboration with Core D (systems serology - Gunn)

HeV and NiV are emerging human and livestock pathogens with a high fatality rate, demonstrated human-to-human transmission, and a worrisome recent history of outbreaks in proximity to densely populated areas, including Kerala, India[10-12]. There is thus an urgent need to develop novel therapeutics directed against these viruses. However, work with HeV and NiV requires BSL-4 containment, severely limiting translational research efforts. Through the development and investigation of this longitudinal BSL-2 animal model of authentic and pathological henipavirus infection, we will greatly facilitate future development and preclinical validation of therapeutics directed against these deadly human pathogens.

Vertebrate Animals

1. The following mouse strains will be used in this proposal:
   - **i)** **B6-albino-Ifnar1-/- mice**. We maintain a breeding colony of B6-albino Ifnar1-/- mice at Charles River Labs (CRL). We generated these mice by crossing the B6-albino strain (Jackson Laboratory B6(Cg)-Tyrc-2J/J, Strain #:000058) with the Ifnar1-/- strain (Jackson Laboratory B6(Cg)-Ifnar1<tm1.2Ees>/J, Strain # 028288), followed by crossing F1 mice back to B6-albino and then intercrossing white progeny that carried the Infar1- allele. This colony is now producing only albino mice that are homozygous Ifnar1-/-.
   - **ii)** **B6-albino-STAT1-/- mice.** We are currently establishing a breeding colony of B6-albino STAT1-/- mice at CRL. We are currently crossing the B6-albino strain (Jackson Laboratory B6(Cg)-Tyrc-2J/J, Strain #:000058) with the STAT1-/- strain (Jackson Laboratory B6.129S(Cg)-Stat1tm1Dlv/J, Strain # 012606), followed by crossing F1 mice back to B6-albino and then intercrossing white progeny that carry the STAT1- allele. We anticipate establishing a colony of the desired genotype by early 2024..

   We will use mice for in vivo infection with rCedVs, with many experiments including bioluminescent imaging. Animals are housed in an approved ABSL-2 room and all laboratory and animal care staff follow BSL-2 safety precautions. The total number of required mice (including all mice produced during breeding) is 5440. Experiments will be performed with male and female mice. The PI has an IACUC-approved experimental protocol and an IBC-approved biosafety protocol that details the use of the above strains of mice for the described experiments.

2. The goal of this project is to validate the mouse as a BSL-2 model for henipavirus pathogenesis, and as a platform for development and testing of countermeasures. For this work, we must use an experimental animal model to allow the course of in vivo infection and the host immune response to be studied. There is no in vitro model which can model the complex in vivo dynamics of viral infection, moving between distinct cell types and anatomical locations, or the host immune response to infection. We have chosen to utilize mice due to their ease of care, the existence of well-characterized inbred strains and knockout models, and our preliminary data with rCedVs showing robust and reproducible infection of mice which is highly amenable to statistical analysis. The number of animals to be used is based on power analyses, as detailed in our approved IACUC protocols.
3. Experiments will involve the euthanasia of infected animals experiencing pain or distress. Our IACUC protocols also define a weight loss threshold or a combined disease score criterion as end points for virus-infected animals requiring euthanasia. Animals not euthanized for the above reasons will be euthanized for tissue collection, at time points described in our IACUC protocol. Euthanasia will be performed as described in part 4, below.
4. Mice reaching criteria/endpoints described in 3, above, will be euthanized by ketamine or ketamine-xylazine overdose. This method is consistent with AMVA guidelines.

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	55,000.00	16,181.00	71,181.00
1	Graduate Students	12.00	0.00	0.00	40,514.00	11,919.00	52,433.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	71,250.00	20,962.00	92,212.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	40,521.00	11,921.00	52,442.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	12,056.00	3,547.00	15,603.00

5 **Total Number Other Personnel**

Total Other Personnel 283,871.00

Total Salary, Wages and Fringe Benefits (A+B) 283,871.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)
Ultralow temp (-80C) Freezer	12,123.00

Additional Equipment: ☐ Add Attachment ☐ Delete Attachment ☐ View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	12,123.00

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,000.00
2.	Foreign Travel Costs	3,000.00
	Total Travel Cost	6,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	
	Total Participant/Trainee Support Costs	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	48,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	34,728.00
9.	Translational Imaging Core (BRIC)	23,400.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	169,416.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	471,410.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	471,410.00	173,196.00
FY23 HJF Companywide G&A	16.70	644,606.00	107,649.00

Total Indirect Costs 280,845.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	752,255.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	752,255.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 03/01/2025 **End Date:** 02/28/2026

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	57,145.00	16,812.00	73,957.00
1	Graduate Students	12.00	0.00	0.00	42,094.00	12,384.00	54,478.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	74,029.00	21,779.00	95,808.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	42,101.00	12,386.00	54,487.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	12,526.00	3,685.00	16,211.00

5 **Total Number Other Personnel**

Total Other Personnel 294,941.00

Total Salary, Wages and Fringe Benefits (A+B) 294,941.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: ☐ Add Attachment ☐ Delete Attachment ☐ View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	48,000.00
2.	Publication Costs	5,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal Costs (procurement and husbandry)	48,046.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	203,334.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	498,275.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	498,275.00	183,067.00
FY23 HJF Companywide G&A	16.70	681,342.00	113,784.00

Total Indirect Costs 296,851.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	795,126.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	795,126.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 03/01/2026 **End Date:** 02/28/2027

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	59,374.00	17,468.00	76,842.00
1	Graduate Students	12.00	0.00	0.00	43,736.00	12,867.00	56,603.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	76,916.00	22,629.00	99,545.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	43,743.00	12,869.00	56,612.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	13,015.00	3,829.00	16,844.00

5 **Total Number Other Personnel**

Total Other Personnel 306,446.00

Total Salary, Wages and Fringe Benefits (A+B) 306,446.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: ☐ Add Attachment | Delete Attachment | View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	43,200.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	48,048.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	193,536.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	499,982.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	499,982.00	183,693.00
FY23 HJF Companywide G&A	16.70	683,673.00	114,173.00

Total Indirect Costs 297,866.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	797,848.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	797,848.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 03/01/2027 **End Date:** 02/28/2028

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	61,689.00	18,149.00	79,838.00
1	Graduate Students	12.00	0.00	0.00	45,441.00	13,369.00	58,810.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	79,916.00	23,511.00	103,427.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	45,449.00	13,371.00	58,820.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	13,522.00	3,978.00	17,500.00

5 **Total Number Other Personnel**

Total Other Personnel 318,395.00

Total Salary, Wages and Fringe Benefits (A+B) 318,395.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: ☐ Add Attachment ☐ Delete Attachment ☐ View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	33,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	48,048.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	183,336.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	501,731.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	501,732.00	184,336.00
FY23 HJF Companywide G&A	16.70	686,068.00	114,573.00

Total Indirect Costs 298,909.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	800,640.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	800,640.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 5

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 5** **Start Date:** 03/01/2028 **End Date:** 02/28/2029

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	64,095.00	18,857.00	82,952.00
1	Graduate Students	12.00	0.00	0.00	47,214.00	13,890.00	61,104.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	83,032.00	24,428.00	107,460.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	47,222.00	13,893.00	61,115.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	14,049.00	4,133.00	18,182.00

5 **Total Number Other Personnel**

Total Other Personnel 330,813.00

Total Salary, Wages and Fringe Benefits (A+B) 330,813.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	20,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	48,048.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	170,336.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	501,149.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	501,149.00	184,122.00
FY23 HJF Companywide G&A	16.70	685,271.00	114,440.00

Total Indirect Costs 298,562.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	799,711.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	799,711.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		0.00
Section B, Other Personnel		1,534,466.00
Total Number Other Personnel	25	
Total Salary, Wages and Fringe Benefits (A+B)		1,534,466.00
Section C, Equipment		12,123.00
Section D, Travel		6,000.00
1. Domestic	3,000.00	
2. Foreign	3,000.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		919,958.00
1. Materials and Supplies	192,200.00	
2. Publication Costs	5,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	226,918.00	
9. Other 2	179,400.00	
10. Other 3	53,040.00	
11. Other 4	263,400.00	
12. Other 5	0.00	
13. Other 6		
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		

Section G, Direct Costs (A thru F)	2,472,547.00
Section H, Indirect Costs	1,473,033.00
Section I, Total Direct and Indirect Costs (G + H)	3,945,580.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	3,945,580.00

No.	Instrument/Software Name	Type
1	10x Genomics Visium SpaceRanger	Software
2	Illumina bcl2fastq	Software
	10x Genomics Chromium CellRanger	Software

Manufacturer/Developer	Purpose	Key Features
10x Genomics	Spatial transcriptomics	Unbiased transcriptomics mapped to subregions of tissue sections
Illumina	bulk transcriptome profiling by RNA-seq	Unbiased transcriptomics
10x Genomics	Single Cell transcriptomics	Cell type/population specific transcriptomic expression values

Website/Contact Info	Notes	Data Output Format	Approximate File Size
https://www.10xgenomics.com/support/software/space-ranger/getting-started	Data will be deposited in NCBI GEO (Gene Expression Omnibus) as filtered gene count matrix, aligned images, spot coordinates from Visium analysis pipeline	Pipeline output, ancillary files and metadata	11 GB
https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software.html	Data will be deposited in NCBI GEO (Gene Expression Omnibus) as raw FASTQs and metabata	FASTQ	2 GB
https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger	Data will be deposited in NCBI GEO (Gene Expression Omnibus) as filtered gene count matrix from CellRanger analysis pipeline	Pipeline output, ancillary files and metadata	6 GB

Number of Files Expected
480
1440
320

No.	Instrument/Software Name	Type
1	CTL S6 Analyzer	Instrument
2	CTL Basic Count software	Software
3	GraphPad Prism 9	Software

Manufacturer/Developer	Purpose	Key Features
Immunospot	Read and quantify GFP foci from BSL-2 rCedV-GFP chimeric FRNT assays	Image and quantify the BSL-2 neutralization plates
Immunospot	Analyze the quantified GFP foci and convert to Neutralization percent	Generate Neutralization percent
GraphPad Software Inc.	Analyze the Neutralization percent to generate IC50 titers	Generate IC50 titers and graph dose response curves

Website/Contact Info	Notes	Data Output Format	Approximate File Size
https://immunospot.com/immunospot-s6-universal-m2.html	Additional Info	TIF image	300 KB
https://immunospot.com/immunospot-s6-universal-m2.html	Additional Info	Tabulated File	49 KB
https://www.graphpad.com/		TIF image and Data files	10,000 KB

Number of Files Expected
300 per year
300 per year
300 per year

utmb Health
Research Services

Research Services
Office of Sponsored Programs

301 University Blvd.
Galveston, TX 77555-0156
O 409.266.9400 F 409.266.9470
E sponsored.research@utmb.edu
W http://research.utmb.edu

Statement of Intent to Establish a Consortium Agreement with NIH Funds

Dates: 03/01/2024–02/28/2029
Grant: RFA-AI-23-020 Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picornaviridae
Application Title: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX)
Total Proposed Amount: $2,938,494
Sponsor (grantee Institution): The University of Texas Medical Branch at Galveston

The appropriate programmatic and administrative personnel of each institution involved in this grant application are aware of the NIH consortium grant policy and are prepared to establish the necessary inter-institutional agreement(s) consistent with that policy.

Subcontractor further certifies that it will comply with NIH public access policy, Division G, Title II, Section 218 of PL 110-161 (Consolidated Appropriations Act, 2008) requiring that any final manuscript resulting from research sponsored by NIH be submitted to the NIH upon acceptance for publication, including all modifications from the peer review process, thereby being made available to the public in PubMed Central as soon as possible but no later than 12 months after publication.

In accordance with the provisions of 42 CFR Part 50 Subpart F, 42 CFR Part 94 and the University of Texas System policy, UTS 175, the University of Texas Medical Branch at Galveston (UTMB Health) has revised its conflicts of interest policy (IHOP 11.1.2) in response to the newly enacted NIH rule. UTMB Health's revised conflicts of interest policy was effective January 1, 2012. As an awardee institution, UTMB Health must ensure that its sub-recipients are complying with all applicable policies, regulations and laws concerning Conflicts of Interest. Please complete and sign the attached Conflicts of Interest Disclosure Form for Sub-recipient Institutions.

The University of Texas Medical Branch
at Galveston UEI: MSPWVMXXMN76

The Henry M. Jackson Fdn for the Adv'mt of Mil
Med, Inc. UEI: UYLKBRENAPG5

(b) (6)

Dr. Thomas W. Geisbert Date
Professor/PI

Dr. Christopher Broder Date
Professor and Chair /Site Principal Investigator

Erika LeGros Date
Research Operations Manager
Office of Sponsored Programs

Lisa Straker Date
Deputy Director
Investigator Initiatives – Business Development
240-694-2262 | aims@hjf.org

*** If awarded, your organization will need to be registered (<u>and registration must be current</u>) with System for Award Management (SAM) before a contract can be issued. Please register at: https://www.sam.gov.***

Conflict of Interest Disclosure Form for Sub-recipient Institutions

Conflict of Interest Disclosure applies to any member of the research team responsible for the design, conduct or reporting of data (each a "Covered Individual")

Grant Award No. RFA-AI-23-020 PI at Institution: Christopher Broder, PhD

Institution Name: The Henry M. Jackson Fdn for the Adv'mt of Mil

Project Title: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX)

UTMB PI: Dr. Thomas W. Geisbert

Sub-recipient organization/institution hereby certifies that it has an active and enforced conflict of interest policy that is consistent with the provisions of 42 CFR Part 50 Subpart F or 42 CFR Part 94.

☒ YES **(If yes, please answer either a or b below.)**

☐ NO, Sub-recipient organization/institution does not have an active and/or enforced conflicts of interest policy and hereby agrees to comply with The University of Texas Medical Branch at Galveston (UTMB Health) policies and procedures relating to financial conflicts of interest. In accordance with this commitment, each Covered Individual as defined in UTMB Health's policy (IHOP 11.2.1) has completed UTMB Health conflict of interest training and disclosure attached hereto.

☒ **(a)** Sub-recipient organization/institution certifies that all personnel involved in the design, conduct and reporting of research data on the above-named project have completed and filed with sub-recipient organization/institution a current Conflict of Interest Disclosure form and none of the personnel involved has an identified Financial Conflict of Interest.

☐ **(b)** Sub-recipient organization/institution certifies that all personnel involved in the design, conduct and reporting of research data on the above-named project have completed and filed with sub-recipient organization/institution a current Conflict of Interest Disclosure form. The personnel listed below have an identified Financial Conflict of Interest which is being managed, reduced or eliminated by the sub-recipient organization/institution.

*In accordance with 42 CFR 50.604, sub-recipient organization/institution certifies that all personnel involved in the design, conduct or reporting of research data on the above-named project will complete training in regard to all applicable policies, regulations and laws before engaging in the research on the above named project.

Sub-recipient organization/institution certifies that the information provided above (if applicable, including any attached Disclosures) is(are) true and accurate to the best of its knowledge and further certifies that site and principal investigator shall comply with 42 CFR 50, Subpart F, Sections 50.601 to 50.607, responsibilities regarding conflicting interests of investigators. Sub-recipient organization/institution will notify UTMB within 30 days of any changes to a covered individual's status as it pertains to Financial Conflict of Interest.

(b) (6)

Signature of Authorized Representative Lisa Straker

Deputy Director Date

Investigator Initiatives-Business Development
Department Line: (240) 694-4016
Email: proposals@hjf.org

LETTER OF INTENT TO COLLABORATE

June 2, 2023

Thomas W. Geisbert, PhD
Center for Biodefense and Emerging Infectious Diseases
The University of Texas Medical Branch
Keiller Bldg., Rm. 1.104D
301 University Blvd.
Galveston, TX 77555-0609

Dear Dr. Geisbert:

This letter confirms The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.'s (HJF) intent to collaborate with The University of Texas Medical Branch (UTMB) in response to the RFA-AI-23-020 and is offered on the condition that the UTMB agrees that if it receives an award for the subject project, it will issue a subaward to HJF for the work hereby proposed.

Confidentiality Notice: *This letter of Intent, including all proposal documents attached hereto, contains HJF confidential and proprietary information and is provided for use solely in connection with HJF's and the (Prime Institution) joint pursuit of the project listed below. Any unauthorized use, disclosure or distribution is prohibited.*

Prime Institution:	The University of Texas Medical Branch	
Prime Principal Investigator:	Thomas W. Geisbert, PhD	
Project Title:	Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX)	
Proposed Principal Investigator:	Christopher Broder, PhD (USU-Principal Investigator)	
Proposed Project Period:	03/01/2024-02/28/2029	
Proposed Budget Amount:	$1,841,442 $1,097,052 $2,938,494	Direct Costs Indirect Costs Total Cost

HJF confirms that it has in place a written and enforced financial conflict of interest policy that applies to all HJF investigators and any other HJF employees responsible for the design, conduct or reporting of the budgeted activities. U.S. Government Principal Investigator(s) (if any) are subject to federal regulations governing conflicts of interest.

Your submission of a prime proposal that includes the subaward activities hereby proposed by HJF will constitute your acknowledgment of, and agreement to, the terms set forth in this letter.

Sincerely, (b) (6)

Lisa Straker-Deputy Director, Investigator Initiatives-Business Development

cc: Christopher Broder, PhD | Brian Schaefer, PhD

Investigator Initiatives-Business Development
Department Line: (240) 694-4016
Email: proposals@hjf.org

LETTER OF INTENT TO COLLABORATE

June 2, 2023

Thomas W. Geisbert, PhD
Center for Biodefense and Emerging Infectious Diseases
The University of Texas Medical Branch
Keiller Bldg., Rm. 1.104D
301 University Blvd.
Galveston, TX 77555-0609

Dear Dr. Geisbert:

This letter confirms The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.'s (HJF) intent to collaborate with The University of Texas Medical Branch (UTMB) in response to the RFA-AI-23-020 and is offered on the condition that the UTMB agrees that if it receives an award for the subject project, it will issue a subaward to HJF for the work hereby proposed.

Confidentiality Notice: *This letter of Intent, including all proposal documents attached hereto, contains HJF confidential and proprietary information and is provided for use solely in connection with HJF's and the (Prime Institution) joint pursuit of the project listed below. Any unauthorized use, disclosure or distribution is prohibited.*

Prime Institution:	The University of Texas Medical Branch	
Prime Principal Investigator:	Thomas W. Geisbert, PhD	
Project Title:	Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX)	
Proposed Principal Investigator:	Brian Schaefer, PhD (USUHS Principal Investigator)	
Proposed Project Period:	03/01/2024-02/28/2029	
Proposed Budget Amount:	$2,472,547	Direct Costs
	$1,473,033	Indirect Costs
	$3,945,580	Total Cost

HJF confirms that it has in place a written and enforced financial conflict of interest policy that applies to all HJF investigators and any other HJF employees responsible for the design, conduct or reporting of the budgeted activities. U.S. Government Principal Investigator(s) (if any) are subject to federal regulations governing conflicts of interest.

Your submission of a prime proposal that includes the subaward activities hereby proposed by HJF will constitute your acknowledgment of, and agreement to, the terms set forth in this letter.

Sincerely, (b) (6)

Lisa Straker-Deputy Director-Investigator Initiatives-Business Development

cc: Brian Schaefer, PhD

utmb Health
Research Services

Research Services
Office of Sponsored Programs

301 University Blvd.
Galveston, TX 77555-0156
O 409.266.9400 F 409.266.9470
E sponsored.research@utmb.edu
W http://research.utmb.edu

Statement of Intent to Establish a Consortium Agreement with NIH Funds

Dates: 03/01/2024-02/28/2029
Grant: RFA-AI-23-020 Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picornaviridae
Application Title: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX)
Total Proposed Amount: $3,945,580
Sponsor (grantee Institution): The University of Texas Medical Branch at Galveston

The appropriate programmatic and administrative personnel of each institution involved in this grant application are aware of the NIH consortium grant policy and are prepared to establish the necessary inter-institutional agreement(s) consistent with that policy.

Subcontractor further certifies that it will comply with NIH public access policy, Division G, Title II, Section 218 of PL 110-161 (Consolidated Appropriations Act, 2008) requiring that any final manuscript resulting from research sponsored by NIH be submitted to the NIH upon acceptance for publication, including all modifications from the peer review process, thereby being made available to the public in PubMed Central as soon as possible but no later than 12 months after publication.

In accordance with the provisions of 42 CFR Part 50 Subpart F, 42 CFR Part 94 and the University of Texas System policy, UTS 175, the University of Texas Medical Branch at Galveston (UTMB Health) has revised its conflicts of interest policy (IHOP 11.1.2) in response to the newly enacted NIH rule. UTMB Health's revised conflicts of interest policy was effective January 1, 2012. As an awardee institution, UTMB Health must ensure that its sub-recipients are complying with all applicable policies, regulations and laws concerning Conflicts of Interest. Please complete and sign the attached Conflicts of Interest Disclosure Form for Sub-recipient Institutions.

The University of Texas Medical Branch
at Galveston UEI: MSPWVMXXMN76

Dr. Thomas W. Geisbert Date
Professor/PI

Erika LeGros Date
Research Operations Manager
Office of Sponsored Programs

The Henry M. Jackson Fdn for the Adv'mt of Mil
Med, Inc. UEI: UYLKBRENAPG5

(b) (6)

Dr. Brian Schaefer Date
Professor /Site Principal Investigator

(b) (6)

Lisa Straker Date
Deputy Director
Investigator Initiatives – Business Development
240-694-2262 | lstraker@hjf.org

*** If awarded, your organization will need to be registered (<u>and registration must be current</u>) with System for Award Management (SAM) before a contract can be issued. Please register at: https://www.sam.gov.***

Conflict of Interest Disclosure Form for Sub-recipient Institutions

Conflict of Interest Disclosure applies to any member of the research team responsible for the design, conduct or reporting of data (each a "Covered Individual")

Grant Award No. RFA-AI-23-020 PI at Institution: Brian Schaefer, PhD

Institution Name: The Henry M. Jackson Fdn for the Adv'mt of Mil

Project Title: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX)

UTMB PI: Dr. Thomas W. Geisbert

Sub-recipient organization/institution hereby certifies that it has an active and enforced conflict of interest policy that is consistent with the provisions of 42 CFR Part 50 Subpart F or 42 CFR Part 94.

☒ YES **(If yes, please answer either a or b below.)**

☐ NO, Sub-recipient organization/institution does not have an active and/or enforced conflicts of interest policy and hereby agrees to comply with The University of Texas Medical Branch at Galveston (UTMB Health) policies and procedures relating to financial conflicts of interest. In accordance with this commitment, each Covered Individual as defined in UTMB Health's policy (IHOP 11.2.1) has completed UTMB Health conflict of interest training and disclosure attached hereto.

☒ **(a)** Sub-recipient organization/institution certifies that all personnel involved in the design, conduct and reporting of research data on the above-named project have completed and filed with sub-recipient organization/institution a current Conflict of Interest Disclosure form and none of the personnel involved has an identified Financial Conflict of Interest.

☐ **(b)** Sub-recipient organization/institution certifies that all personnel involved in the design, conduct and reporting of research data on the above-named project have completed and filed with sub-recipient organization/institution a current Conflict of Interest Disclosure form. The personnel listed below have an identified Financial Conflict of Interest which is being managed, reduced or eliminated by the sub-recipient organization/institution.

*In accordance with 42 CFR 50.604, sub-recipient organization/institution certifies that all personnel involved in the design, conduct or reporting of research data on the above-named project will complete training in regard to all applicable policies, regulations and laws before engaging in the research on the above named project.

Sub-recipient organization/institution certifies that the information provided above (if applicable, including any attached Disclosures) is(are) true and accurate to the best of its knowledge and further certifies that site and principal investigator shall comply with 42 CFR 50, Subpart F, Sections 50.601 to 50.607, responsibilities regarding conflicting interests of investigators. Sub-recipient organization/institution will notify UTMB within 30 days of any changes to a covered individual's status as it pertains to Financial Conflict of Interest.

Signature of Authorized Representative

Lisa Straker

Title

Date

From: Jacquelyne Ford on behalf of Jacquelyne Ford <JFord@hjf.org>
To: Sherry Haller
Cc: Christopher Broder; Thomas Geisbert; Lisa Straker; Kimberly Boxley; Brian Schaefer
Subject: BRODER - SCHAEFER DOCUMENTS TO UTMB-USUHS REVAMPP U19
Date: Friday, June 2, 2023 4:26:51 PM
Attachments: BIOSKETCH - SCHAEFER.pdf
BIOSKETCH - AMAYA.pdf
BIOSKETCH - BRODER.pdf
BIOSKETCH - DIMITROV.pdf
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HJF LETTER OF INTENT - SCHAEFER-USU-UTMBsubaward-6-2-23.pdf
SCHAEFER CONSORTIUM LOI-v3-signed BCS-HJF-USU-SCHAEFER-6-2-2023.pdf
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Hello Dr. Haller,

This email is being sent on behalf of Drs. Broder and Schaefer, Site Principal Investigators for the Uniformed Services University of the Health Sciences to collaborate with Dr. Thomas Geisbert, Prime PI with The University of Texas Medical Branch at Galveston (UTMB). Below is a listing of the attached documents. If you require additional information, please do not hesitate to contact me. Upon receipt of the attachments, please send a confirmation. Thank you for doing business with HJF.

1. Key Person Biosketches – 5 pages, NIH format– please address your role and expertise for this U19 in personal statement
2. Key Person Information (including person to be contacted on matters for this application for your institution)
3. Site Information
4. Abstract/Project Summary (30 lines)
5. Facilities & Resources (Word doc)
6. Equipment (Word doc)
7. Resource Sharing Plan (Word doc)
8. Vertebrate Animals (Word doc as applicable)
9. Select Agent Research Plan (as applicable)
10. Authentication of Key Biological/Chemical Resources
11. Budget (SF424) & Justification (Word doc) – please include justification for Core A effort as well Project Leads at least 1.2 person months effort
12. Signed Letter of Intent/Consortium Agreement- for projects that include only effort but no salary or other costs, we will provide a collaborative agreement for signature instead.
13. Specific Aims- 1 page

14. Research Strategy: 12 pages for Research Project
15. Letters of Support

Yours in good health,

Jacquelyne M. Ford
Sr. Proposal Specialist, Domestic Portfolio
Investigator Initiatives – Business Development
Strategic Initiatives Department

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SPECIFIC AIMS

The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparednessThreats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches **which can be adapted across members of each viral family in a "plug-and-play" manner**. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a concerns tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GP co-delivered with LASV NP subunits or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed in or monoclonal antibody PREP strategies which can provide protective immunity against Old and New World arenaviruses or emerging nairoviruses. (2) Develop reverse genetics tools to evaluate emerging arenaviruses and nairoviruses for which no isolates are available. Specifically, we will:

Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:

1.1 Subunit immunogens and MNP vaccination: Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches. We will then utilize lessons learned to address related "test case" arenaviruses and nairoviruses to develop a subunit/MNP approach for an old world arenavirus (LUJV) a new world arenavirus (CHPV), and an emerging bat borne nairovirus, Kasokero virus.

1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments: Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.

Milestone 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:

We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.

RESEARCH STRATEGY:

1. SIGNIFICANCE:

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens (REF) where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness (REF). Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Research Project 1(RP1) proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- **Old Word Mammarenaviruses (OWAV):** RP1 will utilize **LASV as the prototype OWAV** due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with **XX** deaths across West Africa(REF). LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to over **XX** countries (REFS). Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) (REFS). Most of these vaccines utilize the LASV (glycoprotein) GP or the LASV GP + Nucleoprotein (NP) as the target immunogens in these vectors (REFs).
- **New World Mamarenaviruses (NWAV):** A live attenuated vaccine for JUNV is currently in use in Argentina [2], but there is conflicting evidence of whether cross protection with other NWAV is possible [3, 4] and there are safety concerns related to reversion to pathogenic versions. This proposal will use **MACV and JUNV as the prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- **Orthonairoviruses:** Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated **XXX** cases per year across Europe, Africa, and Asia. CCHFV outbreaks are often very large list some recent examples. A suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974, but has numerous safety concerns and limited demonstrated efficacy(**REFS**). Since then, a number of vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages(REF); however only an MVA-CCHFV vaccine has begun to advance towards clinical trials (REF).

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e. the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success (**REFS**). The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability(REFS). Members of PABVAX, have developed protective

protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments [5]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from the each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Once a candidate subunit-patch vaccine approach is selected, this approach will use lessons learned to produce plug-and-play approach to develop vaccines against a representative human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, Lujo virus was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [6]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, underscoring the need for antiviral tools against this emerging threat.
- First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [7, 8]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [8]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective, but the phylogenetic divergence of CHAPV of XX% from JUNV suggests it may not be.
- In 1977, Kasokero virus (KASV), a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [9]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for *Ornithodoros faini* ticks to participate in enzootic maintenance of KASV [10]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised.

Pre-exposure prophylaxis (PREP) and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high-~~-~~risk individuals for infections such as Respiratory syncytial virus (palivizumab for prophylaxis), rabies (polyclonal immunoglobulin for post-exposure prophylaxis) or COVID-19 (tixagevimab/cilgavimab for prophylaxis (REFS). The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will ~~also~~ perform pilot studies to examine the feasibility of PREP ~~utilizing~~ with a serum half-life extended mAb against JUNV~~s~~. Clinically validated Fc mutations can result in an improvement in serum half-life from 3-4 weeks to 2-4 months ~~which can increase the instant barrier immunity from 30-45 days to 60+ days~~ ([44, 45][46] [47]~~refs~~).

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens

The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[11] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[11]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[12-17]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[18]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine(REFS). Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

Commented [CRW1]: Need to confirm

Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens

CCHFV is a divergent virus with up to seven genetic lineages (REFS). This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on (REF). Conversely, NP is highly conserved but not exposed on the virus surface cellular surface (REFs). Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [19]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control mammarenaviruses

For situations where a vaccine does not exist or is not appropriate (*e.g.* immunocompromised individuals or first responders who do not have the time to wait for natural immunity to develop from vaccination), mAbs with Fc mutations to extend serum-half-life offer an appealing alternative that can provide immediate protection lasting greater than 6 months. For this PABVAX proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE [44, 45] and LA [46] [47] Fc point mutations to determine the potential for delivering instantaneous, long-lasting (> 6 months) barrier immunity using monoclonal antibodies.

Commented [CRW2]: Larry, feel free to refine and/or elaborate.

Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses

Add blurb discussing unique access to field samples from across the world and established expertise to generate infectious clones from emerging arenaviruses and nairoviruses:

3. APPROACH

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

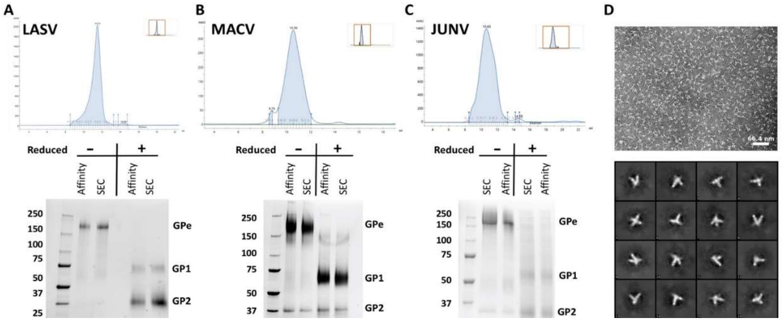


Figure XX. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide-linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[20]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[20] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure xx)**. Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 3)**. Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure xx)**. Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

Our trimeric arenavirus GPs support efforts to isolate novel ultra-potent mAbs and to develop a second-generation arenavirus immunotherapeutic with reduced dosing requirements and broader reactivity compared to current LASV mAb candidates[21]. This significantly lowers the cost of goods while increasing ease of use for clinicians, both essential for a product used in developing nations where arenaviruses are prevalent.

LASV subunit vaccine protects guinea pigs against lethal challenge

While classical vaccine approaches have largely focused on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[12-17] suggests that the cellular response, mediated by T cells, can play a highly important and complementary role in the long-term protection against pathogens. Based on literature reports that T cell responses highly correlate with LASV recovery, and that LASV NP plays a significant role in inducing those responses[12, 22], we also generated LASV NP antigens. We chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle (**Figure xx**) and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks.

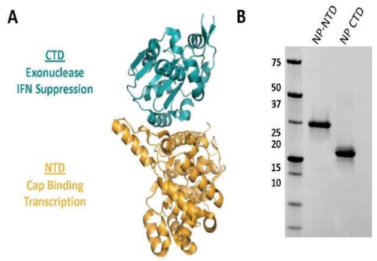
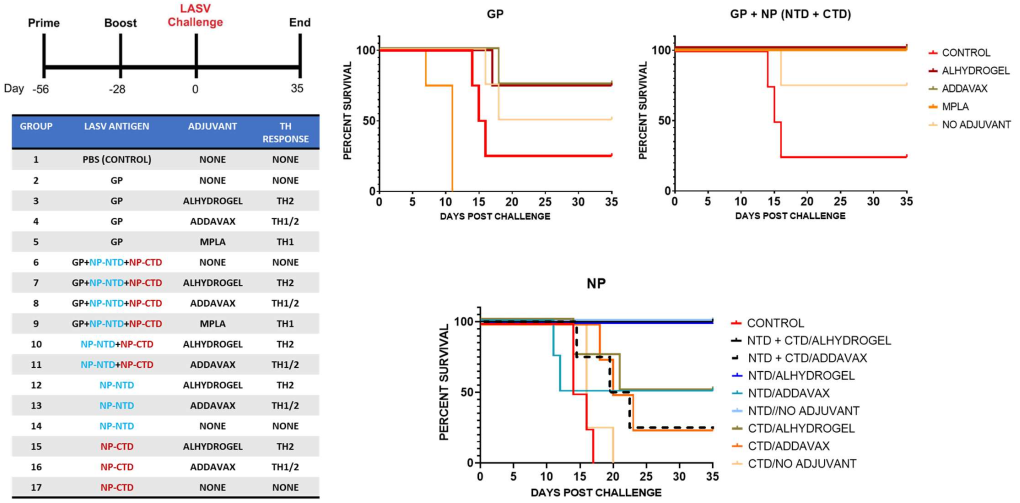


Figure xx. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B)



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

Figure 6. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 μg of GP, or 2x50 μg doses of GP + 2x25μg of NP-NTD + 2x25 μg of NP-CTD, or either 2x25 μg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

We evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 6)** to assess whether immune responses to GP only, or GP+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking (**Figure 6**), and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP but no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of

NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

*Engineering glycoproteins antigens for the CCHFV*The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to test serum reactivity in CCHF survivors (Figure XX). We will use established protocols [23] as a template to optimize expression of CCHFV and Kasokero NP proteins.

Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses

Rescue of recombinant viruses. An important goal of the PABVAX Center is to generate tools for pandemic preparedness. An important component of both RP1 and RP2 is to generate and recombinant viruses to fill gaps and provide tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with RP1 Core E will assist in the development and rescue of New World arenaviruses and the henipavirus Langya virus as detailed in the Research Strategies for RP1 and RP2.

Commented [CRW3]: VERBIAGE FROM CORE C, NEED TO ADAPT AND ELABORATE HERE.

APPROACH

Overall Hypothesis:FILL THIS IN

Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:

Rationale: The choice of Mammarenavirus Prototype Pathogens is justified as follows:

- *Old World Mammarenaviridae:* We propose LASV Josiah strain (Lineage 4) as the prototype pathogen due to the depth of available research data, availability of established rodent (**REF**) and nonhuman primate(NHP) models (**REFS**), frequency of exportation (**REFS**), and large number of annual cases throughout West Africa where the case fatality rate can be up to 60% in hospitalized cases (**REF**).
- *New World Mammarenaviridae:* Due to the availability of a JUNV vaccine in the endemic region where JUNV circulates and the lack of cross protection against challenge with other NWAV, MACV, the causative agent of Bolivian hemorrhagic fever, will be used as the prototype pathogen for NWAV for studies outlined in Milestone 1.1. There are established guinea pig (**REF**) and NHP (**REF**) models for MACV. JUNV is the New World arenavirus with the largest knowledge base at present [**REF**]. For a pilot efficacy study outlined in Milestone 1.2, we will use JUNV Espindola Strain as a natural extension of previous published work demonstrating protective efficacy of the template PREP mAb (**REF**).
- *Nairoviridae*. We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].

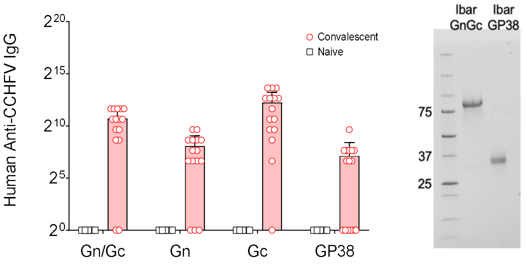


Figure XX: Engineered CCHFV proteins are reactive with serum from CCHFV survivors. SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification

Paragraph about subunit vaccines and PREP study.

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in table XX using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in Table XX. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core C** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group (REFS). After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-LASV-GPC)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of LASV Vaccination Schedule for Milestone 1.1.1.A

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (JUNV-Candid-1)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table XX** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain **(REF).** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV **(REFs).**

1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs. Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core C** as outlined in Table XX. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (VSV-LASV-GPC)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (JUNV-Candid-1)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

Similarly, after down selecting the optimal adjuvant and vaccination route, we will assess the potential for our MACV subunit vaccines to confer heterologous protection against thre lineages of MACV endemic to genetically distinct from the prototype strain (Chicava-Lineage 2). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core C** as outlined in Table XX. We will also utilize JUNV (Romero strain)(**REF**) challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP: 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals (Table XX). Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for LASV Milestone 1.1.1.B

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table XX** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain **(REF).** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will confirm protection in NHPs if demonstrated in guinea pigs **(REFs).**

2.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHFV-M)	8		8	
	TOTALS	128	TOTALS	128

2.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHF-M)	8		8	
	TOTALS	128	TOTALS	128

2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

2.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses using optimized subunit-patch vaccination in guinea pigs and NHP

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LASSA NP-NTD	8
LASSA GPe	8
LASSA GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

2.1.2.E: Determination of protective efficacy against novel, emerging nairovirus using optimized subunit-patch vaccination in STAT-1KO mice and NHP

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

1.1.1.F: Determination of cellular and humoral immune correlates associated with protection

1.1.1.F.a: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups.

We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard whole blood flow cytometric staining protocols will be used for surface and intracellular staining markers, briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye for 20 minutes at room temperature. Cells are then washed again, surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS.

Stained, fixed cells will then either be acquired directly. Cell populations are then quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments:. PBMC stimulations will be set up according to modifications of well established, previously published protocols. [24, 25] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37ºC for 1 hour. A protein transport inhibitor such as brefeldinA will then be added at a concentration of 10mg/mL and incubated at 37ºC for until flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37ºC. Stimulations to assess secreted analytes by multiplex-bead based assays will be set up in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye for 20 minutes at room temperature, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are incubated in BD Cytofix/Cytoperm solution for 20 minutes followed by a wash with BD Perm/Wash Buffer. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a 4-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated for 3 hours at room temperature, protected from light. 1mL of wash buffer will be added and beads will be pelleted by centrifugation at 200g for 5 minutes. Wash buffer will be removed and discarded. Beads will be resuspended in 300uL wash buffer and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37ºC. Wells will be washed manually 3-times, followed by the addition of detection antibody and incubated either overnight at 4ºC or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes at room temperature, protected from light. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37ºC), and spot forming units (SPU) will be read on an **need model** ELISPOT Fluorescence Microplate Reader.

Data Analysis: We will look at the differences in immune responses across groups between each timepoint and baseline. Model assumptions will be checked for a one-way ANOVA to compare the three groups. we will run the analysis after checking model assumptions, if those are not met then we will adjust to a nonparametric model or transform the outcome appropriately. To look at the longitudinal aspect of the data, we will investigate measurements over time between groups, the interaction of group and time can be analyzed using covariance pattern models (or linear mixed models), where the correlation between observations measured over time can be handled more flexibly. As a more exploratory analysis we will also look at adjusting for possible confounding variables such as sex and age. For categorical variables, comparisons will be made using Chi-Square tests. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

1.1.1.F.b: Systems serology ***Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups.***

We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform developed by Dr. Gunn in Core D[26-28] to define the Fc-mediated humoral antiviral functions associated with vaccination. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV,LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens utilized for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages. We will use a multiplexed analysis to determine the levels of antibodies against the viral antigens using on MagPix xMAP instrument as described.[27, 29] Specifically, each viral antigen will be coupled to coded magnetic beads (Luminex), mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will make a standard using pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry at KGH.

ADCC: Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates for 5 hours at 37C 5% CO_2, and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses were performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction was used to determine statistical significance between groups. A network analysis will be performed to identify significant antibody features. Associations between antibody features will be determined using nonparametric Spearman correlation coefficient. Statistically significant associations after Bonferroni correction for multiple comparisons (adjusted $P < .05$) were used to generate networks in Cytoscape (version 3.4.0).

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and additional clinical information (ie: PES symptoms). These will be included in the network analysis.

1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine. In conjunction with ~~RP1~~ Cores D and ~~Core~~ E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an existing anti-Junin virus mAb ~~with~~ whose potent therapeutic efficacy [39, 43] ~~(guinea pig and NHP references)~~ we have previously described ~~by Abelson, Geisbert and Zeitlin~~ will be produced with YTE and LA Fc point mutations to extend the serum half-life of the mAb~~(Table 4)~~. The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (~~Bioqual, Inc.; Rockville, MD~~see Core D) to select a lead candidate for testing in NHPs against Junin Romero challenge in Core E. ~~For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference).~~ We will select t~~T~~he variant with the most favorable pharmacokinetics ~~PK will be selected~~ for testing with ~~by~~ ~~**RP1/**~~**Core E**. Briefly, groups of 4 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV (~~Espindola or~~ Romero~~?~~) delivered IV. For further details of the NHP testing, please see **Core E**. These data will be used by the Scientific Advisory ~~Committee~~ Board to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes against other viruses.

Commented [CRW4]: From Larry, adapt from this.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC

Please note: Discussion of expected results, other alternative approaches and criteria for "go" or "no go" decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the **Project Milestones and Timelines** *section.*

JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,

AIM 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC

Please note: Discussion of expected results, other alternative approaches and criteria for "go" or "no go" *decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the* **Project Milestones and Timelines** *section.*

JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,

Milestones-Timelines-GANT CHART

Summary statement impact

1. Abudurexiti, A., et al., *Taxonomy of the order Bunyavirales: update 2019.* Arch Virol, 2019. **164**(7): p. 1949-1965.
2. Saito, T., et al., *Vaccine Candidates against Arenavirus Infections.* Vaccines, 2023. **11**(3): p. 635.
3. Koma, T., et al., *Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic.* J Virol, 2016. **90**(3): p. 1290-7.
4. Jahrling P, T.R., Barrero O. . *Crossprotection against Machupo virus with Candid 1 Junin virus vaccine*. in *2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region*. 1988. Mar del Plata, Argentina.
5. Balmert, S.C., et al., *A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity.* iScience, 2022. **25**(10): p. 105045.
6. Sewlall, N.H., et al., *Clinical features and patient management of Lujo hemorrhagic fever.* PLoS Negl Trop Dis, 2014. **8**(11): p. e3233.
7. Delgado, S., et al., *Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia.* PLOS Pathogens, 2008. **4**(4): p. e1000047.
8. Loayza Mafayle, R., et al., *Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019.* New England Journal of Medicine, 2022. **386**(24): p. 2283-2294.
9. Kalunda, M., et al., *Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda.* The American Journal of Tropical Medicine and Hygiene, 1986. **35**(2): p. 387-392.
10. Schuh, A.J., et al., *Human-Pathogenic Kasokero Virus in Field-Collected Ticks.* Emerg Infect Dis, 2020. **26**(12): p. 2944-2950.
11. Brisse, M.E. and H. Ly, *Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors.* Front Immunol, 2019. **10**: p. 372.
12. Baize, S., et al., *Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys.* J Virol, 2009. **83**(11): p. 5890-903.
13. Fisher, D.G., G.M. Coppock, and C.B. Lopez, *Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination.* Vaccine, 2018. **36**(28): p. 4039-4045.
14. Meyer, B. and H. Ly, *Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses.* J Virol, 2016. **90**(8): p. 3810-3818.
15. ter Meulen, J., et al., *Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein.* J Virol, 2000. **74**(5): p. 2186-92.

16. Meulen, J., et al., *Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones.* Virology, 2004. **321**(1): p. 134-43.
17. Goicochea, M.A., et al., *Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model.* Vaccine, 2012. **30**(8): p. 1445-52.
18. Sullivan, B.M., et al., *High crossreactivity of human T cell responses between Lassa virus lineages.* PLoS Pathog, 2020. **16**(3): p. e1008352.
19. Leventhal, S.S., et al., *Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge.* EBioMedicine, 2022. **82**: p. 104188.
20. Hastie, K.M., et al., *Structural basis for antibody-mediated neutralization of Lassa virus.* Science, 2017. **356**(6341): p. 923-928.
21. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nat Med, 2017. **23**(10): p. 1146-1149.
22. McElroy, A.K., et al., *A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses.* J Infect Dis, 2017. **215**(12): p. 1862-1872.
23. Carter, S.D., et al., *Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein.* J Virol, 2012. **86**(20): p. 10914-23.
24. Meddows-Taylor, S., et al., *Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome.* J Virol Methods, 2007. **144**(1-2): p. 115-21.
25. Kagina, B.M., et al., *Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry.* J Immunol Methods, 2015. **417**: p. 22-33.
26. Gunn, B.M., et al., *A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus.* Immunity, 2021. **54**(4): p. 815-828.e5.
27. Gunn, B.M., et al., *Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses.* J Infect Dis, 2020. **221**(1): p. 156-161.
28. Saphire, E.O., et al., *Antibody-mediated protection against Ebola virus.* Nat Immunol, 2018. **19**(11): p. 1169-1178.
29. Brown, E.P., et al., *Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles.* J Immunol Methods, 2017. **443**: p. 33-44.

SPECIFIC AIMS

The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparednessThreats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches **which can be adapted across members of each viral family in a "plug-and-play" manner**. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a concerns tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GP co-delivered with LASV NP subunits or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed in or monoclonal antibody PREP strategies which can provide protective immunity against Old and New World arenaviruses or emerging nairoviruses. (2) Develop reverse genetics tools to evaluate emerging arenaviruses and nairoviruses for which no isolates are available. Specifically, we will:

Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:

1.1 Subunit immunogens and MNP vaccination: Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches. We will then utilize lessons learned to address related "test case" arenaviruses and nairoviruses to develop a subunit/MNP approach for an old world arenavirus (LUJV) a new world arenavirus (CHPV), and an emerging bat borne nairovirus, Kasokero virus.

1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments: Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.

Milestone 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:

We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.

RESEARCH STRATEGY:

1. SIGNIFICANCE:

The viral order *Bunyavirales* is composed of 13 families of which five (*Peribunyaviridae*, *Phenuiviridae Hantaviridae*, *Arenaviridae, and Nairoviridae)* are known to contain several high priority human pathogens associated with sever fever, encephalitis, respiratory, and/or hemorrhagic disease[1]. Notably, Arenaviridae and Nairoviridae families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens (REF) where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens. This classification stems is owed to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness (REF). Threats to public health are further heightened due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these viruses.

Research Project 1(RP1) proposes preclinical research that will support Investigational New Drug Application (IND)-applications to develop vaccine approaches against prototype viruses from two Bunyavirales viral families, the Mammarenaviruses and the Orthonairoviruses. Generation of effective vaccines against members of both has potential great global benefit as both viral families have sprawling geographic endemic ranges ranges which have resulted in marked genetic diversity within each viral family further challenging effective vaccine approaches.

- **Old Word Mammarenaviruses (OWAV):** RP1 will utilize **LASV as the prototype OWAV** due to its elevated public health burden throughout West Africa with hundreds of thousands of estimated cases annually with **XX** deaths across West Africa(REF). LASV has is the most imported viral hemorrhagic fever (VHF) next to dengue virus and has been imported to over **XX** countries (REFS). Case fatality rates (CFR) up to 60% in hospitalized cases and marked long term sequelae are the result of no approved vaccines or therapeutics, though several live attenuated virus candidates are approaching clinical evaluation with the assistance of Coalition for Epidemic Preparedness Initiative (CEPI) (REFS). Most of these vaccines utilize the LASV (glycoprotein) GP or the LASV GP + Nucleoprotein (NP) as the target immunogens in these vectors (REFs).
- **New World Mamarenaviruses (NWAV):** A live attenuated vaccine for JUNV is currently in use in Argentina [2], but there is conflicting evidence of whether cross protection with other NWAV is possible [3, 4] and there are safety concerns related to reversion to pathogenic versions. This proposal will use **MACV and JUNV as the prototype NWAVs** as there are no approved vaccines or therapeutics available for MACV and the safety concerns associated with Candid-1 vaccine suggest other approaches are warranted.
- **Orthonairoviruses:** Here, **CCHFV will serve as the prototype virus** for vaccine development as is responsible for the largest global public health burden within the orthonairovirus family with an estimated **XXX** cases per year across Europe, Africa, and Asia. CCHFV outbreaks are often very large list some recent examples. A suckling mouse brain derived, CHCl2/heat-inactivated aluminum hydroxide adsorbed whole virus vaccine for CCHFV has been approved for use only in Bulgaria since 1974, but has numerous safety concerns and limited demonstrated efficacy(**REFS**). Since then, a number of vaccine approaches have been examined ranging from viral vectored approaches to DNA based vaccines with varying efficacy, advantages, and disadvantages(REF); however only an MVA-CCHFV vaccine has begun to advance towards clinical trials (REF).

Many of the vaccine approaches targeting the prototype viruses chosen for this proposal utilize live-attenuated viruses, which have many advantages in terms of being able to rapidly induce protective and often durable protection from debilitating disease and death. However, as the global human population grows, the number of special populations with depressed immune systems (i.e. the elderly, immunocomprimised, and/or pregnant) grows, which call in to the question the safety of live-attenuated vaccines and suggests more pathologically benign approaches may have value. The use of subunit vaccines designed from immunologically critical epitopes from different pathogens has been a central driver for a number of successful and approved vaccinee strategies including Tetanus, HPV, Hepatitis B, a suite of bacterial pathogens and is the basis for mRNA vaccine success (**REFS**). The success of many subunit vaccines is owed to targeted immunogenicity, ability to rapidly scale production, and potential for long-term, shelf stability(REFS). Members of PABVAX, have developed protective

protein-based subunit vaccines against LASV which will serve as the template to develop vaccines against other mammarenaviruses and orthonairoviruses.

Subunit vaccines have several advantages including potential for shelf stable formulations and utility in alternative delivery methods such as **microneedle skin patches (MNP).** Skin patch vaccination has several advantages over traditional IM vaccination which include needle free operation, compatibility with shelf stable subunit vaccines, and a delivery approach that harnesses the potently immunoresponsive cutaneous microenvironments [5]. RP1 will capitalize on PABVAX member expertise to couple protective subunit vaccines to MNPs targeting key antigens associated with survival from infection from the each prototype pathogen group, namely viral glycoproteins and nucleoproteins with the goal of eliciting robust, strong, and long-lasting protective immunity against the target pathogen.

Once a candidate subunit-patch vaccine approach is selected, this approach will use lessons learned to produce plug-and-play approach to develop vaccines against a representative human pathogen from each prototype virus group for which no research grade vaccines have been created and tested in animal models.

- In 2008, Lujo virus was isolated from a case of severe hemorrhagic fever originating in Zambia but quickly causing nosocomial infections in care givers after the index case's medical evacuation to South Africa [6]. The case fatality rate for this incredibly infectious virus was 80% in the small outbreak of 5 people. There are currently no approved or therapeutics available for this virus and its genetic divergence from LASV suggest countermeasures with antigenic targets may be of limited use, underscoring the need for antiviral tools against this emerging threat.
- First reported in 2003 in Bolivia, Chapare virus (CHAPV) is a rodent borne mammarenavirus associated with development of severe hemorrhagic fever with case fatality rates up to 60% [7, 8]. Recent studies have demonstrated clear human-to-human transmission risk during the acute phase evidenced by several nosocomial infections but also detection of viable virus from semen at 86 days after symptom onset demonstrating a clear need to specialized management and treatment [8]. It is unknown if the JUNV Candid-1 vaccine employed for control of JUNV in Argentina would be protective, but the phylogenetic divergence of CHAPV of XX% from JUNV suggests it may not be.
- In 1977, Kasokero virus (KASV), a bat borne Orthonairovirus, was first isolated from Ugandan bats and responsible for causing several cases of acute febrile illness of laboratory workers from the same lab that processed infected bats [9]. Serosurveys of 109 laboratory workers from the same facility the virus was isolated and worked with demonstrated a 13% seropositivity where medical surveys revealed clinical symptoms in 4 to 15 months after the virus was isolated, suggesting either continued laboratory associated infections or chains of ongoing person-to-person transmission. Recently, tick surveys have demonstrated the potential for *Ornithodoros faini* ticks to participate in enzootic maintenance of KASV [10]. While most chiropteran ticks exhibit host specificity, humans entering caves where these bats are have reported bites from *O.faini* ticks as well. The highly infectious potential for this virus in humans coupled to a complete lack of vaccines or therapeutics make suggest investment in countermeasure development is well advised.

Pre-exposure prophylaxis (PREP) and Post exposure prophylaxis (PEP) using monoclonal antibodies or immunoglobulins has been employed for providing instant barrier immunity to high risk individuals for infections such as rabies or COVID-19 (REFS). The PABVAX consortium has a long track record of success developing monoclonal antibody (mAb) therapies against VHFs and emerging viruses. Building on this success, RP1 will also perform pilot studies to examine the feasibility PREP utilizing half-life extended mAbs which can increase the instant barrier immunity from 30-45 days to 60+ days (refs).

The PABVAX consortium will build on over a decade of successful collaboration generating vaccines and therapeutics for emerging, high priority viruses where collective expertise in virology, immunology, biological manufacturing and vaccine device innovation to develop cutting edge vaccination approaches using plug-and-play approaches tailored from prototype mammarenaviruses and orthonairoviruses and applicable to related members of these viral families.

2. INNOVATION

Unique arenavirus subunit vaccine strategy utilizing GP and NP immunogens

The Coalition for Epidemic Preparedness Innovations (CEPI) is currently funding development of multiple LASV vaccine candidates[11] with four out of five candidates based on viral vector backbones. The remaining candidate is a DNA-based vaccine in active development by Inovio Pharmaceuticals. Of significance, very little pre-clinical data concerning the safety and efficacy of these vaccine candidates is currently available[11]. Further, four of the five deliver LASV GP as the sole immunogen, potentially limiting long-term durability and potential of the immune response.

In contrast, we are developing arenavirus subunit vaccines that co-deliver GP and NP immunogens. When engineering viral immunogens to be used as subunit vaccines, the recombinant antigens must structurally and antigenically represent the authentic viral antigen. Importantly, we have engineered a recombinant trimeric LASV GP spike antigen (the primary structural antigen on the surface of arenavirus), and to our knowledge, ours is the first LASV subunit vaccine candidate to utilize trimeric LASV GP. The trimeric GP immunogen, representative of the authentic viral antigen, induces a highly potent and protective anti-GP humoral immune response. In conjunction with humoral immunity, cellular immunity, mediated by a robust T cell response, is known to be highly predictive of recovery from a LASV infection[12-17]. Epitopes within LASV NP, and to a lesser extent GP, are known to substantially contribute to T cell mediated immunity[18]. Exploiting highly conserved N-terminal immunodominant epitopes on of LASV NP, we find that vaccination with only NP epitopes is sufficient to confer 100% protection in a lethal, outbred guinea pig model. Sequence conservation across NP is higher than GP across LASV lineages suggesting that vaccine strategies coupling NP and GP may provide a more broadly protective vaccine(REFS). Indeed, we have found that an immunogenic cocktail comprised of LASV GP and NP offered complete protection against LASV challenge in guinea pigs.

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Unique nairovirus subunit vaccine strategy utilizing GP and NP immunogens

CCHFV is a divergent virus with up to seven genetic lineages (REFS). This diversity is evident in the variation observed most clearly in glycoprotein sequences across lineages. Given the glycoprotein is a pivotal protein dictating viral entry and immune recognition, it is little wonder why CCHFV vaccines have struggled with heterologous challenge with strains of viruses outside of the clade the vaccine was based on (REF). Conversely, NP is highly conserved but not exposed on the virus surface cellular surface (REFs). Indeed, a recent report has demonstrated that an NP expressing replicating RNA vaccine was able to confer protection against heterologous challenge [19]. Taken together, a similar strategy of coupling GP and NP into a vaccine strategy likely has the greatest potential for potent, cross-clade protection.

PREP "vaccination" as a measure to control mammarenaviruses

For this proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by PABVAX researchers will be produced with YTE and LA Fc point mutations to determine the potential for delivering instantaneous barrier immunity using monoclonal antibodies.

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Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses

Add blurb discussing unique access to field samples from across the world and established expertise to generate infectious clones from emerging arenaviruses and nairoviruses:

3. APPROACH

PRELIMINARY RESULTS

Engineering trimeric glycoprotein antigens for the arenaviruses

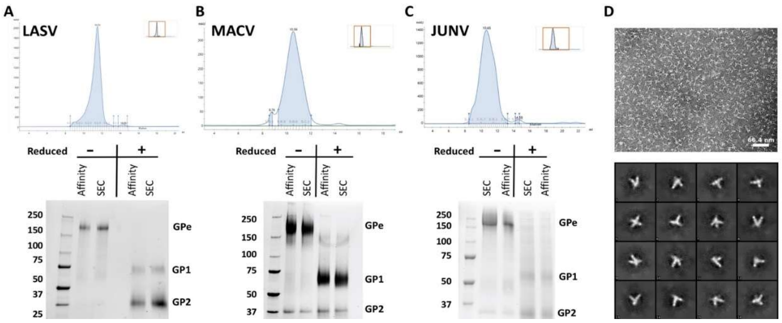


Figure XX. Arenavirus immunogens. (A) LASV, (B) MACV and (C) JUNV GP immunogens were expressed and purified and the trimeric nature confirmed with SEC and SDS-PAGE. (D) Negative stain EM and 2D class averages of JUNV GP-J199 Fab complex. EM courtesy of Dr. J. Mclellan, UT Austin.

Production of recombinant trimeric LASV GP has been particularly challenging within the LASV research community. The LASV GP ectodomain (GP) consists of two non-covalently associated subunits, GP1 and GP2, which readily disassociate in solution, historically hindering the expression and purification of a trimeric GPe. A recent study introduced a pair of cysteine mutations into GP1 and GP2, resulting in a stable disulfide-linked GP1/GP2 monomer amenable to structural studies when stabilized by a fiduciary antibody Fab fragment[20]. Mapp has since made structure-based improvements to generate a stable GP immunogen representative of the authentic viral GP spike and suitable for use as a vaccine. We reverted the E329P mutant, hypothesized to stabilize heptad repeat 1 in GP2[20] and added an additional trimerization motif to stabilize the GP spike in the absence of Fabs. The tagged trimeric LASV GP is expressed and secreted from Drosophila S2 cells and purified from supernatant via affinity chromatography using a StrepTrap HP column (GE Healthcare). SDS-PAGE and Size-exclusion chromatography (SEC) offer verification of trimerization **(Figure xx)**. Pilot stability studies inclusive of freeze thaw cycles and storage at 4˚C are supportive of the stability required for a vaccine candidate.

The engineering methodologies employed for LASV GP translated well to the NWA GPs from JUNV and MACV **(Figure 3)**. Negative stain EM of JUNV GP in complex with the neutralizing and protective Fab form of J199, reveals monodispersed trimeric GP/Fab complexes **(Figure xx)**. Both the interaction with a well-characterized mAb and the EM data suggest our GP antigens closely recapitulate the authentic viral GP trimeric spike and support their use as a vaccine immunogen.

Our trimeric arenavirus GPs support efforts to isolate novel ultra-potent mAbs and to develop a second-generation arenavirus immunotherapeutic with reduced dosing requirements and broader reactivity compared to current LASV mAb candidates[21]. This significantly lowers the cost of goods while increasing ease of use for clinicians, both essential for a product used in developing nations where arenaviruses are prevalent.

LASV subunit vaccine protects guinea pigs against lethal challenge

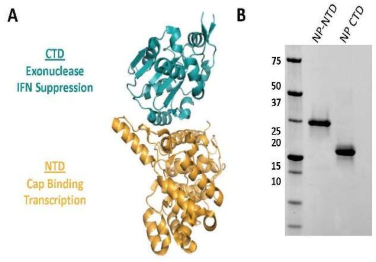
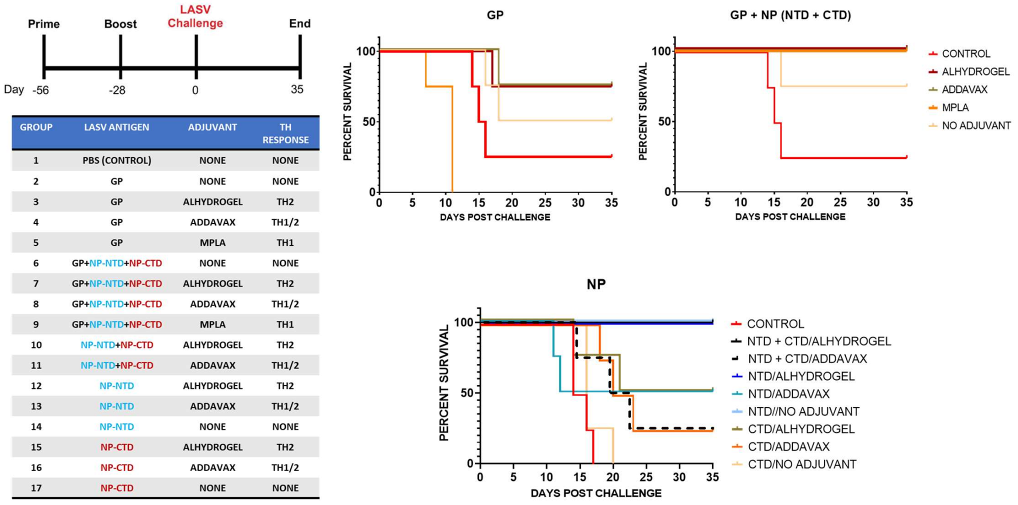


Figure xx. LASV NP subdomains and expression. (A) Crystal structure of LASV NP highlighting the two functional subdomains. (B) SDS-PAGE gel showing highly purified LASV NP antigens for vaccine study.

While classical vaccine approaches have largely focused on evoking a strong humoral response and high titers of nAbs to a target pathogen, increasing evidence[12-17] suggests that the cellular response, mediated by T cells, can play a highly important and complementary role in the long-term protection against pathogens. Based on literature reports that T cell responses highly correlate with LASV recovery, and that LASV NP plays a significant role in inducing those responses[12, 22], we also generated LASV NP antigens. We chose to deliver the C-terminal domain (CTD) and N-terminal domain (NTD) of LASV NP separately as they have distinct functions in the virus lifecycle (**Figure xx**) and express to high levels (50-100 mg/L) as two independent domains in bacteria using standard laboratory shaker flasks.

We evaluated our LASV antigens as vaccine immunogens in the lethal Hartley guinea pig model of LASV infection. Our study was designed **(Figure 6)** to assess whether immune responses to GP only, or GP+NP would offer protective immunity from lethal LASV challenge. We chose to investigate several



GROUP	LASV ANTIGEN	ADJUVANT	TH RESPONSE
1	PBS (CONTROL)	NONE	NONE
2	GP	NONE	NONE
3	GP	ALHYDROGEL	TH2
4	GP	ADDAVAX	TH1/2
5	GP	MPLA	TH1
6	GP+NP-NTD+NP-CTD	NONE	NONE
7	GP+NP-NTD+NP-CTD	ALHYDROGEL	TH2
8	GP+NP-NTD+NP-CTD	ADDAVAX	TH1/2
9	GP+NP-NTD+NP-CTD	MPLA	TH1
10	NP-NTD+NP-CTD	ALHYDROGEL	TH2
11	NP-NTD+NP-CTD	ADDAVAX	TH1/2
12	NP-NTD	ALHYDROGEL	TH2
13	NP-NTD	ADDAVAX	TH1/2
14	NP-NTD	NONE	NONE
15	NP-CTD	ALHYDROGEL	TH2
16	NP-CTD	ADDAVAX	TH1/2
17	NP-CTD	NONE	NONE

Figure 6. A LASV subunit vaccine provides complete protection in the guinea pig model of LASV infection. (Left) Groups of 4 guinea pigs were immunized intraperitoneally in a prime-boost strategy with either PBS vehicle control, 2x50 µg of GP, or 2x50 µg doses of GP + 2x25µg of NP-NTD + 2x25 µg of NP-CTD, or either 2x25 µg NP antigen in combination or separately. Immunized animals were challenged with a lethal dose of LASV/Josiah 28 days post-boost. (Right) Animals were monitored for 35 days post-challenge, and survival curves and weight change are plotted.

commercially available adjuvants with known properties for driving Th1 (MPLA) and Th2 (Alhydrogel) responses selectively or in combination (Addavax). All 3 adjuvants are currently utilized in either FDA- or EMA-approved vaccines, thus supporting the long-term feasibility of the approach for potential human use. The results of our vaccination/challenge study were quite striking (**Figure 6**), and indicate a clear benefit to a combination immunization strategy with LASV GP and NP. The maximum survival benefit in the GP only groups were observed in groups using Alhydrogel and Addavax as adjuvants (75% survival). Half of the animals in the unadjuvanted group survived, while the control group had one survivor. Surprisingly, animals that were administered GP with MPLA as an adjuvant displayed rapid onset of clinical signs and succumbed to infection early in the experiment. In contrast, **all animals that were immunized with LASV GP + NP-NTD + NP-CTD subunits and any of the adjuvants were completely protected from challenge with LASV.** Only one animal succumbed to infection in the group receiving GP+NP but no adjuvant. In a follow-up study, we further examined the potential for NTD and/or CTD of LASV NP to confer protection to lethal challenge. While combination of NTD and CTD resulted in uniform protection when administered with Alhydryogel, the cohort supplemented with Addavax only resulted in 25% protection. Correspondingly, NTD vaccination with Allhydrogel resulted in 100% survival where as Addavax only conferred 50% protection. Interestingly, NP NTD vaccination with no adjuvant resulted in 100% survival. Conversely, the best protection from the LASV CTD was conferred when using Alhydrogel at 50% where both Addavax or no adjuvant groups were not protected.

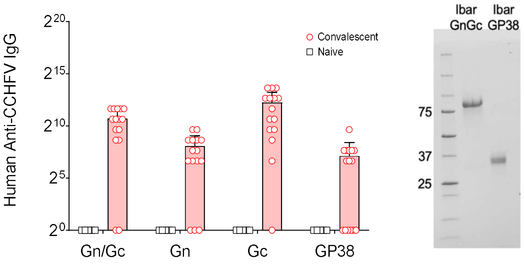


Figure XX: Engineered CCHFV proteins are reactive with serum from CCHFV survivors. SDS-PAGE gel of CCHF Ibar proteins GnGc and GP38 after 2 step purification

*Engineering glycoproteins antigens for the CCHFV*The CCHF virus has a tripartite negative-sense RNA genome,composed of the the small (S), medium (M), and large (L) segments. The M segment encodes the viral glycoprotein precursor (GPC) complex, which is subsequently cleaved by host proteases into multiple proteins, including the envelope glycoprotein GnGc and secreted GP38 protein. GnGc is composed of Gn and Gc subunits, which are linked together by a multiple membrane spanning region. These transmembrane domains complicate the *in vitro* expression of soluble GnGc, but we have designed a construct that replaces the transmembrane domains with a linker and have generated a stable drosophila cell line that expresses up to 3mg/L. We have also generated stable cell lines expressing Gn, Gc, and GP38 proteins. We used these proteins to test serum reactivity in CCHF survivors (Figure XX). We will use established protocols [23] as a template to optimize expression of CCHFV and Kasokero NP proteins.

Generation of Infectious clones and reporter viruses of emergining Arenaviruses and Nairoviruses

Rescue of recombinant viruses. An important goal of the PABVAX Center is to generate tools for pandemic preparedness. An important component of both RP1 and RP2 is to generate and recombinant viruses to fill gaps and provide tools where wild type viruses do not exist either because 1) only sequence information is available or 2) isolates exist but viruses are unable to be shared with the research community. In collaboration with RP1 Core E will assist in the development and rescue of New World arenaviruses and the henipavirus Langya virus as detailed in the Research Strategies for RP1 and RP2.

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APPROACH

Overall Hypothesis:FILL THIS IN

Milestone 1: Develop novel arenavirus and nairovirus vaccination approaches:

Rationale: The choice of Mammarenavirus Prototype Pathogens is justified as follows:

- *Old World Mammarenaviridae:* We propose LASV Josiah strain (Lineage 4) as the prototype pathogen due to the depth of available research data, availability of established rodent (**REF**) and nonhuman primate(NHP) models (**REFS**), frequency of exportation (**REFS**), and large number of annual cases throughout West Africa where the case fatality rate can be up to 60% in hospitalized cases (**REF**).
- *New World Mammarenaviridae:* Due to the availability of a JUNV vaccine in the endemic region where JUNV circulates and the lack of cross protection against challenge with other NWAV, MACV, the causative agent of Bolivian hemorrhagic fever, will be used as the prototype pathogen for NWAV for studies outlined in Milestone 1.1. There are established guinea pig (**REF**) and NHP (**REF**) models for MACV. JUNV is the New World arenavirus with the largest knowledge base at present [**REF**]. For a pilot efficacy study outlined in Milestone 1.2, we will use JUNV Espindola Strain as a natural extension of previous published work demonstrating protective efficacy of the template PREP mAb (**REF**).
- *Nairoviridae.* We propose CCHFV Hoti strain (Clade 5) as the nairovirus prototype pathogen. UTMB has established mouse models (**REF**) and NHP (**REF**) models to enable testing of CCHFV countermeasures against a number of the available clades. CCHF is the most important member of the *Nairoviridae* family with a major public-health impact across Western Asia, the Balkans, Southern Europe, and most of Africa [**REF**].

Paragraph about subunit vaccines and PREP study.

Milestone 1.1 Subunit immunogens and MNP vaccination

Milestone 1.1.1.A: Development and optimization of patch vaccination approach employing arenavirus subunits in guinea pigs: Building on the success of our GPe + NP-NTD LASV vaccine, we will compare the protective efficacy of two adjuvants coupled to our LASV GPe and NP-NTD proteins developed and manufactured in **Core D** either as individual subunit vaccines or combinations as outlined in table XX using an intramuscular (IM) vaccination approach. Eight outbred, 350 gram Hartley guinea pigs (balanced sex) will be randomly assigned to groups as outlined in Table XX. Blood will be collected prior to immunization and then on days 28 and 56-days post-immunization to evaluate humoral immune responses by ELISA and virus neutralization assays. After 28 days immunized animals will be boosted with an equivalent dose as the priming vaccination IM. On day 56 post-immunization, animals will be transferred to **BSL4 evaluation Core C** and challenged with 5000 pfu of a guinea pig adapted LASV-Josiah developed by our group (REFS). After virus challenge, animals will be monitored clinically a minimum of twice daily. Hands-on physical and visual assessment of infected animals may occur outside scheduled times, if required. Following challenge, additional biosamples collected at 1, 4, 7, 10, 14, 21 and 28 days will be processed for measurement of viremia by plaque assay and qRT-PCR. Results from the IM vaccination then be compared head-to-head against an equivalent vaccination/challenge cohort where the subunit vaccines will be administered using the MNP patches prepared by MNP Patch **CORE C**. Patches are administered after shaving a small section of the abdomen and applied using gentle pressure for 10-15 minutes so that the microneedles can fully dissolve.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe + NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-LASV-GPC)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of LASV Vaccination Schedule for Milestone 1.1.1.A

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (JUNV-Candid-1)	8		8	
	TOTALS	64	TOTALS	64

Table: XX outline of MACV Vaccination Schedule for Milestone 1.1.1.A

Similarly, we will determine the protective efficacy using MACV GPe and NP-NTD proteins developed and manufactured in **Core D** following the grouping described in **Table XX** using the identical experimental approach as outlined above. The challenge virus will be the established MACV Chicava strain **(REF).** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will assess its utility as a positive control vaccine against lethal challenge with MACV **(REFs).**

1.1.1.B: Determination of pan-LASV and Pan-MACV protective efficacy using optimized subunit-patch vaccination in guinea pigs. Using the adjuvant and vaccination route down selected in 1.1.1.A, we will assess the potential for our LASV subunit vaccines to confer heterologous protection against four lineages of LASV endemic to geographically distinct from the prototype strain (Josiah-lineage 4, Sierra Leone). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 2 (Nigeria), 3 (Nigeria), 5 (Mali) and 6(Togo) established in the **BSL4 evaluation Core C** as outlined in Table XX. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 2	LINEAGE 3	LINEAGE 5	LINEAGE 6
LASSA NP-NTD	8	8	8	8
LASSA Gpe	8	8	8	8
LASSA Gpe +NP-NTD	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (VSV-LASV-GPC)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-LASV Vaccine Efficacy Schedule for Milestone 1.1.1.B

VACCINE	PAN-LINEAGE PROTECTION			
	LINEAGE 1	LINEAGE 5	LINEAGE 8	JUNV
MACV CHICAVA NP NTD	8	8	8	8
MACV CHICAVA GPe	8	8	8	8
MACV CHICAVA NP + GPe	8	8	8	8
Control animals (NO VACCINE)	8	8	8	8
Control vaccine (JUNV-Candid-1)	8	8	8	8
TOTALS	40	40	40	40

Table: XX outline of PAN-MACV Vaccine Efficacy Schedule for Milestone 1.1.1.B

Similarly, after down selecting the optimal adjuvant and vaccination route, we will assess the potential for our MACV subunit vaccines to confer heterologous protection against thre lineages of MACV endemic to genetically distinct from the prototype strain (Chicava-Lineage 2). Employing optimized strain 13 or outbred, Hartley guinea pig models for lineages 1, 5, and 8 established in the **BSL4 evaluation Core C** as outlined in Table XX. We will also utilize JUNV (Romero strain)(**REF**) challenge to assess heterologous protection against a related but distinct NWAV to begin to assess the potential for cross protection beyond MACV strains. The vaccination, challenge, and sample collection will be identical to that outlined in 1.1.1.A above.

1.1.1.C: Determination of LASV and MACV protective efficacy using optimized subunit-patch vaccination in NHP: 15 cynomolgus monkeys will be randomized into two experimental vaccine groups of 5 animals per group, a control vaccine (VSV-LASV-GPC) with 5 animals, and a control group of five unvaccinated animals (Table XX). Animals in vaccine groups 1 and 2 will be vaccinated using the down selected LASV antigens, adjuvants, and vaccination route identified in 1.1.1.A. Animals in vaccine group 1 will receive only the initial single administration of vaccine while animals in vaccine group 2 will receive the same vaccine as the prime vaccine 14 days post prime. At 35 days post prime (21 days post boost for boosted animals), all animals will be challenged with 3000 PFU of LASV-Josiah. The survival of infected macaques will be assessed, and clinical signs consistent with VHF (as measured by hematology, serum biochemistry, and blood coagulation assays) will be documented. Surviving animals will be euthanized at the study endpoint 4-5 weeks after LASV challenge. In addition, blood will be collected before and after vaccination and at the study endpoint for determination of binding antibody and neutralizing antibody titers. Blood and tissues will also be taken after virus challenge for histopathology, immunohistochemistry, virus isolation and genome detection. Peripheral blood mononuclear cells (PBMCs) will be isolated and cryopreserved for subsequent cellular phenotyping and functional analysis as outline in 1.1.1F.

VACCINE	LASV CHALLENGE
LASSA NP-NTD	10 (TWO GROUPS OF 5)
LASSA Gpe	
LASSA Gpe + NP-NTD	
Control animals (NO VACCINE)	5
Control vaccine (VSV-LASV-GPC)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for LASV Milestone 1.1.1.B

Similarly, we will also determine the protective efficacy of MACV following the grouping described in **Table XX** using the identical experimental approach as outlined above for LASV challenge. The challenge virus will be the established MACV Chicava strain **(REF).** Given the lack of clarity on the potential for the JUNV Candid-1 vaccine to afford protection from challenge with MACV, we will confirm protection in NHPs if demonstrated in guinea pigs **(REFs).**

VACCINE	MACV CHALLENGE
MACV CHICAVA NP NTD	10 (TWO GROUPS OF 5)
MACV CHICAVA GPe	
MACV CHICAVA NP + GPe	
Control animals (NO VACCINE)	5
Control vaccine (JUNV-Candid-1)	5
TOTALS	15

Table: XX outline of NHP efficacy studes for MACV Milestone 1.1.1.B

2.1.2.A: Development and optimization of patch vaccination approach employing nairovirus subunits.

VACCINE	IM VACCINATION		PATCH VACCINATION	
	ALHYDROGEL	PATCH ADJUVANT	ALHYDROGEL	PATCH ADJUVANT
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHFV-M)	8		8	
	TOTALS	128	**TOTALS**	128

2.1.2.B: Determination of pan-CCHF protective efficacy using optimized subunit-patch vaccination in STAT-1KO mice

VACCINE	PAN-LINEAGE PROTECTION			
	CLADE 1	CLADE 3	CLADE 4	CLADE 6
CCHF GnGc	8	8	8	8
CCHF GP38	8	8	8	8
CCHF NP	8	8	8	8
CCHF GnGc+GP38+NP	8	8	8	8
CCHF GnGc+GP38	8	8	8	8
CCHF GP38+NP	8	8	8	8
CCHF GnGc+NP	8	8	8	8
Control animals (NO VACCINE)	8		8	
Control vaccine (VSV-CCHF-M)	8		8	
	TOTALS	128	TOTALS	128

2.1.2.C: Determination of protective efficacy using optimized subunit-patch vaccination in NHP

VACCINE	CCHF CHALLENGE
CCHF GnGc	10 (TWO GROUPS OF 5)
CCHF GP38	
CCHF NP	
CCHF GnGc+GP38+NP	
CCHF GnGc+GP38	
CCHF GP38+NP	
CCHF GnGc+NP	
Control animals (NO VACCINE)	5
Control vaccine (VSV-CCHF-M)	5
	15

2.1.2.D: Determination of protective efficacy against novel, emerging arenaviruses using optimized subunit-patch vaccination in guinea pigs and NHP

VIRUS	GUINEA PIG CHALLENGE
	LUJV
LASSA NP-NTD	8
LASSA GPe	8
LASSA GPe + NP-NTD	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	GUINEA PIG CHALLENGE
	CHAPV
CHAPV NP NTD	8
CHAPV GPe	8
CHAPV NP + GPe	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	LUJV NHP CHALLENGE
LUJV NP-NTD	10 (TWO GROUPS OF 5)
LUJV GPe	
LUJV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

VACCINE	CHAPV NHP CHALLENGE
CHAPV NP NTD	10 (TWO GROUPS OF 5)
CHAPV GPe	
CHAPV GPe + NP-NTD	
Control animals (NO VACCINE)	5
TOTALS	15

2.1.2.E: Determination of protective efficacy against novel, emerging nairovirus using optimized subunit-patch vaccination in STAT-1KO mice and NHP

VIRUS	MOUSE CHALLENGE
	KASOKERO VIRUS
KASV GnGc	8
KASV NP	8
KASV GnGc+NP	8
Control animals (NO VACCINE)	8
TOTALS	32

VACCINE	KASOKERO NHP CHALLENGE
KASV GnGc	10 (TWO GROUPS OF 5)
KASV NP	
KASV GnGc +NP	
Control animals (NO VACCINE)	5
TOTALS	15

1.1.1.F: Determination of cellular and humoral immune correlates associated with protection

1.1.1.F.a: Define the phenotype and function of Arenavirus or Nairovirus specific T-cell responses in in subunit/patch vaccinated NHP compared to unvaccinated groups.

We will perform a targeted analysis of T-cell phenotype and function in study participants with or without subunit-patch vaccination using established methods for measurement of cellular immune responses (flow cytometry, LegendPLex, ELISPOT). Particular interest will be paid to CD4+ and CD8+ T-cells due to their respective role as important correlates of protection in the NHP vaccine model and long-lasting response in natural immunity. Thus, we will measure the strength and diversity of arena or nairo-specific CD4 and CD8 T-cell responses and expect responses in vaccinated EVD survivors to be superior to natural immunity.

Immune subset phenotyping: We will develop and optimize multi-color flow cytometry panels phenotyping a range of immune subsets (B-cells, Monocytes, Dendritic Cells, and NK cells) but primarily focused on T-cell phenotype (CD3, CD4, CD8, CD95, CD28). Standard whole blood flow cytometric staining protocols will be used for surface and intracellular staining markers, briefly cryopreserved PBMCs no older than 3 months old are gently thawed then washed with 2% FBS/PBS and stained with a viability dye for 20 minutes at room temperature. Cells are then washed again, surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS. Stained, fixed cells will then either be acquired directly. Cell populations are then quantified using a Cytek Aurora spectral flow cytometer using an NHP optimized panel of antibodies.

Viral protein stimulation experiments:. PBMC stimulations will be set up according to modifications of well established, previously published protocols. [24, 25] PBMCs will be directly stimulated with purified recombinant vaccine proteins or relevant GP or NP peptide pools using optimized concentrations. Samples will then be mixed incubated at 37°C for 1 hour. A protein transport inhibitor such as brefeldinA will then be added at a concentration of 10mg/mL and incubated at 37°C for until flow cytometry staining. Cells will then be washed and stained according to the flow cytometry protocol outlined below. Alternatively, isolated PBMC may be stimulated with purified recombinant GP and NP proteins or peptide pools using optimized concentrations diluted in R10 media (RPMI/1%Hepes/10%FBS). Cells will then be incubated overnight at 37°C. Stimulations to assess secreted analytes by multiplex-bead based assays will be set up in 96-well plates and supernatants will be harvested for further analysis.

Intracellular cytokine staining: We will utilize NHP optimized multi-color flow cytometry panels measuring a range of functional responses including important T-cell functional markers (ie: IFNg, TNFa, IL-2, IL-4, IL-6, PD-1, CTLA-4). Cells are stained with a viability dye for 20 minutes at room temperature, then washed with 2% FBS/PBS. Surface stain cocktail is added to the tubes, incubated for 20 minutes, and washed. Cells are incubated in BD Cytofix/Cytoperm solution for 20 minutes followed by a wash with BD Perm/Wash Buffer. Intracellular antibodies are then added, incubated, and washed. Cells are fixed for 1 hour with Stabilizing Fixative (BD Biosciences, Franklin Lakes, NJ) then washed in PBS. Stained, fixed cells will then be acquired using a Cytek Aurora spectral flow cytometer.

Multiplex Bead based assays: As a measure of global response to arena or nairo-specific T-cell activation, we will measure secreted cytokines in supernatants of cells stimulated with or without viral antigens or control (Staphylococcal enterotoxin B, SEB) proteins. We will develop a 4-color multiplex panel using BioLegend LegendPlex technology. Samples will be assayed according to manufacturer's instructions. Briefly, cytokine capture beads will be mixed and added to assay tubes followed by addition of prepared standards and controls, or participants samples and incubated for 3 hours at room temperature, protected from light. 1mL of wash buffer will be added and beads will be pelleted by centrifugation at 200g for 5 minutes. Wash buffer will be removed and discarded. Beads will be resuspended in 300uL wash buffer and data acquired on a MagPix xMAP instrument.

IFNg/TNFa ELISPOT: ELISPOT assays will be performed utilizing classical ELISPOT approaches. Briefly, cells with or without GP or NP antigens or peptide pools or control (SEB) antigen will be incubated overnight at 37ºC. Wells will be washed manually 3-times, followed by the addition of detection antibody and incubated either overnight at 4ºC or for 2 hours at room temperature. Wells will be washed again and incubated with the development substrate for 1 hour at room temperature protected from light. Wells will then be decanted, rinsed with deionized water and Chromagen Solution will be added to each well and incubated for 20 minutes at room temperature, protected from light. Finally, wells will be decanted and rinsed, dried thoroughly (60-90 minutes at room temperature or 15-30minutes at 37ºC), and spot forming units (SPU) will be read on an **need model** ELISPOT Fluorescence Microplate Reader.

Data Analysis: We will look at the differences in immune responses across groups between each timepoint and baseline. Model assumptions will be checked for a one-way ANOVA to compare the three groups. we will run the analysis after checking model assumptions, if those are not met then we will adjust to a nonparametric model or transform the outcome appropriately. To look at the longitudinal aspect of the data, we will investigate measurements over time between groups, the interaction of group and time can be analyzed using covariance pattern models (or linear mixed models), where the correlation between observations measured over time can be handled more flexibly. As a more exploratory analysis we will also look at adjusting for possible confounding variables such as sex and age. For categorical variables, comparisons will be made using Chi-Square tests. Significance will be set at 0.05 and GraphPad/PRISM (Carlsbad, CA), SAS software (Cary, NC), and R will be used for analyses. Flow cytometry data will be analyzed in FlowJo version 10. Diversity of T-cell responses will be analyzed by t-SNE in R.

Alternative Strategies: Although unlikely based on the available literature, differences in T-cells dynamics across groups may not be prevalent predictors in this context. We are also including measures of innate immunity in our flow cytometry panels to assess the relative importance of these subsets. Additionally, serum samples will be banked to assess the presence of biomarkers associated with differences between groups.

1.1.1.F.b: Systems serology *Characterization of Arenavirus and Nairovirus specific Ig subtypes and Fc-mediated innate effector function profiles in subunit/patch vaccinated NHP compared to unvaccinated groups.*

We will perform a comprehensive analysis of the humoral immune response using an Viral Systems Serology platform developed by Dr. Gunn in Core D[26-28] to define the Fc-mediated humoral antiviral functions associated with vaccination. Protective Fc-mediated humoral immune profiles have been identified in natural immunity to LASV and may serve as an effective baseline. Sera will be analyzed for 1) levels of LASV, MACV, CCHFV,LUJV, and CHAPV specific or cross-reactive antibodies; and 2) innate immune effector functions (phagocytosis, NK cell activation, and complement fixation).

Analysis of antibody levels by Multiplex immunoassay: Serum samples will be tested for antibody reactivity against a panel of viral antigens utilized for vaccination. To evaluate cross-reactive antibody responses against the GP of other LASV, MACV, or CCHFV lineages. We will use a multiplexed analysis to determine the levels of antibodies against the viral antigens using on MagPix xMAP instrument as described.[27, 29] Specifically, each viral antigen will be coupled to coded magnetic beads (Luminex), mixed together, and incubated with serial dilutions of patient samples. Beads are washed and the levels of different antibody isotypes/subclasses specific for each antigen will be determined using labeled secondary antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgM. For quantitation of bound antibody, we will make a standard using pooled banked serum from NHP survivors.

Analysis of Fc-mediated effector functions: We will characterize plasma samples for induction of innate immune effector functions including antibody-dependent phagocytosis (ADP), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent complement deposition (ADCD). All assays will include an NHP derived seropositive standard curve, and seronegative/isotype control.

ADP/ADCD: Recombinant EBOV GP protein will be biotinylated and coupled to fluorescent Neutravidin (Life Tech) beads, and serial dilutions of plasma samples/mAbs will be incubated with beads. To measure ADP, primary human monocytes, neutrophils, or THP-1 cells will be incubated with immune complexes, and uptake of beads will be measured by flow cytometry. To measure ADCD, freshly reconstituted guinea pig complement will be incubated with immune complexes. C3 deposition onto beads will be detected using an anti-C3 antibody conjugated to FITC (MP Biomedicals) and measured by flow cytometry at KGH.

ADCC: Primary human NK cells or NK92 cells with CD16 overexpression (ATCC) will be incubated with EBOV GP immune complexes bound to MaxiSorp ELISA plates for 5 hours at 37C 5% CO_2, and surface expression of CD107a will be measured to identify level of degranulation, a surrogate of ADCC, and intracellular cytokine staining will be used to detect production of pro-inflammatory cytokines and chemokines IFNg, MIP-1b, and TNFa by flow cytometry.

Data Analysis: Univariate analyses were performed using Prism 9.5.0 software. Kruskal–Wallis with Dunn multiple correction test or Mann–Whitney with Bonferroni correction was used to determine statistical significance between groups. A network analysis will be performed to identify significant antibody features. Associations between antibody features will be determined using nonparametric Spearman correlation coefficient. Statistically significant associations after Bonferroni correction for multiple comparisons (adjusted $P < .05$) were used to generate networks in Cytoscape (version 3.4.0).

Alternative Strategies: If we are unable to define clear immune profiles associated with each group, we will integrate our humoral immune profiing data with the larger dataset that includes antibody neutralization, cellular and inflammatory immune profiles, genetic markers (HLA-type), and additional clinical information (ie: PES symptoms). These will be included in the network analysis.

1.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments

PREP	JUNV NHP CHALLENGE
5 mg/kg	5
15mg/kg	5
Control animals (NO PREP)	5
TOTALS	15

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine. In conjunction with RP1 and Core E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by Abelson, Geisbert and Zeitlin will be produced with YTE and LA Fc point mutations (Table 4). The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (Bioqual, Inc.; Rockville, MD) to select a lead candidate for testing in NHPs against Junin Romero challenge in Core E. For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference). The variant with the most favorable PK will be selected for testing by **RP1/Core E**. Briefly, groups of 4 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV (Espindola or Romero?) delivered

IV. For further details of the NHP testing, please see **Core E**. These data will be used by the Advisory Committee to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes.

Commented [CRW4]: From Larry, adapt from this.

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC

Please note: Discussion of expected results, other alternative approaches and criteria for "go" or "no go" decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the **Project Milestones and Timelines** *section.*

JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,

AIM 2: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:

Expected results, alternative approaches and Go-No-Go decisions (brief summary – see also Project Milestones and Timelines). We expect to be able to construct an immunogenic and protective subunit-patch targeting LASV, MACV, and CCHFV ……. If this is not the case any of these three viruses by year 2 we will refocus effects on the remaining virus families adjusting the requested budgets accordingly after consulation with NIAID and SAC

Please note: Discussion of expected results, other alternative approaches and criteria for "go" or "no go" decisions for MILESTONES 1-4 are discussed as required in RFA-AI-20-023 in the **Project Milestones and Timelines** *section.*

JUNV GP/NP as alternative strategy, mRNA as an alternative strategy,

Milestones-Timelines-GANT CHART

Summary statement impact

1. Abudurexiti, A., et al., *Taxonomy of the order Bunyavirales: update 2019.* Arch Virol, 2019. **164**(7): p. 1949-1965.

2. Saito, T., et al., *Vaccine Candidates against Arenavirus Infections.* Vaccines, 2023. **11**(3): p. 635.
3. Koma, T., et al., *Machupo Virus Expressing GPC of the Candid#1 Vaccine Strain of Junin Virus Is Highly Attenuated and Immunogenic.* J Virol, 2016. **90**(3): p. 1290-7.
4. Jahrling P, T.R., Barrero O. . *Crossprotection against Machupo virus with Candid 1 Junin virus vaccine*. in *2nd Int Conf Impact Viral Dis Dev Latin Am Countries Caribbean Region*. 1988. Mar del Plata, Argentina.
5. Balmert, S.C., et al., *A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity.* iScience, 2022. **25**(10): p. 105045.
6. Sewlall, N.H., et al., *Clinical features and patient management of Lujo hemorrhagic fever.* PLoS Negl Trop Dis, 2014. **8**(11): p. e3233.
7. Delgado, S., et al., *Chapare Virus, a Newly Discovered Arenavirus Isolated from a Fatal Hemorrhagic Fever Case in Bolivia.* PLOS Pathogens, 2008. **4**(4): p. e1000047.
8. Loayza Mafayle, R., et al., *Chapare Hemorrhagic Fever and Virus Detection in Rodents in Bolivia in 2019.* New England Journal of Medicine, 2022. **386**(24): p. 2283-2294.
9. Kalunda, M., et al., *Kasokero Virus: a New Human Pathogen from Bats (Rousettus Aegyptiacus) in Uganda.* The American Journal of Tropical Medicine and Hygiene, 1986. **35**(2): p. 387-392.
10. Schuh, A.J., et al., *Human-Pathogenic Kasokero Virus in Field-Collected Ticks.* Emerg Infect Dis, 2020. **26**(12): p. 2944-2950.
11. Brisse, M.E. and H. Ly, *Hemorrhagic Fever-Causing Arenaviruses: Lethal Pathogens and Potent Immune Suppressors.* Front Immunol, 2019. **10**: p. 372.
12. Baize, S., et al., *Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys.* J Virol, 2009. **83**(11): p. 5890-903.
13. Fisher, D.G., G.M. Coppock, and C.B. Lopez, *Virus-derived immunostimulatory RNA induces type I IFN-dependent antibodies and T-cell responses during vaccination.* Vaccine, 2018. **36**(28): p. 4039-4045.
14. Meyer, B. and H. Ly, *Inhibition of Innate Immune Responses Is Key to Pathogenesis by Arenaviruses.* J Virol, 2016. **90**(8): p. 3810-3818.
15. ter Meulen, J., et al., *Characterization of human CD4(+) T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein.* J Virol, 2000. **74**(5): p. 2186-92.
16. Meulen, J., et al., *Old and New World arenaviruses share a highly conserved epitope in the fusion domain of the glycoprotein 2, which is recognized by Lassa virus-specific human CD4+ T-cell clones.* Virology, 2004. **321**(1): p. 134-43.
17. Goicochea, M.A., et al., *Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model.* Vaccine, 2012. **30**(8): p. 1445-52.
18. Sullivan, B.M., et al., *High crossreactivity of human T cell responses between Lassa virus lineages.* PLoS Pathog, 2020. **16**(3): p. e1008352.
19. Leventhal, S.S., et al., *Replicating RNA vaccination elicits an unexpected immune response that efficiently protects mice against lethal Crimean-Congo hemorrhagic fever virus challenge.* EBioMedicine, 2022. **82**: p. 104188.
20. Hastie, K.M., et al., *Structural basis for antibody-mediated neutralization of Lassa virus.* Science, 2017. **356**(6341): p. 923-928.
21. Mire, C.E., et al., *Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever.* Nat Med, 2017. **23**(10): p. 1146-1149.
22. McElroy, A.K., et al., *A Case of Human Lassa Virus Infection With Robust Acute T-Cell Activation and Long-Term Virus-Specific T-Cell Responses.* J Infect Dis, 2017. **215**(12): p. 1862-1872.
23. Carter, S.D., et al., *Structure, function, and evolution of the Crimean-Congo hemorrhagic fever virus nucleocapsid protein.* J Virol, 2012. **86**(20): p. 10914-23.
24. Meddows-Taylor, S., et al., *Development of a whole blood intracellular cytokine staining assay for mapping CD4(+) and CD8(+) T-cell responses across the HIV-1 genome.* J Virol Methods, 2007. **144**(1-2): p. 115-21.
25. Kagina, B.M., et al., *Qualification of a whole blood intracellular cytokine staining assay to measure mycobacteria-specific CD4 and CD8 T cell immunity by flow cytometry.* J Immunol Methods, 2015. **417**: p. 22-33.
26. Gunn, B.M., et al., *A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus.* Immunity, 2021. **54**(4): p. 815-828.e5.
27. Gunn, B.M., et al., *Survivors of Ebola Virus Disease Develop Polyfunctional Antibody Responses.* J Infect Dis, 2020. **221**(1): p. 156-161.
28. Saphire, E.O., et al., *Antibody-mediated protection against Ebola virus.* Nat Immunol, 2018. **19**(11): p. 1169-1178.

29. Brown, E.P., et al., *Multiplexed Fc array for evaluation of antigen-specific antibody effector profiles.* J Immunol Methods, 2017. **443**: p. 33-44.

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Cc: Geisbert, Thomas W.; Gunn, Bronwyn Mei; Antony Dimitrov
Subject: Re: ReVAMPP Bunyavirales Project
Date: Friday, June 2, 2023 4:10:24 PM
Attachments: 01JUN23_RP1-BUNYAVIRALES VACCINES--RESEARCH STRATEGY_RWC_LZ.docx

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I added some language for the mAb as alternative to a vaccine sections…

From: Cross, Robert W. <rwcross@UTMB.EDU>
Date: Thursday, June 1, 2023 at 11:56 PM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Larry Zeitlin <larry.zeitlin@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Getting there, here is the update to now. I hope to finish filling out missing sections by tomorrow evening. Welcome any feedback

Bob

From: Cross, Robert W.
Sent: Monday, May 29, 2023 1:49 PM
To: Broder, Christopher <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Hi Chris,

Some thoughts…

On the PREP, this is just the one study being proposed as proof of concept. Will let Larry elaborate, but we will need to wordsmith to suggest that if successful this may be an avenue to look at with other viruses with the support of SAC and program.

On the cellular and humoral part. Space is a big issue here and I'm not sure we will need to describe in detail in every RP or core, but it needs to be outlined somewhere clearly and that will eat up some space. I put it in my RP since there was no room in the animal core and Larry's core didn't detail it too much either (so far). My version will likely need to be whittled down as well to make everything fit, so but not so much that the gist is lost. I wonder if we could find a small amount of space to describe the immunology work up I described will be carried out in the scientific cores (and in collaboration with RP1) as outlined in RP1? That said, we can maybe refer to methods used in RP1 for the core as well since that write-up is already having issues with space? Also note that I'm not doing any immunology on rodents since the patch core mentioned they would do rudimentary serology and ELISPOTs, all this advanced immunology work will be done with primates only due to lack of reagents.

Bob

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Monday, May 29, 2023 1:09 PM
To: Dafna Abelson <dafna.abelson@mappbio.com>
Cc: Cross, Robert W. <rwcross@UTMB.EDU>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

hi all
RP2 is not looking anything like this post-vaccination subject analysis on the CD4/CD8 and cellular responses or the serological studies.

We were allowing the Upitt core to explore the mice with cellular responses as the pilot.
The plan for the MNP vaccines is to test efficacy in ferrets then proceed to AGMs.

Not sure what to do or how to proceed here! Are there analysis text sections from what the animal core can or should propose here or outline here, as Bob laid out? I dont have anything like this in hand,

also, i saw the comment on PREP
We dont have any plans for PREP for NiV/HeV included here either, our best candidates are already in Mapp's pipeline, or in the CETR

chris

On Sun, May 28, 2023 at 3:35 PM Dafna Abelson <dafna.abelson@mappbio.com> wrote:

> Hey Bob
> Looks great! I added some language to use or lose in the Engineering Glycoproteins for CCHF section and the gel we used in the Core D preliminary results.
> Best,
> Dafna
>
> ---
>
> **From:** Cross, Robert W. <rwcross@UTMB.EDU>

Date: Saturday, May 27, 2023 at 10:26 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

A ways to go on the text, but studies mapped out fairly well at this point, working on filling in text for areas highlighted in blue. Sending now to give the group an idea on direction for aims and subaims

Will work on fleshing out significance/innovation sections/and approach text tomorrow. Tables will be shrunk to fit, but followed Larry's color scheme to be consistent for now.

- Bonnie I adapted the immunology support sections from our pilot grant to start with. Will very likely need to majorly consolidate, but better to have too much and refine to essentials once all the content is there.

- Larry, I just included the pilot study for the PREP study assuming you and Tom already budgeted for it. You mentioned go-no-go, but I did not budget for any "go" studies, so maybe you mean PREP for henipaviruses?

- Dafna and Larry, open to thoughts on the CCHF proteins for prelimary data or innovation. I had some serology which we did using your proteins on human survivors to demonstrate reactivity which I think is helpful. Open to other thoughts.

- Tom, I added another group for each of the NHP studies to allow for 2 groups to be evaluated either with different doses or different vax formulations. Was thinking this might be ideal for grantsmanship to have at least another option. Thinking was that we would not use all 5 controls for all studies and may be able to make these up?

Bob

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Wednesday, May 17, 2023 2:51 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Bob,

This is what is in Core D so far:

A mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine. In conjunction with RP1 and Core E, an IM long-acting mAb prototype will be generated as a Go/No Go decision as to whether this product format will be an option for development with any of the other viruses being targeted by PABVAX. For this proof-of-concept effort, an anti-Junin virus mAb with potent therapeutic efficacy (guinea pig and NHP references) previously described by Abelson, Geisbert and Zeitlin will be produced with YTE and LA Fc point mutations (Table 4). The pharmacokinetics (PK) of these two variants will be evaluated in NHPs (Bioqual, Inc.; Rockville, MD) to select a lead candidate for testing in NHPs against Junin Romero challenge in Core E. For the PK study, two doses (15 and 5 mg/kg) of each mAb will be administered IM to groups of 3 cynomolgus macaques (mixed sex). Serum will be collected (pre-dosing, 15 min, 6 hours and at days 1, 2, 3, 7, 10, 14, 21, 28, 42, 56) and tested via ELISA and Octet to quantitate serum antibody concetrations. PK parameters will be modeled with WinNonlin Phoenix software (reference). The variant with the most favorable PK will be selected for testing by **RP1/Core E**. Briefly, groups of 4 cynomolgus macaques (mixed sex) will receive 15 or 5 mg/kg of the lead variant IM and the animals will be challenged one month later with 5000 pfu of JUNV (Espindola or Romero?) delivered IV. For further details of the NHP testing, please see **Core E**. These data will be used by the Advisory Committee to inform decision making around possible additional IM mAb prophylaxis plug and play prototypes.

Feel free to tweak so it fits with what you are thinking about. I can also give you some innovation language if you'd like on this topic.

Best
LZ

From: Cross, Robert W. <rwcross@UTMB.EDU>
Date: Wednesday, May 17, 2023 at 3:47 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

I'm trying to shoe horn the prophy into RP1, will ciruculate to the group as a whole shortly. If we add this PEP study into RP1, we will probably need to renaim RP1 to something like: **Novel Vaccine Approaches for Emerging Arenaviruses and Nairoviruses: (Cross, Abelson, Gunn)**

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Saturday, May 13, 2023 11:25 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Cross, Robert W. <rwcross@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Tom,

I like the idea of it as a sub-aim. Core D will refer to it (manufacturing the mAb as well as performing PK to select the best Fc mutant). Depending on how much you are mentioning about the protocols in the Animal Core, you could also mention it there (e.g. "For studies looking at long-acting mAbs as an alternative to a vaccine, NHPs will receive a single dose 1 month prior to challenge…).

I can also if preferred, describe the study in Core D rather than RP1. I'm certainly setting up the idea of it with the PK work…

Best
LZ

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Date: Saturday, May 13, 2023 at 12:15 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Larry Zeitlin <larry.zeitlin@mappbio.com>, Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Larry, Dafna

How do you want to handle the Junin NHP prophylaxis study? At the very least I think RP1 has to mention it somewhere. Does it go into RP1 as a subaim? I don't think we can say it is alternate strategy as that route is usually taken when something proposed fails. Does it get pitched as a subaim to derisk the project? Not quite sure here.

Many thanks!

Tom

From: Cross, Robert W. <rwcross@UTMB.EDU>
Sent: Saturday, May 13, 2023 11:10 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Great, thanks!

Yeah it was challenging to add a lot into the SA page. But we can refine to make it more inclusive of all points addressed. Definitely will stretch out in the Research strategy.

We can maybe add some reverse genetics, especially for missing MACV lineages…maybe CCHF for the same reason using the cassette approach. Need to think about it a little today, but this may work.

Do we need to add the JUNV prophy study into RP1? Or is that in the core?

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Saturday, May 13, 2023 9:38 AM
To: Cross, Robert W. <rwcross@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: ReVAMPP Bunyavirales Project

Thanks Bob! One thing that we need to think about as Chris outlined for the rescue of Langya in the RP2 Aims is are we going to tackle reverse genetics at all for arenaviruses and CCHF? If so will need to tie that into the Animal Core in terms of rescue. Other thing is tie into Mapp Core for the Junin NHP prophylaxis study in Year 1 as an alternative approach to vaccination. And then need to think about if we need an approach to tie into RP4 and RP5. All of this of course does not need to be on the Aims page and can be addressed in the 12 page Research Strategy. If you tackle reverse genetics it could be its own Aim along the lines of what Chris has in RP2.

From: Cross, Robert W. <rwcross@UTMB.EDU>
Sent: Friday, May 12, 2023 3:52 PM
To: Dafna Abelson <dafna.abelson@mappbio.com>; Larry Zeitlin <larry.zeitlin@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Gunn, Bronwyn Mei <bronwyn.gunn@wsu.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: RE: ReVAMPP Bunyavirales Project

Here is the first attempt at the specific aims page. Kept it simple to start.

From: Dafna Abelson <dafna.abelson@mappbio.com>
Sent: Thursday, May 4, 2023 1:31 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>; Cross, Robert W. <rwcross@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Hi Bob

Here’s a draft of an SBIR that Crystal wrote in case that’s helpful. It only has data from the first Guinea pig study.

Best,

Dafna

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Date: Thursday, May 4, 2023 at 11:18 AM
To: Cross, Robert W. <rwcross@UTMB.EDU>, Dafna Abelson <dafna.abelson@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Bob,

Please have at it and let us know how we can help.

Best

LZ

From: Cross, Robert W. <rwcross@UTMB.EDU>
Date: Thursday, May 4, 2023 at 2:07 PM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, Dafna Abelson <dafna.abelson@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: RE: ReVAMPP Bunyavirales Project

Agree, this SA page from Crystal is super helpful. Was going to start putting pen to paper to get a rough draft started for the Bunya RP. Unless you guys have already started?

Is there a format we need to follow for writing the proposal? I think we had 12 pages correct?

Assuming the standard NIH format?

- Significance
- Innovation
- Preliminary data
- Approach/SpecificAim 1-?
- Alternative strategies
- Milestones-Timelines
- Summary statement impact

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Tuesday, April 18, 2023 3:43 PM
To: Dafna Abelson <dafna.abelson@mappbio.com>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Oh, and here is the specific aims page of Crystal's proposal from a few years ago…looks like it would fit pretty well

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From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Date: Tuesday, April 18, 2023 at 4:34 PM
To: Dafna Abelson <dafna.abelson@mappbio.com>, Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Cross, Robert W. <rwcross@UTMB.EDU>
Subject: Re: ReVAMPP Bunyavirales Project

Just a few thoughts:

1. It looks like we can have multiple prototypes per family if justified. I think lassa and junin would be great prototypes for old and new world arenas…seems easy to justify as we don't know if a single product could cover both and pursuing both could help to answer the question.
2. Might be good to confirm with program, but as I initially read it, it looks like if we start with CCHF we'd be expected in Y4-Y5 to expand to other nairos. Hantas may be a little easier – Jim has some mAbs and Mapp does as well, but those are encumbered a bit with Kartik's CETR so we'd have to have some conversations to make sure that would be kosher.
3. The RFA has a bit of mixed message on vaccines and mAbs. I do think they go well together and mAbs can be an alternative option to a vaccine.
4. Do the microneedle people have some experience transitioning to the clinic? I'm looking at this language: "Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine expertise in manufacturing, clinical development, and regulatory pathways." Mapp can fill that role for mAb vaccines, but not so well for traditional vaccines. It looks like we don't have to have them lined up yet, but might be nice to have a letter of support if needed. DVC is an option as is IBT.

From: Dafna Abelson <dafna.abelson@mappbio.com>
Date: Tuesday, April 18, 2023 at 4:15 PM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>, Cross, Robert W. <rwcross@UTMB.EDU>, Larry Zeitlin <larry.zeitlin@mappbio.com>

Subject: Re: ReVAMPP Bunyavirales Project

Hi Tom

We do have the Junin PBMCs you sent and I like the idea of including Junin in the proposal because we already have cell lines in house to express Machupo, Junin and Lassa GPs. I think we could hit the ground running on arenavirus antibody discovery.

I don't have strong feelings on Hanta vs CCHF. Larry?

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Date: Tuesday, April 18, 2023 at 12:53 PM
To: Cross, Robert W. <rwcross@UTMB.EDU>, Larry Zeitlin <larry.zeitlin@mappbio.com>, Dafna Abelson <dafna.abelson@mappbio.com>
Subject: ReVAMPP Bunyavirales Project

All,

We need to think about the ReVAMPP Bunyavirales Project and what viruses and how to integrate with all of the other projects. I know it is tough to schedule a Zoom call so maybe we can kick this around on email a little. I think we all agree that the Mapp Lassa vaccine data is great. So maybe Lassa is the prototype even though we know CEPI is funding Lassa vaccines and it has a lot more attention than other things. But prototype with a plan to transition to other arenaviruses. So move the Lassa vaccine to the microneedle patch which ties in nicely with that Core. I guess we need to think about whether to have pan arenavirus vaccines or individual. I think Junin is a good choice as well. To that end I think we had sent PBMC from Junin Candid 1 vaccinees to Mapp right before COVID-19 hit. Do you still have those? If we go with Lassa do we have a plan for Lassa MAbs to support? Then for another family I think CCHF for Nairoviridae makes the most sense as we have already been working on it for MAbs as has Jim Crowe. We would need to sort out the vaccine angle that ties into the microneedle patch. So I guess then for arenaviruses we would have Lassa and maybe Junin as prototypes and for nairoviruses CCHFV. Where I get confused and am struggling is the transition in Years 4 and 5 that NIAID/NIH has to approve where I think the expectation is that you move from prototypes to more broad coverage. So do we say for example for Lassa since there are so many lineages that the prototype is lineage IV Josiah but in the out years we show that it protects against all lineages? Same thing for CCHF. Or is the expectation that you go beyond the prototype virus so for example from Lassa and Junin to also cover say Machupo, Guanarito, Chapare, etc.? Or is it better instead of CCHF say to start with Andes as the prototype and then move in the out years to Hantaan or vice versa? Thoughts?

Tom

Project Summary

Among viruses that cause disease in humans viral zoonoses within the family Paramyxoviridae and order Bunyavirales contain pathogens that cause significant lethality. Importantly, within the Paramyxovirdae the zoonotic henipavirus Hendra virus (HeV) is on the World Health Organizations (WHO) Priority Pathogens list. Notably, henipaviruses stand out for their impressive lethality with case fatality rates averaging about 75%. The Arenaviridae and Phelboviridae families contain viruses that cause severe hemorrhagic disease in humans. These viruses include the arenaviruses Lassa virus (LASV) and Junin virus (JUNV) and the phleboviruses Rift Valley fever virus (RVFV) and severe fever with thrombocytopenia syndrome virus (SFTSV). LASV and RVFV are also included among the WHO List of Priority Pathogens and pose threats to global public health due to the absence of approved vaccines or therapeutics. There is a clear unmet need for medical countermeasures to combat the threat of natural outbreaks, epidemics, or deliberate release. The Machine Learning-(ML)-Enabled Design of Prototype Pathogen Vaccines and Antibodies Center is a well-integrated consortium conducting a set of discovery and translational research programs composed of an Administrative Core, a Data Management Core, X Scientific Cores, and X Research Projects (RPs). The synergistic activities of the Center partners will be focused on developing novel vaccines, treatments, and tools to support pandemic preparedness. Federal law requires that the paramyxoviruses, arenaviruses, and phleboviruses used in the Center be handled in approved Biosafety Level (BSL)-3 and BSL-4 containment laboratories. Core D provides approved BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities and a trained and highly experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2. The services provided by Core D will include 1) a secure repository of well characterized seed stocks of BSL-3 and BSL-4 paramyxoviruses, arenaviruses, and phleboviruses 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5); development and/or optimization of animal models for strains or variants of Sosuga virus (SOSV) and JUNV to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BSL-3 and BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-3 and BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies.

Relevance

The Animal Models and Vaccine Evaluation Scientific Core (Core D) provides BSL-3 and BSL-4 resources and expertise for RP1 and RP2. The goal of Core D is to work closely with Research Project and Scientific Core Leaders and staff, the Scientific Advisory Board, and NIAID to advance the development of countermeasures against Paramyxoviridae and Bunyavlrales.

ANIMAL MODELS AND VACCINE EVALUATION SCIENTIFIC CORE (CORE D)

1. SPECIFIC AIMS

The main objective of the Animal Models and Vaccine Evaluation Scientific Core is to test and characterize the host response and efficacy of the vaccines and monoclonal antibody (mAb) based treatments identified in RP1 and RP2 against paramyxoviruses, arenaviruses, and phleboviruses under BSL-3/ABSL-3 and BSL-4/ABSL-4 containment. The Core will also develop select reporter viruses and conduct cell-based assays to characterize vaccine-induced neutralizing antibody responses and mAbs.

The Specific Aims of the Animal Models and Vaccine Evaluation Scientific Core are to:

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses.

Aim 2: Provide technical expertise and conduct *in vitro* assays.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, immunology assays, virology assays, necropsies, and histopathological analysis.

Aim 4: Develop and/or optimize animal models for Sosuga virus and Junin virus.

The Aims will be achieved by the following specific functions to be carried out by the Animal Models and Vaccine Evaluation Scientific Core Leader, Co-Core Leaders, and personnel:

1. Maintain records of well characterized BSL-3 and BSL-4 Hendra virus (HeV), Sosuga virus (SOSV), Lassa virus (LASV), Junin virus (JUNV), Rift valley fever virus (RVFV), and severe fever with thrombocytopenia syndrome virus (SFTSV) seed stocks including background history, passage history, genomic information, periodic monitoring of titer, mycoplasma testing, endotoxin testing, and sterility testing.
2. Perform *in vitro* antiviral activity assays. Including high-throughput BSL-2 based neutralization testing of henipavirus vaccine responses and mAbs.
3. Assist Center Investigators in designing animal studies, prepare animal protocols, and obtain IACUC approval of BSL-3 and BSL-4 animal studies.
4. Perform animal challenges using well characterized HeV, SOSV, LASV, JUNV, RVFV, and SFTSV seed stocks for RP1 and RP2.
5. Perform vaccination and treatment of animals with vaccines and treatments provided by RP1 and RP2.
6. Provide samples from animals collected during the vaccination portion of studies to RP1 for immunological analysis.
7. Perform all duties associated with animal challenge studies including monitoring of animals, collection of samples, and necropsies.
8. Perform clinical pathology assays including hematology and clinical chemistry assays.
9. Perform virology assays on blood and/or tissue samples collected from infected animals including viral load by plaque assay and RT-PCR.
10. Perform gross pathology analysis on all animals at necropsy.
11. Perform histopathologic analysis on tissues from animals collected at necropsy.
12. Develop and/or optimize animal models for SOSV and JUNV to fill gaps; make these models available to Center Research Projects and Scientific Cores as well as external ReVAMPP Centers
13. Provide all data generated in Core D to Data Management Core B.

RESEARCH STRATEGY

A. SIGNIFICANCE

Among viruses that cause disease in humans viral zoonoses within the family Paramyxoviridae and order Bunyavirales contain pathogens that cause significant lethality. Importantly, within the Paramyxovirdae the zoonotic henipavirus Hendra virus (HeV) is on the World Health Organizations (WHO) Priority Pathogens list. Notably, henipaviruses stand out for their impressive lethality with case fatality rates averaging about 75%. The Arenaviridae and Phelboviridae families contain viruses that cause severe hemorrhagic disease in humans. These viruses include the arenaviruses Lassa virus (LASV) and Junin virus (JUNV) and the phleboviruses Rift Valley fever virus (RVFV) and severe fever with thrombocytopenia syndrome virus (SFTSV). LASV and RVFV are also included among the WHO List of Priority Pathogens and pose threats to global public health due to the absence of approved vaccines or therapeutics. There is a clear unmet need for medical countermeasures to combat the threat of natural outbreaks, epidemics, or deliberate release. The Machine Learning-(ML)-Enabled Design of Prototype Pathogen Vaccines and Antibodies Center is a well-integrated consortium conducting a set of discovery and translational research programs composed of an Administrative Core, a Data Management Core, X Scientific Cores, and X Research Projects (RPs). The synergistic activities of the Center partners will be focused on developing novel vaccines, treatments, and tools to support pandemic preparedness. Federal law requires that the paramyxoviruses, arenaviruses, and phleboviruses used in the Center be handled in approved Biosafety Level (BSL)-3 and BSL-4 containment laboratories. Core D provides approved BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities and a trained and highly experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2. The services provided by Core D will include 1) a secure repository of well characterized seed stocks of BSL-3 and BSL-4 paramyxoviruses, arenaviruses, and phleboviruses 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5); development and/or optimization of animal models for strains or variants of Sosuga virus (SOSV) and JUNV to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BSL-3 and BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-3 and BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies.

All X of the RPs within the Center focus on developing vaccines or monoclonal antibody (mAb) based therapies against paramyxoviruses, arenaviruses, and phleboviruses. RP1 and RP2 require that countermeasures be evaluated in animals against infectious paramyxoviruses, arenaviruses, and phleboviruses. As HeV, LASV, JUNV, and RVFV are CDC and/or USDA select agents Federal regulations require that these viruses be handled in Biosafety Level (BSL)-3 or BSL-4 containment. Core D provides approved BSL-3/ABSL-3 and BSL-4/BSL-4 facilities and a trained and experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2.

B. INNOVATION

Scientific Core D will be co-located within the Galveston National Laboratory (GNL) and Robert Shope Laboratory at the University of Texas Medical Branch (UTMB) at Galveston. The GNL is a high and maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda and stands ready to assist local, state and national public health efforts in the event of a public health emergency. The facility is unique in that it is one of two operational BSL-4 facilities in the United States that is located on an academic campus. This unique location benefits the Center in several ways. Regarding physical resources the GNL is a state-of-the-art facility with many specialized assets including BSL-4 imaging, telemetry, and aerobiology equipment. In addition, being located on an academic campus Drs. Geisbert (Core D, Project Leader (PL), Cross, Freiberg, and Ikegami are faculty members in the Departments of Microbiology and Immunology and Pathology. This academic environment is a major strength, that fosters creativity and collaboration with other UTMB investigators which will be beneficial to the overall success of Core D. Dr. Broder, is faculty member of the Department of Microbiology and Immunology at Uniformed Services University (USU). Drs. Broder and Geisbert have a long-standing emerging virus research collaboration which has included the development and characterization the first nonhuman primate (NHP) models for HeV and Nipah virus (NiV) that will be employed by the Center's RPs. Another strength within the Core is Drs. Geisbert, Broder, Cross, and Veesler (Center Co-PI) currently collaborate and lead active henipavirus projects that are focused on translational countermeasure development which includes more traditional vaccine and mAb therapeutics approaches, and the shared knowledge base and experience in these areas will greatly facilitate the Core's abilities in supporting the Center's research goals in applying advanced machine learning-enabled designs of effective prototype pathogen vaccines and antibodies. Along with animal model development and animal modeling expertise, many of the unique virological tools including recombinantly generated RNA viruses, reporter gene-encoding viruses, and other pseudotype virus technologies, that will be employed by the Core, have been developed from UTMB-USU collaboration.

C. APPROACH

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses.

Paramyxovirus, arenavirus, and phlebovirus seed stocks. UTMB will provide fully equipped BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities that are required to work with HeV, SOSV, LASV, JUNV, RVFV, and SFTSV. UTMB will also provide and maintain all required approvals to work these viruses. The BSL-3 and BSL-4 paramyxovirus, arenavirus, and phlebovirus seed stocks employed for RP1 and RP2 have been or will be titered by conventional standard plaque assay and qRT-PCR as detailed in Aim 2 below. The virus strain identity of each seed stock has been or will be confirmed by sequence analysis. In addition, RNA has been or will be deep sequenced to verify the complete viral genome of each virus seed stock used for animal challenge studies. We have or will test each seed stock for mycoplasma contamination. Endotoxin testing has been or will be performed on paramyxovirus, arenavirus, and phlebovirus stocks using the Endosafe PTS system (Charles River Laboratories) following the manufacturer’s instructions. The Endosafe PTS system is a cartridge-based testing system utilizing FDA-licensed cartridges (0.1 EU/ml, 0.05 EU/ml, 0.01 EU/ml, 0.05 EU/ml) for ultrasensitive testing. Seed stocks will also be evaluated for morphological identity by transmission electron microscopy (TEM). Virus particle counts will be determined using a Virus Counter and confirmed by conventional TEM methods if needed. The bacteriological and mycological sterility of the product will be determined by selection of aliquots of the dispensed stock, inoculation of a sterile 500 ml bottle of Dulbecco's high glucose MEM with 10% FBS and then after 3 days subcultured to FTM and TBS media. paramyxovirus, arenavirus, and phlebovirus seed stocks will be inventoried in the UTMB BSL-4 and BSL-4 virus stock databases and secured in dedicated controlled access alarmed minus 80°C freezers in the GNL or Robert Shope Laboratory. Paramyxovirus, arenavirus, and phlebovirus seed stocks are monitored periodically for any changes in virus titer.

Plan for staffing, managing, and prioritizing Core use. The Core Leader and Co-Leaders have a cadre of staff that are trained and highly experienced in all BSL-3 and BSL-4 procedures and assays needed to conduct the proposed work. In addition to the Core Leader and Co-Leaders this staff includes an ACVP board certified veterinary pathologist, two BSL-4 contributing scientists, a BSL-4 Technical Director, a BSL-4 trained postdoctoral fellow, six BSL-4 experienced technical staff, a BSL-4 trained histotechnologist, and a BSL-4 trained graduate student. We do not anticipate any BSL-3 or BSL-4 scheduling problems regarding studies proposed in RP1 or RP2. However, we have developed a mitigation strategy if unforeseen events cause any prioritization of resources. A committee consisting of the Core D Leader, the PI of the Center, and the members of the external Scientific Advisory Committee (SAC) will review the status of RP1 and RP2 and any circumstances causing a prioritization of work. The Committee will also hold a meeting or teleconference (Zoom, Teams) with the Project Leaders and key staff of RP1 and RP2 to gather additional information. After careful review of all available information the Committee will then make a recommendation on prioritization to the Center PI. The Center PI will then consult with NIAID/NIH program staff and make the final determination on prioritization.

Aim 2: Provide technical expertise and conduct *in vitro* assays.

In vitro antiviral activity assays: Core D will perform conventional virus neutralization assays for RP1 and RP2 to determine the ability of sera/plasma from immunized animals from RP1 and antibodies from RP2 to inhibit the replication of paramyxoviruses, arenaviruses, and phleboviruses *in vitro* using infectious viruses in the UTMB BSL-3 and BSL-4 laboratories. In brief, cultures of Vero, Vero E6, SW13, or other cell types will be infected with a range of m.o.i. of paramyxoviruses, arenaviruses, and bunyaviruses and treated with various concentrations of the provided sera/plasma or antibodies. Cells and/or culture fluids will be harvested at various time points after infection and infectious virus enumerated by conventional plaque assay as detailed below.

Develop chimeric reporter-gene encoding viruses and virus neutralization assays for BSL-2 use. We have previously used reverse genetics to generate several types of replication competent VSV-GFP reporter viruses including those encoding Australian bat lyssavirus (ABLV) G glycoproteins (VSV-ABLV G-GFP)[1,2] , as well as employed reverse genetics to rescue authentic ABLV and various reporter gene-encoding viruses[3,4]. In addition, we have generated a reverse genetics platform for generating recombinant Cedar virus (rCedV) and rCedV chimeras whereby the fusion (F) and attachment (G) glycoprotein genes of rCedV were replaced with those of NiV-Bangladesh (NiV-B) or HeV, generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV), both with and without reporter genes[4-8]. The rCedV chimeras are a replication competent authentic henipavirus platform that is a high-throughput surrogate neutralization assay that is rapid, cost-effective and can be utilized outside high containment. Similar approaches will be applied here to generate new reporter viruses for Ghana bat virus (GhV). We will design, construct and rescue both VSV and rCedV chimeras that encode the envelope glycoproteins (fusion (F) and attachment (G) glycoproteins) of GhV. Two reporter-gene encoding versions will be attempted for VSV-GhV: one encoding GFP (VSV-GhV-GFP) and the other encoding the *Photinus pyralis* (firefly) luciferase gene (VSV-GhV-Luc). We will also construct and rescue GFP and Luc reporter gene encoding versions of rCedV chimeras encoding the GhV F and G genes (rCedV-GhV-GFP and rCedV-GhV-Luc) (**Fig. 1**). The rescued viruses will be characterized using cell-based infection tropism and growth kinetics assays.

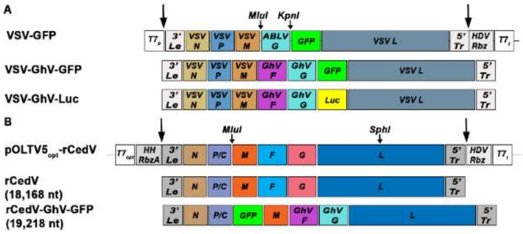

Figure 1. Schematic of chimeric reporter viruses. (**A**)The VSV-ABLV G-GFP plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and KpnI to construct VSV-GhV-GFP and VSV-GhV-Luc. (**B**) pOLTV5$_{opt}$-rCedV plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and SphI used to construct rCedV-GhV-GFP.

The rCedV (NiV-B and HeV) chimeric virus platform serves as a robust surrogate neutralization assay system for quantifying antibody neutralization potency of anti-NiV and anti-HeV antisera and mAbs[8]. The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G specific mAbs against rCedV-NiV-B-GFP and rCedV-HeV-GFP are highly correlated with those obtained using authentic NiV-B and HeV when tested in parallel by plaque reduction neutralization test (PRNT). Pearson correlation of rCedV chimeras BSL-2 PRNT to the NiV-B or HeV BSL-4 PRNT is shown in **Fig. 2a**. A high-throughput quantitative assay based on reduction of GFP foci (fluorescent reduction neutralization test (FRNT)) was also developed. Correlation analysis with mAb neutralization values by PRNT vs FRNT (**Fig. 2b)**, Pearson's r ≥ 0.9 and p-value < 0.001).

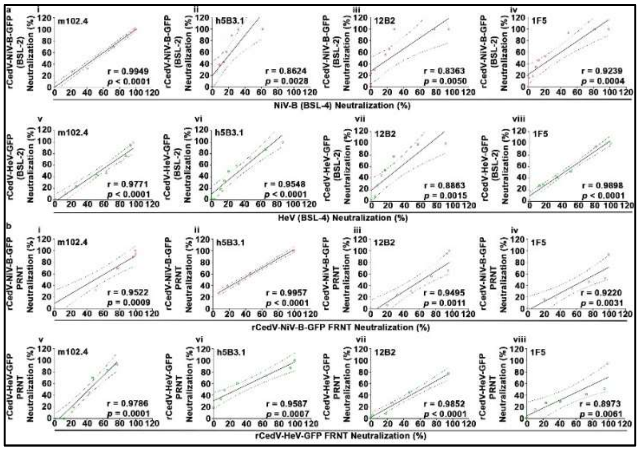

Figure 2. Panel a: Pearson correlation for BSL-2 PRNT vs BSL-4 PRNT (rCedV-NiV-B-GFP versus NiV-B (**i-iv**) and rCedV-HeV-GFP versus HeV (**v-viii**)). Panel b: Pearson correlation for PRNT vs FRNT ((rCedV-NiV-B-GFP (**i-iv**) and rCedV-HeV-GFP (**v-viii**)).

Rescue and characterize reporter viruses encoding GhV envelope glycoproteins.

Generation of a VSV encoding GhV envelope glycoproteins. We will construct reporter gene encoding versions of a replication competent VSV encoding the GhV F and G glycoproteins (GFP and Luc) (**Fig. 1A)**. A large gene cassette comprising VSV untranslated intergenic regions flanking the GhV F and G coding sequences will be synthesized. The GhV F and G coding sequences will be based on the GhV isolate Eid_hel/GH-M74a/GHA/2009 (GenBank: NC_025256.1). The GenBank accession number for GhV F is YP_009091837.1 and that for the GhV G protein is YP_009091838.1. Unique restriction enzyme sites, MluI and KpnI will facilitate the insertion of the GhV F and G gene cassette into the VSV backbone plasmid. The VSV-GhV-Luc will be similarly made. Cloning procedures are performed in *E. coli* Stbl2 and all plasmids verified by sequencing. Rescue will be performed in BSR-T7/5 cells, transfected helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-GhV-GFP or VSV-GhV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus is performed using HEK293T cells by inoculation with the supernatant from step 1. When GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above and the virus stocks stored at -80°C. Larger viral stocks are prepared in T-150 flasks in HEK293T cells, and virus supernatants are purified by buffered sucrose cushion centrifugation and virus pellets resuspended in 10% sucrose/TNE buffer, and small volume single use aliquots stored at -80°C. All virus stocks are deep sequenced. Virus stocks are tittered by serial dilution on HEK293T cells by plaque assay[9].

Generation of rCedV encoding GhV envelope glycoproteins. rCedV-GhV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the GhV F and G coding sequences. The CedV non-coding intergenic regions are retained as is adherence to the genomic "rule of six". Unique restriction sites, MluI and SphI will facilitate the insertion of the GhV F and G gene cassette into the genome clone (pOLTV5$_{opt}$-rCedV) generating rCedV-GhV-GFP (**Fig. XB**). All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-GhV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (henipavirus CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. At maximal syncytia and GFP (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C[7,10,11].

Characterization of the recombinant reporter viruses in cell-based infection assays: The replication kinetics of the VSV-based and rCedV-based GhV F/G encoding viruses will be assayed by Vero cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in several 24hr intervals and tittered. VSV-GhV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G[1]. Similarly, rCedV-GhV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Unlike NiV and HeV, GhV G engages only the ephrin-B2 ligand to trigger membrane fusion[12]. The GhV F/G bearing reporter viruses will be verified for this specific ephrin ligand tropism in cell infection and/or syncytia formation experiments and comparisons made to wildtype VSV or VSV-ABLV G and also alternatively to rCedV, and rCedV NiV/HeV chimeras. We have generated murine mAbs to GhV F and G (10C4-LY and 10D5-LY to GhV G; and 5B7-LY and 7C11-LY to GhV F) and these will be used in neutralization tests. The VSV-GhV-GFP assays will be conducted by FRNTs and VSV-GhV-Luc in luciferase assays.

Evaluating neutralization potential of vaccine platforms from other research projects. Our rCeV chimeras (rCedV-NiV-B-GFP and rCedV-HeV-GFP) will allow this Core to conduct large numbers of neutralization tests by FRNT, to the many ML-derived prototype henipavirus vaccine antigen constructs, mAbs, and nanobinders, that will be generated by the Center's RPs. In Center years 1-3 the focus will be on HeV and NiV prototypes, and in years 4-5 the Core is expected to shift to GhV test case. The FRNT assay is rapid (36hrs), high throughput, and performed in 96-well plates to establish typical 7-point dose response virus neutralization data and performed at BSL-2. Typically, immune sera, mAbs, or other entry inhibitors are analyzed by serially dilution and mixed with virus (rCedV-NiV-B-GFP or rCedV-HeV-GFP) for 2hrs then samples used in triplicate to infect confluent Vero 76 cells in black-walled clear bottom 96-well plates and incubated 24hrs, and then plates are fixed and imaged and fluorescent foci quantitated with CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) is determined by 50% reduction in fluorescent foci versus untreated controls.

Anticipated Results, Potential Pitfalls and Alternative Strategies. We anticipate the reporter viruses will replicate to titers fit for purpose. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. If we encounter unexpected limitations in rescuing VSV-based or rCedV-based GhV F/G bearing reporter viruses; we will 1) test different ratios of helper plasmids and antigenome plasmids; 2) perform rescue using different cell lines; 3) construct alternate versions of the GhV F and G genes within the antigenome plasmids, such as truncating the GhV G protein which has been shown to enhance GhV mediated F/G membrane fusion[13]. Alternatively, fusion of the GhV F and G ectodomains with the transmembrane and cytoplasmic tail domains of the corresponding CedV F and G elements may facilitate virus particle formation and membrane fusion activity.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, immunology assays, virology assays, necropsies, and histopathological analysis.

The Core D Leader and Co-Leaders will work closely with the Project Leaders and key staff of RP1 and RP2 to develop vaccination, treatment and sampling regimens, study protocols, and animal protocols that utilize countermeasures identified in RP1 and RP2. The Core Leader and Co-Leaders will write and obtain UTMB IACUC approval for all animal protocols performed in Core D. The animal models and clinical pathology, immunology, virology, and histopathology assays employed for Aim 3 are detailed below. All raw data as well as graphed or tabulated data generated by Core D will be handled and analyzed in coordination with the Core B.

Animal models: Core D will employ established animal models for HeV, LASV, JUNV, RVFV, and SFTSV. While animal models exist for SOSV and JUNV we will optimize these models to fill gaps and provide new tools as detailed in Aim 4 below. Mice, guinea pigs, hamsters, ferrets, and cats have been used as small animal models of HeV infection[14]. This project will employ Syrian golden hamsters as the small animal model of HeV disease because they are more suitable than other small animals for screening large numbers of vaccines and mAbs. Using hamsters will allow us to select optimal vaccine and mAb formulations and regimens prior to efficacy testing in NHPs. In 2010 Drs. Geisbert and Broder developed the first highly lethal NHP model for henipavirus infection (Nipah virus) using African green monkeys (AGM)[15]. They subsequently developed a lethal AGM model for HeV infection[16]. Henipavirus NHP models mirror human disease with animals showing severe respiratory pathology, neurological disease, and generalized vasculitis. For LASV and JUNV, Core D will use established lethal outbred guinea pig models that cover several lineages of LASV[17-21] and several genetically distinct strains of JUNV[22-25] as guinea pigs are the only immunocompetent small animal model for LASV and JUNV disease. Core D will also use established cynomolgus monkey models that cover several genetically distinct lineages of LASV[26-29] and strains of JUNV[30].

Animal vaccinations: All animals will be obtained from commercial vendors and allowed to acclimate prior to vaccination (~ 3-7 days for mice, hamsters, and guinea pigs and ~ 14 days for ferrets and NHPs). Mice, hamsters, and guinea pigs will be vaccinated by intramuscular (i.m.) or intraperitoneal (i.p.) injection of the vaccines provided by RP1. Ferrets and NHPs will be vaccinated by i.m. injection of the vaccines provided by RP1. All animals will be vaccinated in ABSL-2. Animals will be monitored daily for any reaction or adverse events caused by the vaccination. Blood will be collected for plasma, serum, and PBMC isolations before vaccination and at times after vaccination for assessment of the host immune response.

Animal challenges: Hamsters, ferrets, and NHPs will be challenged with infectious HeV or SOSV as previously described[31-33]. After appropriate acclimation, hamsters and ferrets will be exposed to HeV or SOSV by intranasal (i.n.) or i.p. administration of virus. NHPs will be challenged with HeV by the intratracheal (i.t.) and/or i.n. routes. Mice, guinea pigs, and ferrets will be exposed to arenaviruses and phleboviruses by i.p. injection[19,24,25,34,35]. NHPs will be exposed to LASV or JUNV by i.m. injection[28-30] while marmosets will be exposed to RVFV as previously described[36]. For all animals, blood will be collected with or without anticoagulants for plasma and serum isolations, respectively, at times before paramyxovirus, arenavirus, or phlebovirus challenge, on the day of challenge, and at various times after challenge as detailed in RP1 and RP2. Animals will be monitored for clinical signs daily. We will apply a scoring sheet approved by the UTMB IACUC depicted in the Vertebrate Animals section to assist in determining the time of euthanasia depending on clinical signs (e.g., respiratory distress, anorexia, activity, appearance, neurologic signs, etc.). Animals will be euthanized according to the 2013 AVMA Guidelines on Euthanasia. Any surviving animals will be euthanized on day 35-42 days after paramyxovirus, arenavirus, or phlebovirus challenge.

Clinical pathology assays: *Hematology on EDTA whole blood*: For NHP studies, we will measure the following parameters at time points before and after HeV, LASV, or JUNV challenge: total white blood cell counts (WBC), percent lymphocytes (LY), percent monocytes (MO), percent neutrophils (NEU), percent eosinophils (EOS), percent basophils (BAS), total lymphocyte counts (LY#), total monocyte counts (MO#), total neutrophil counts (NEU#), total eosinophil counts (EOS #), total basophil counts (BAS #), red blood cell counts (RBC), total hemoglobin (Hgb), hematocrit values (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV), and Plateletcrit (PCT). Values will be obtained from the Abaxis VetScan HM5 following manufacturer's directions.

Clinical chemistry on serum: For NHP studies, the following parameters will be measured at time points before and after HeV, LASV, and JUNV challenge: Albumin (ALB), amylase (AMY), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), glucose (GLU), total protein (TP), blood urea nitrogen (BUN), and creatinine (CRE), C-reactive protein (CRP), Calcium (Ca2+), and uric acid (UA). Values will be obtained from the Abaxis Piccolo chemistry analyzer following manufacturer's directions.

Viral load assays: *PCR Assay:* To assess viral loads in whole blood and tissues, RNA will be extracted using a virus RNA isolation kit (Qiagen, Valencia, CA) followed by TaqMan PCR for the viral genome being studied. Qiagen buffers have been safety tested for inactivation of paramyxoviruses, arenaviruses, and phleboviruses. PCR assays employed for HeV, SOSV, LASV, JUNV, RVFV, and SFTSV have been previously described[28-30,33,37-39].

Plaque Assay: The plaque assays employed for paramyxoviruses, arenaviruses, and phleboviruses have previously been described[28-30,33,40]. Briefly, 10-fold dilutions (10-1 to 10-6) of serum, plasma, or whole blood samples are prepared in cell maintenance medium (DMEM/10%FBS/gentamicin) and adsorbed to 70-90% confluent monolayers of Vero E6 cells in 6-well plates, adding 0.2 mL/well. The plates are incubated at 37°C, 5% CO2 for 45 min, with periodical rocking every 10-15 min. The incubation period is followed by addition of 2 mL 1% agarose-containing medium (2X EBME/Hepes/10% FBS/gentamicin) to each well. After the agarose has solidified, the plates are returned to the C02 incubator and maintained for 2-14 days until plaques are visualized. At this time, 2 mL PBS containing 5% neutral red and 5% FBS is added to each well, the plates returned to the CO2 incubator and held overnight. The following day, the liquid stain overlay is removed from each well and plaques are counted.

Bead-based multiplex assays: Plasma inflammatory mediators, thrombosis markers, and fibrinolysis analytes were measured by flow cytometry using Biolegend LegendPlex assays and a FACS Canto-II cytometer (Becton Dickson). Samples will be processed in duplicate for each Nonhuman Primate Inflammation 13-plex (1:4 dilution), Human Thrombosis (1:100), and Human Fibrinolysis (1:40,000) panel as previously described[41-43].

Gross pathology and histopathology: A necropsy will be performed on all NHPs. We will examine the external surfaces of the body, all orifices, and the thoracic and peritoneal cavities and their contents. Core D will prepare a written report of the gross pathology findings of each NHP. The following NHP tissues will be collected at necropsy, fixed in 10% neutral-buffered formalin, embedded in paraffin, and stained by H&E: liver, spleen, lung, kidney, adrenal gland, pancreas, axillary lymph node, inguinal lymph node, mesenteric lymph node, duodenum, ileum, colon, brain (frontal cortex, cerebellum, brain stem), cervical spinal cord, urinary bladder, ovary or testis, prostate or uterus, and eye. A microscopic evaluation will be performed on H&E slides of all tissues collected from all NHPs by an ACVP board certified veterinary pathologist. Reports of the histologic findings of each tissue from each animal will be provided to RP1 Leaders. In consultation with the RP1 Leaders, Core D may also perform immunohistochemical and/or special stains such as PTAH for fibrin as previously described[28-30,33].

Quality systems management of all records and data collected from animal studies: Although the animal studies conducted by Core D will not be required to be performed under GLP compliance, UTMB will provide Quality Assurance that will achieve data quality and integrity when conducting animal experiments. Quality refers to whether the study was conducted in accordance with the protocol, standard operating procedures, and applicable standards of research. Integrity refers to assurance that the raw data and documentation are consistent with reported results. All animal study personnel will be trained by the UTMB Office of Regulated Nonclinical Studies (ORNcS). on Good Documentation Practices (GDP). All animal study data generated by Core D will be provided to Core B as detailed in the Core B Research Strategy.

Aim 4: Develop and/or optimize animal models for Sosuga virus and Junin virus.

For HeV and LASV Core D will employ animal models that have already been developed and are well established. However, for SOSV, JUNV, RVFV, SFTSV while animal models exist there are gaps that need to be addressed. Recently, it was shown that i.n. or i.p. infection of hamsters with SOSV resulted in viremia and mild clinical disease[34] but there is no lethal model for SOSV. Core B will further explore the hamster model for SOSV by 1) using higher challenge doses of SOSV which is often required to produce lethal disease in other paramyxovirus hamster models[32,44] or 2) serially passage of tissues in hamsters. Core D will also assess the pathogenic potential of ferrets for SOSV as they are often used as animal models for paramyxoviruses. For arenaviruses such as JUNV, serial adaptation is often required to produce lethal disease in outbred guinea pigs. While adapted viruses have utility in assessing the protecting efficacy of medical countermeasures in animal models non adapted wild type viruses are preferred to better assess vaccines and treatments. We and others recently showed that for Ebolaviruses while serial adaptation is required to produce lethality in mice, hamsters, and guinea pigs, infection of ferrets with *Zaire ebolavirus*, *Sudan ebolavirus*, *Bundibugyo ebolavirus*, or *Reston ebolavirus* resulted in uniform lethality with wild type viruses and did not require adaptation[45-48]. Therefore, Core D will assess the pathogenic potential of JUNV in ferrets.

BIBLIOGRAPHY AND REFERENCES CITED

1. Weir DL, Smith IL, Bossart KN, Wang LF, Broder CC. Host cell tropism mediated by Australian bat lyssavirus envelope glycoproteins. Virology 2013;444:21-30. PMCID: PMC6183051.
2. Weir DL, Laing ED, Smith IL, Wang LF, Broder CC. Host cell virus entry mediated by Australian bat lyssavirus G envelope glycoprotein occurs through a clathrin-mediated endocytic pathway that requires actin and Rab5. Virol J 2014;11:40. PMCID: PMC3946599.
3. Mastraccio KE, Huaman C, Warrilow D, Smith GA, Craig SB, Weir DL, Laing ED, Smith IL, Broder CC, Schaefer BC. Establishment of a longitudinal pre-clinical model of lyssavirus infection. J Virol Methods 2020;281:113882. PMCID: PMC8056983.
4. Laing ED, Sterling SL, Weir DL, Beauregard CR, Smith IL, Larsen SE, Wang LF, Snow AL, Schaefer BC, Broder CC. Enhanced Autophagy Contributes to Reduced Viral Infection in Black Flying Fox Cells. Viruses 2019;11:260. PMCID: PMC6466025.
5. Wang Z, Dang HV, Amaya M, Xu Y, Yin R, Yan L, Hickey AC, Annand EJ, Horsburgh BA, Reid PA, Smith I, Eden JS, Xu K, Broder CC, Veesler D. Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. Proc Natl Acad Sci USA 2022;119:e2122769119. PMCID: PMC9295758.
6. Laing ED, Navaratnarajah CK, Cheliout Da Silva S, Petzing SR, Xu Y, Sterling SL, Marsh GA, Wang LF, Amaya M, Nikolov DB, Cattaneo R, Broder CC, Xu K. Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. Proc Natl Acad Sci USA 2019;116:20707-20715. PMCID: PMC6789926.
7. Laing ED, Amaya M, Navaratnarajah CK, Feng YR, Cattaneo R, Wang LF, Broder CC. Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. Virol J 2018 Mar 27;15:56. PMCID: PMC5869790.
8. Amaya M, Yin R, Yan L, Borisevich V, Adhikari BN, Bennett A, Malagon F, Cer RZ, Bishop-Lilly KA, Dimitrov AS, Cross RW, Geisbert TW, Broder CC. A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. Viruses 2023;15:1077. PMCID: PMC10223282.
9. Abdelmageed AA, Ferran MC. The Propagation, Quantification, and Storage of Vesicular Stomatitis Virus. Curr Protoc Microbiol 2020;58:e110. PMCID: PMC7449588.
10. Amaya M, Cheng H, Borisevich V, Navaratnarajah CK, Cattaneo R, Cooper L, Moore TW, Gaisina IN, Geisbert TW, Rong L, Broder CC. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral Res 2021;193:105084. PMCID: PMC8631057.
11. Weingartl HM, Berhane Y, Caswell JL, Loosmore S, Audonnet JC, Roth JA, Czub M. Recombinant nipah virus vaccines protect pigs against challenge. J Virol 2006;80:7929-38. PMCID: PMC1563797.
12. Weis M, Behner L, Hoffmann M, Krüger N, Herrler G, Drosten C, Drexler JF, Dietzel E, Maisner A. Characterization of African bat henipavirus GH-M74a glycoproteins. J Gen Virol 2014;95:539-548. PMID: 24296468.
13. Voigt K, Hoffmann M, Drexler JF, Müller MA, Drosten C, Herrler G, Krüger N. Fusogenicity of the Ghana Virus (*Henipavirus*: *Ghanaian bat henipavirus*) Fusion Protein is Controlled by the Cytoplasmic Domain of the Attachment Glycoprotein. Viruses 2019;11:800. PMCID: PMC6784138.
14. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. Curr Top Microbiol Immunol 2012;359:197-223. PMID: 22476556.
15. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, Smith MA, Chan YP, Wang LF, Mattapallil JJ, Geisbert JB, Bossart KN, Broder CC. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010;5:e10690. PMCID: PMC2872660.
16. Rockx B, Bossart KN, Feldmann F, Geisbert JB, Hickey AC, Brining D, Callison J, Safronetz D, Marzi A, Kercher L, Long D, Broder CC, Feldmann H, Geisbert TW. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010;84:9831-9. PMCID: PMC2937751.
17. Carrion R Jr, Patterson JL, Johnson C, Gonzales M, Moreira CR, Ticer A, Brasky K, Hubbard GB, Moshkoff D, Zapata J, Salvato MS, Lukashevich IS. A ML29 reassortant virus protects guinea pigs against a distantly related Nigerian strain of Lassa virus and can provide sterilizing immunity. Vaccine 2007;25:4093-102. PMCID: PMC1892204.
18. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A recombinant vesicular stomatitis virus-based Lassa fever vaccine protects guinea pigs and macaques against challenge with geographically and genetically distinct Lassa viruses. PLoS Negl Trop Dis 2015;9:e0003736. PMCID: PMC4401668.

19. Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M, Khan SH, Robinson JE, Geisbert TW, Garry RF. Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies. Antiviral Res 2016;133:218-222. PMCID: PMC5032844.
20. Maruyama J, Manning JT, Mateer EJ, Sattler R, Bukreyeva N, Huang C, Paessler S. Lethal Infection of Lassa Virus Isolated from a Human Clinical Sample in Outbred Guinea Pigs without Adaptation. mSphere 2019;4:e00428-19. PMCID: PMC6763766.
21. Deschambault Y, Soule G, Klassen L, Sloan A, Audet J, Azaransky K, Musa AS, Ahmad A, Akinpelu AM, Mba N, Stein DR, Ranson M, Almiski M, Tierney K, Fischer G, Chan M, Safronetz D. An Outbred Guinea Pig Disease Model for Lassa Fever Using a Host-Adapted Clade III Nigerian Lassa Virus. Viruses 2023;15:769. PMCID: PMC10052409.
22. Kenyon RH, Green DE, Maiztegui JI, Peters CJ. Viral strain dependent differences in experimental Argentine hemorrhagic fever (Junin virus) infection of guinea pigs. Intervirology 1988;29:133-43. PMID: 2846464.
23. Yun NE, Linde NS, Dziuba N, Zacks MA, Smith JN, Smith JK, Aronson JF, Chumakova OV, Lander HM, Peters CJ, Paessler S. Pathogenesis of XJ and Romero strains of Junin virus in two strains of guinea pigs. Am J Trop Med Hyg 2008;79:275-82. PMCID: PMC2700623.
24. Zeitlin L, Geisbert JB, Deer DJ, Fenton KA, Bohorov O, Bohorova N, Goodman C, Kim D, Hiatt A, Pauly MH, Velasco J, Whaley KJ, Altmann F, Gruber C, Steinkellner H, Honko AN, Kuehne AI, Aman MJ, Sahandi S, Enterlein S, Zhan X, Enria D, Geisbert TW. Monoclonal antibody therapy for Junin virus infection. Proc Natl Acad Sci USA 2016;113:4458-63. PMCID: PMC4843420.
25. Sorvillo TE, Cross RW, Johnson DM, Dobias NS, Fenton KA, Mire CE, **Geisbert TW**. Single dose rVSVΔG-JUNVGP vaccine protects guinea pigs against lethal Junin virus challenge. NPJ Vaccines. 2021 Aug 9;6(1):96. PMCID: PMC8352877.
26. Baize S, Marianneau P, Loth P, Reynard S, Journeaux A, Chevallier M, Tordo N, Deubel V, Contamin H. Early and strong immune responses are associated with control of viral replication and recovery in lassa virus-infected cynomolgus monkeys. J Virol 2009;83:5890-903. PMCID: PMC2681932.
27. Hensley LE, Smith MA, Geisbert JB, Fritz EA, Daddario-DiCaprio KM, Larsen T, Geisbert TW. Pathogenesis of Lassa fever in cynomolgus macaques. Virol J 2011;8:205. PMCID: PMC3104370.
28. Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M, Boisen ML, Grant DS, Fullah M, Khan SH, Fenton KA, Robinson JE, Branco LM, Garry RF, Geisbert TW. Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever. Nat Med 2017;23:1146-1149. PMCID: PMC5719877.
29. Cross RW, Xu R, Matassov D, Hamm S, Latham TE, Gerardi CS, Nowak RM, Geisbert JB, Ota-Setlik A, Agans KN, Luckay A, Witko SE, Soukieh L, Deer DJ, Mire CE, Feldmann H, Happi C, Fenton KA, Eldridge JH, Geisbert TW. Quadrivalent VesiculoVax vaccine protects nonhuman primates from viral-induced hemorrhagic fever and death. J Clin Invest 2020;130:539-551. PMCID: PMC6934204.
30. Zeitlin L, Cross RW, Geisbert JB, Borisevich V, Agans KN, Prasad AN, Enterlein S, Aman MJ, Bornholdt ZA, Brennan MB, Campbell L, Kim D, Mlakar N, Moyer CL, Pauly MH, Shestowsky W, Whaley KJ, Fenton KA, Geisbert TW. Therapy for Argentine hemorrhagic fever in nonhuman primates with a humanized monoclonal antibody. Proc Natl Acad Sci USA. 2021;118:e2023332118. PMCID: PMC7980402.
31. Mire CE, Chan YP, Borisevich V, Cross RW, Yan L, Agans KN, Dang HV, Veesler D, Fenton KA, Geisbert TW, Broder CC. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets Against Lethal Nipah Virus and Hendra Virus Infection. J Infect Dis 2020;221(Suppl 4):S471-S479. PMCID: PMC7199785.
32. Doyle MP, Kose N, Borisevich V, Binshtein E, Amaya M, Nagel M, Annand EJ, Armstrong E, Bombardi R, Dong J, Schey KL, Broder CC, Zeitlin L, Kuang EA, Bornholdt ZA, West BR, Geisbert TW, Cross RW, Crowe JE Jr. Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. Cell Rep 2021;36:109628. PMCID: PMC8527959.
33. Geisbert TW, Bobb K, Borisevich V, Geisbert JB, Agans KN, Cross RW, Prasad AN, Fenton KA, Yu H, Fouts TR, Broder CC, Dimitrov AS. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. NPJ Vaccines 2021;6:23. PMCID: PMC7870971.
34. Barbeau DJ, Albe JR, Nambulli S, Tilston-Lunel NL, Hartman AL, Lakdawala SS, Klein E, Duprex WP, McElroy AK. Rift Valley Fever Virus Infection Causes Acute Encephalitis in the Ferret. mSphere 2020;5:e00798-20. PMCID: PMC7593599.
35. Johnson KN, Kalveram B, Smith JK, Zhang L, Juelich T, Atkins C, Ikegami T, Freiberg AN. Tilorone-Dihydrochloride Protects against Rift Valley Fever Virus Infection and Disease in the Mouse Model. Microorganisms 2021;10:92. PMCID: PMC8781158.

36. Smith DR, Bird BH, Lewis B, Johnston SC, McCarthy S, Keeney A, Botto M, Donnelly G, Shamblin J, Albariño CG, Nichol ST, Hensley LE. Development of a novel nonhuman primate model for Rift Valley fever. J Virol 2012;86:2109-20. PMCID: PMC3302397.
37. Welch SR, Ritter JM, Schuh AJ, Genzer SC, Sorvillo TE, Harmon JR, Coleman-McCray JD, Jain S, Shrivastava-Ranjan P, Seixas JN, Estetter LB, Fair PS, Towner JS, Montgomery JM, Albariño CG, Spiropoulou CF, Spengler JR. Tissue replication and mucosal swab detection of Sosuga virus in Syrian hamsters in the absence of overt tissue pathology and clinical disease. Antiviral Res 2023;209:105490. PMID: 36521633
38. Lokugamage N, Ikegami T. Genetic stability of Rift Valley fever virus MP-12 vaccine during serial passages in culture cells. NPJ Vaccines 2017;2:20. PMCID: PMC5627234.
39. Yoshikawa T, Fukushi S, Tani H, Fukuma A, Taniguchi S, Toda S, Shimazu Y, Yano K, Morimitsu T, Ando K, Yoshikawa A, Kan M, Kato N, Motoya T, Kuzuguchi T, Nishino Y, Osako H, Yumisashi T, Kida K, Suzuki F, Takimoto H, Kitamoto H, Maeda K, Takahashi T, Yamagishi T, Oishi K, Morikawa S, Saijo M, Shimojima M. Sensitive and specific PCR systems for detection of both Chinese and Japanese severe fever with thrombocytopenia syndrome virus strains and prediction of patient survival based on viral load. J Clin Microbiol 2014;52:3325-33. PMCID: PMC4313158.
40. Ikegami T, Won S, Peters CJ, Makino S. Rescue of infectious rift valley fever virus entirely from cDNA, analysis of virus lacking the NSs gene, and expression of a foreign gene. J Virol 2006;80:2933-40. PMCID: PMC1395455.
41. Woolsey C, Borisevich V, Prasad AN, Agans KN, Deer DJ, Dobias NS, Heymann JC, Foster SL, Levine CB, Medina L, Melody K, Geisbert JB, Fenton KA, Geisbert TW, Cross RW. Establishment of an African green monkey model for COVID-19 and protection against re-infection. Nat Immunol 2021;22:86-98. PMCID: PMC7790436.
42. Woolsey C, Borisevich V, Agans KN, Fenton KA, Cross RW, Geisbert TW. *Bundibugyo ebolavirus* Survival Is Associated with Early Activation of Adaptive Immunity and Reduced Myeloid-Derived Suppressor Cell Signaling. mBio 2021;12:e0151721. PMCID: PMC8406165.
43. Woolsey C, Fears AC, Borisevich V, Agans KN, Dobias NS, Prasad AN, Deer DJ, Geisbert JB, Fenton KA, Geisbert TW, Cross RW. Natural history of *Sudan ebolavirus* infection in rhesus and cynomolgus macaques. Emerg Microbes Infect 2022;11:1635-1646. PMCID: PMC9225728.
44. Rockx B, Brining D, Kramer J, Callison J, Ebihara H, Mansfield K, Feldmann H. Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. J Virol 2011;85:7658-71. PMCID: PMC3147900.
45. Cross RW, Mire CE, Borisevich V, Geisbert JB, Fenton KA, Geisbert TW. The domestic ferret (*Mustela putorius furo*) as a lethal infection model for 3 species of Ebolavirus. J Infect Dis 2016;214:565-9. PMCID: PMC4957446.
46. Kozak R, He S, Kroeker A, de La Vega MA, Audet J, Wong G, Urfano C, Antonation K, Embury-Hyatt C, Kobinger GP, Qiu X. Ferrets Infected with Bundibugyo Virus or Ebola Virus Recapitulate Important Aspects of Human Filovirus Disease. J Virol 2016;90:9209-23. PMCID: PMC5044830.
47. Kroeker A, He S, de La Vega MA, Wong G, Embury-Hyatt C, Qiu X. Characterization of Sudan Ebolavirus infection in ferrets. Oncotarget 2017;8:46262-46272. PMCID: PMC5542265.
48. Yan F, He S, Banadyga L, Zhu W, Zhang H, Rahim MN, Collignon B, Senthilkumaran C, Embury-Hyatt C, Qiu X. Characterization of Reston virus infection in ferrets. Antiviral Res 2019;165:1-10. PMID: 30836107.

From: Geisbert, Thomas W. on behalf of Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
To: Neil King
Cc: Cross, Robert W.; David Veesler; Freiberg, Alexander N.; Ikegami, Tetsuro; Lynda Stuart; kherrera; CHRISTOPHER BRODER
Subject: Re: UTMB update on REVAMPP materials.
Date: Friday, June 2, 2023 11:05:00 AM
Attachments: UTMB Core D-Project Summary and Relevance-2June23.docx
Specific Aims Core D-2June23.docx
UWashington-Core D-Research Strategy-2June23.docx

Here you go. We need you to add a little that is highlighted as we were not sure of the final Center structure. Alex, Tetsuro, I did not think they you were proposing new reporter viruses or animal models for RVFV or SFTSV. If that is not correct we still have some room left in the Research Strategy for additions.

From: Neil King <neilking@uw.edu>
Sent: Thursday, June 1, 2023 8:38 PM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Cc: Cross, Robert W. <rwcross@UTMB.EDU>; David Veesler <dveesler@uw.edu>; Freiberg, Alexander N. <anfreibe@UTMB.EDU>; Ikegami, Tetsuro <teikegam@utmb.edu>; Lynda Stuart <lyndast@uw.edu>; kherrera <kherrera@uw.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: UTMB update on REVAMPP materials.

Gentlemen,

We really need completed science documents.

Thanks,
Neil

On Thu, Jun 1, 2023 at 9:08 AM Geisbert, Thomas W. <twgeisbe@utmb.edu> wrote:

> It is mostly written. Chris is in the process of integrating some preliminary data.
>
> ---
>
> **From:** Cross, Robert W. <rwcross@UTMB.EDU>
> **Sent:** Thursday, June 1, 2023 10:28 AM
> **To:** David Veesler <dveesler@uw.edu>; Neil King <neilking@uw.edu>
> **Cc:** Stutz, Sonja J. <sjstutz@utmb.edu>; Freiberg, Alexander N. <anfreibe@UTMB.EDU>; Ikegami, Tetsuro <teikegam@utmb.edu>; Lynda Stuart <lyndast@uw.edu>; kherrera <kherrera@uw.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Haller, Sherry <shhaller@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
> **Subject:** RE: UTMB update on REVAMPP materials.
>
> Looping Tom in here for an update.

From: David Veesler <dveesler@uw.edu>
Sent: Thursday, June 1, 2023 9:02 AM
To: Neil King <neilking@uw.edu>
Cc: Stutz, Sonja J. <sjstutz@utmb.edu>; Cross, Robert W. <rwcross@UTMB.EDU>; Freiberg, Alexander N. <anfreibe@UTMB.EDU>; Ikegami, Tetsuro <teikegam@utmb.edu>; Lynda Stuart <lyndast@uw.edu>; kherrera <kherrera@uw.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Haller, Sherry <shhaller@UTMB.EDU>
Subject: Re: UTMB update on REVAMPP materials.

Hi Bob et al,

Hope all is well.

I am checking in to confirm that you will be able to please send us a near final draft of the science write-up for your Core in the next few hours?

Thanks in advance
David

On Tue, May 30, 2023 at 12:27 PM Neil King <neilking@uw.edu> wrote:

> Thank you Sonja!
>
> On Tue, May 30, 2023, 11:46 AM Stutz, Sonja J. <sjstutz@utmb.edu> wrote:
>
>> Hello Dr. King,
>>
>> We have received internal approval on the budget, and I am working to get the info loaded into the SF424 form now. I expect to have the budget, justification and LOI to you by the end of the day.
>>
>> Thanks,
>> Sonja
>>
>> ---
>>
>> **From:** Neil King <neilking@uw.edu>
>> **Sent:** Tuesday, May 30, 2023 12:25 PM
>> **To:** Cross, Robert W. <rwcross@UTMB.EDU>
>> **Cc:** Freiberg, Alexander N. <anfreibe@UTMB.EDU>; Ikegami, Tetsuro <teikegam@utmb.edu>; Lynda Stuart <lyndast@uw.edu>; kherrera <kherrera@uw.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; David Veesler <dveesler@uw.edu>; Haller, Sherry <shhaller@UTMB.EDU>; Stutz, Sonja J. <sjstutz@utmb.edu>
>> **Subject:** Re: UTMB update on REVAMPP materials.

> > > Thanks Bob -- please find responses in-line below.
> > >
> > > Best,
> > > Neil
> > >
> > > > - The admin documents were submitted to our OSP officials on Friday and we have checked for an update. Sonja is working on this and will respond when more info is available.
> > >
> > > Thank you. Do we have an estimated ETA for the arrival of the documents at UW?
> > >
> > > > - Milestones/Goals for the Overall document. Per the RFA, there are no milestones required for cores, but we can make mention of the core's role in supporting RP milestones if that is what was meant?
> > >
> > > Correct, thanks!
> > >
> > > > - We are still passing the core's science document around and adding needed needed supporting data, hope to have this in a sharable form by this Thursday.
> > >
> > > **Could we please get a draft Specific Aims page today?** This is necessary to allow us to prepare to weave Core D into the rest of the proposal.
>
> --
> David Veesler, PhD
> Investigator, Howard Hughes Medical Institute
> Hans Neurath Endowed Chair in Biochemistry
> Associate Professor, Department of Biochemistry
> University of Washington, Seattle
> veeslerlab.com
> @veeslerlab

RESEARCH STRATEGY

A. SIGNIFICANCE

Among viruses that cause disease in humans viral zoonoses within the family Paramyxoviridae and order Bunyavirales contain pathogens that cause significant lethality. Importantly, within the Paramyxovirdae the zoonotic henipavirus Hendra virus (HeV) is on the WHO Priority Pathogens list. Notably, henipaviruses stand out for their impressive lethality with case fatality rates averaging about 75%. The Arenaviridae and Phelboviridae families contain viruses that cause severe hemorrhagic disease in humans. These viruses include the arenaviruses Lassa virus (LASV) and Junin virus (JUNV) and the phlebovirus Rift Valley fever virus (RVFV). All of these viruses are also included among the World Health Organization's (WHO) List of Priority Pathogens and pose threats to global public health due to the absence of approved vaccines or therapeutics. There is a clear unmet need for medical countermeasures to combat the threat of natural outbreaks, epidemics, or deliberate release. The Machine Learning-(ML)-Enabled Design of Prototype Pathogen Vaccines and Antibodies Center is a well-integrated consortium conducting a set of discovery and translational research programs composed of an Administrative Core, a Data Management Core, X Scientific Cores, and X Research Projects (RPs). The synergistic activities of the X Center partners will be focused on developing novel vaccines, treatments, and tools to support pandemic preparedness. Federal law requires that the paramyxoviruses, arenaviruses, and phleboviruses used in the X Center be handled in approved Biosafety Level (BSL)-3 and BSL-4 containment laboratories. Core D provides approved BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities and a trained and highly experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2. The services provided by Core D will include 1) a secure repository of well characterized seed stocks of BSL-3 and BSL-4 paramyxoviruses, arenaviruses, and phleboviruses 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5); development and/or optimization of animal models for strains or variants of Sosuga virus (SOSV), JUNV, RVFV, and severe fever with thrombocytopenia syndrome virus (SFTSV) to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies.

All X of the research projects within the X Center focus on developing vaccines or monoclonal antibody (mAb) based therapies against paramyxoviruses, arenaviruses, and phleboviruses. All X of these research projects require that countermeasures be evaluated in animals against infectious paramyxoviruses, arenaviruses, and phleboviruses. As HeV, LASV, JUNV, and RVFV are CDC and/or USDA select agents Federal regulations require that these viruses be handled in Biosafety Level (BSL)-3 or BSL-4 containment. Core D provides approved BSL-3/ABSL-3 and BSL-4/BSL-4 facilities and a trained and experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2.

B. INNOVATION

Scientific Core D will be co-located within the Galveston National Laboratory (GNL) and Robert Shope Laboratory at the University of Texas Medical Branch (UTMB) at Galveston. The GNL is a high and maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda and stands ready to assist local, state and national public health efforts in the event of a public health emergency. The facility is unique in that it is one of two operational BSL-4 facilities in the United States that is located on an academic campus. This unique location benefits the X Center in several ways. Regarding physical resources the GNL is a state-of-the-art facility with many specialized assets including BSL-4 imaging, telemetry, and aerobiology equipment. In addition, being located on an academic campus Drs. Geisbert (Core D, Project Leader (PL), Cross, Freiberg, and Ikegami are faculty members in the Departments of Microbiology and Immunology and Pathology. This academic environment is a major strength, that fosters creativity and collaboration with other UTMB investigators which will be beneficial to the overall success of Core D. Dr. Broder, is faculty member of the Department of Microbiology and Immunology at Uniformed Services University (USU). Drs. Broder and Geisbert (PL) have a long-standing emerging virus research collaboration which has included the development and characterization the first nonhuman primate (NHP) models for HeV and Nipah virus (NiV) that will be employed by the Center's RPs. Another strength within the Core is Drs. Geisbert, Broder, Cross, and Veesler (Center Co-PI) currently collaborate and lead active henipavirus projects that are focused on translational countermeasure development which includes more traditional vaccine and mAb therapeutics approaches, and the shared knowledge base and experience in these areas will greatly facilitate the Core's abilities in supporting the Center's research goals in applying advanced machine learning-enabled

designs of effective prototype pathogen vaccines and antibodies. Along with animal model development and animal modeling expertise, many of the unique virological tools including recombinantly generated RNA viruses, reporter gene-encoding viruses, and other pseudotype virus technologies, that will be employed by the Core, have been developed from UTMB-USU collaboration.

C. APPROACH

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses.

Paramyxovirus, arenavirus, and phlebovirus seed stocks. UTMB will provide fully equipped BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities that are required to work with HeV, SOSV, LASV, JUNV, RVFV, and SFTSV. UTMB will also provide and maintain all required approvals to work these viruses. The BSL-3 and BSL-4 paramyxovirus, arenavirus, and phlebovirus seed stocks employed for RP1 and RP2 have been or will be titered by conventional standard plaque assay and qRT-PCR as detailed in Aim 2 below. The virus strain identity of each seed stock has been or will be confirmed by sequence analysis. In addition, RNA has been or will be deep sequenced to verify the complete viral genome of each virus seed stock used for animal challenge studies. We have or will test each seed stock for mycoplasma contamination. Endotoxin testing has been or will be performed on paramyxovirus, arenavirus, and phlebovirus stocks using the Endosafe PTS system (Charles River Laboratories) following the manufacturer’s instructions. The Endosafe PTS system is a cartridge-based testing system utilizing FDA-licensed cartridges (0.1 EU/ml, 0.05 EU/ml, 0.01 EU/ml, 0.05 EU/ml) for ultrasensitive testing. Seed stocks will also be evaluated for morphological identity by transmission electron microscopy (TEM). Virus particle counts will be determined using a Virus Counter and confirmed by conventional TEM methods if needed. The bacteriological and mycological sterility of the product will be determined by selection of aliquots of the dispensed stock, inoculation of a sterile 500 ml bottle of Dulbecco's high glucose MEM with 10% FBS and then after 3 days subcultured to FTM and TBS media. paramyxovirus, arenavirus, and phlebovirus seed stocks will be inventoried in the UTMB BSL-4 and BSL-4 virus stock databases and secured in dedicated controlled access alarmed minus 80°C freezers in the GNL or Robert Shope Laboratory. Paramyxovirus, arenavirus, and phlebovirus seed stocks are monitored periodically for any changes in virus titer.

Plan for staffing, managing, and prioritizing Core use. The Core Leader and Co-Leaders have a cadre of staff that are trained and highly experienced in all BSL-3 and BSL-4 procedures and assays needed to conduct the proposed work. In addition to the Core Leader and Co-Leaders this staff includes an ACVP board certified veterinary pathologist, two BSL-4 contributing scientists, a BSL-4 Technical Director, a BSL-4 trained postdoctoral fellow, six BSL-4 experienced technical staff, a BSL-4 trained histotechnologist, and a BSL-4 trained graduate student. We do not anticipate any BSL-3 or BSL-4 scheduling problems regarding studies proposed in RP1 or RP2. However, we have developed a mitigation strategy if unforeseen events cause any prioritization of resources. A committee consisting of the Core D Leader, the PI of the Center, and the members of the external Scientific Advisory Committee (SAC) will review the status of RP1 and RP2 and any circumstances causing a prioritization of work. The Committee will also hold a meeting or teleconference (Zoom, Teams) with the Project Leaders and key staff of RP1 and RP2 to gather additional information. After careful review of all available information the Committee will then make a recommendation on prioritization to the Center PI. The X Center PI will then consult with NIAID/NIH program staff and make the final determination on prioritization.

Aim 2: Provide technical expertise and conduct *in vitro* assays.

In vitro antiviral activity assays: Core D will perform conventional virus neutralization assays for RP1 and RP2 to determine the ability of sera/plasma from immunized animals from RP1 and antibodies from RP2 to inhibit the replication of paramyxoviruses, arenaviruses, and phleboviruses *in vitro* using infectious viruses in the UTMB BSL-3 and BSL-4 laboratories. In brief, cultures of Vero, Vero E6, SW13, or other cell types will be infected with a range of m.o.i. of paramyxoviruses, arenaviruses, and bunyaviruses and treated with various concentrations of the provided sera/plasma or antibodies. Cells and/or culture fluids will be harvested at various time points after infection and infectious virus enumerated by conventional plaque assay as detailed below.

Develop chimeric reporter-gene encoding viruses and virus neutralization assays for BSL-2 use.
We have previously used reverse genetics to generate several types of replication competent VSV-GFP reporter viruses including those encoding Australian bat lyssavirus (ABLV) G glycoproteins (VSV-ABLV G-GFP) (PMID: 23849788 and PMID: 24576301), as well as employed reverse genetics to rescue authentic ABLV and various reporter gene-encoding viruses (PMID: 32407866 and PMID: 30875748). In addition, we have generated a reverse genetics platform for generating recombinant Cedar virus (rCedV) and rCedV chimeras whereby the

fusion (F) and attachment (G) glycoprotein genes of rCedV were replaced with those of NiV-Bangladesh (NiV-B) or HeV, generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV), both with and without reporter genes (PMID: 35617431, PMID: 31548390, PMID: 29587789, PMID: 37243163). The rCedV chimeras are a replication competent authentic henipavirus platform that is a high-throughput surrogate neutralization assay that is rapid, cost-effective and can be utilized outside high containment. Similar approaches will be applied here to generate new reporter viruses for Ghana bat virus (GhV). We will design, construct and rescue both VSV and rCedV chimeras that encode the envelope glycoproteins (fusion (F) and attachment (G) glycoproteins) of GhV. Two reporter-gene encoding versions will be attempted for VSV-GhV: one encoding GFP (VSV-GhV-GFP) and the other encoding the *Photinus pyralis* (firefly) luciferase gene (VSV-GhV-Luc). We will also construct and rescue GFP and Luc reporter gene encoding versions? of rCedV chimeras encoding the GhV F and G genes (rCedV-GhV-GFP and rCedV-GhV-Luc) (**Fig. 1**). The rescued viruses will be characterized using cell-based infection tropism and growth kinetics assays.

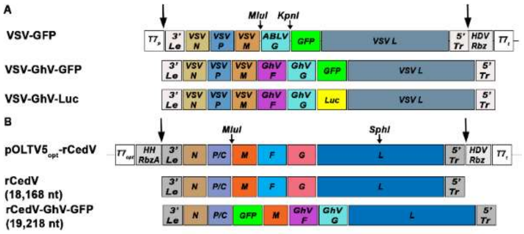

Figure 1. Schematic of chimeric reporter viruses. (**A**)The VSV-ABLV G-GFP plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and KpnI to construct VSV-GhV-GFP and VSV-GhV-Luc. (**B**) pOLTV5$_{opt}$-rCedV plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and SphI used to construct rCedV-GhV-GFP.

The rCedV (NiV-B and HeV) chimeric virus platform serves as a robust surrogate neutralization assay system for quantifying antibody neutralization potency of anti-NiV and anti-HeV antisera and mAbs [Amaya et al, Viruses 2023 see comment box]. The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G specific mAbs against rCedV-NiV-B-GFP and rCedV-HeV-GFP are highly correlated with those obtained using authentic NiV-B and HeV when tested in parallel by plaque reduction neutralization test (PRNT). Pearson correlation of rCedV chimeras BSL-2 PRNT to the NiV-B or HeV BSL-4 PRNT is shown in **Fig. 2a**. A high-throughput quantitative assay based on reduction of GFP foci (fluorescent reduction neutralization test (FRNT)) was also developed. Correlation analysis with mAb neutralization values by PRNT vs FRNT (**Fig. 2b**), Pearson's r ≥ 0.9 and p-value < 0.001).

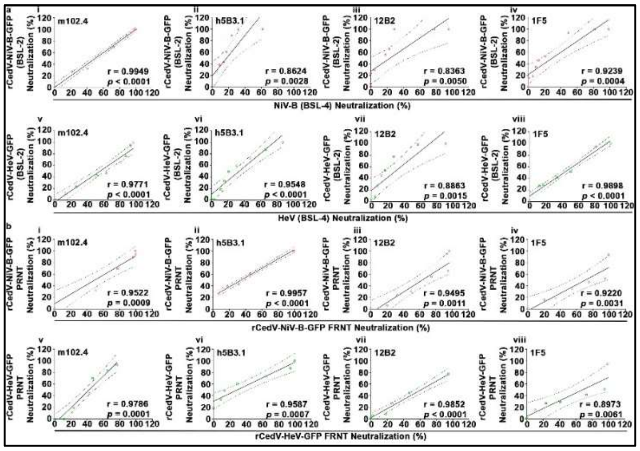

Figure 2. Panel a: Pearson correlation for BSL-2 PRNT vs BSL-4 PRNT (rCedV-NiV-B-GFP versus NiV-B (**i-iv**) and rCedV-HeV-GFP versus HeV (**v-viii**)). Panel b: Pearson correlation for PRNT vs FRNT ((rCedV-NiV-B-GFP (**i-iv**) and rCedV-HeV-GFP (**v-viii**)).

Rescue and characterize reporter viruses encoding GhV envelope glycoproteins.

Generation of a VSV encoding GhV envelope glycoproteins. We will construct reporter gene encoding versions of a replication competent VSV encoding the GhV F and G glycoproteins (GFP and Luc) (**Fig. 1A)**. A large gene cassette comprising VSV untranslated intergenic regions flanking the GhV F and G coding sequences will be synthesized. The GhV F and G coding sequences will be based on the GhV isolate Eid_hel/GH-M74a/GHA/2009 (GenBank: NC_025256.1). The GenBank accession number for GhV F is YP_009091837.1 and that for the GhV G protein is YP_009091838.1. Unique restriction enzyme sites, MluI and KpnI will facilitate the insertion of the GhV F and G gene cassette into the VSV backbone plasmid. The VSV-GhV-Luc will be similarly made. Cloning procedures are performed in *E. coli* Stbl2 and all plasmids verified by sequencing. Rescue will be performed in BSR-T7/5 cells, transfected helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-GhV-GFP or VSV-GhV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus is performed using HEK293T cells by inoculation with the supernatant from step 1. When GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above and the virus stocks stored at -80°C. Larger viral stocks are prepared in T-150 flasks in HEK293T cells, and virus supernatants are purified by buffered sucrose cushion centrifugation and virus pellets resuspended in 10% sucrose/TNE buffer, and small volume single use aliquots stored at -80°C. All virus stocks are deep sequenced. Virus stocks are tittered by serial dilution on HEK293T cells by plaque assay (PMID: 32833351).

Generation of rCedV encoding GhV envelope glycoproteins. rCedV-GhV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the GhV F and G coding sequences. The CedV non-coding intergenic regions are retained as is adherence to the genomic "rule of six".

Unique restriction sites, MluI and SphI will facilitate the insertion of the GhV F and G gene cassette into the genome clone ($pOLTV5_{opt}$-rCedV) generating rCedV-GhV-GFP (**Fig. XB**). All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and $pOLTV5_{opt}$-rCedV-GhV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (henipavirus CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. At maximal syncytia and GFP (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C (PMCID: PMC8631057, PMCID: PMC5869790, PMCID: PMC1563797).

Characterization of the recombinant reporter viruses in cell-based infection assays: The replication kinetics of the VSV-based and rCedV-based GhV F/G encoding viruses will be assayed by Vero cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in several 24hr intervals and tittered. VSV-GhV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G (PMID: 23849788). Similarly, rCedV-GhV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Unlike NiV and HeV, GhV G engages only the ephrin-B2 ligand to trigger membrane fusion (PMID: 24296468). The GhV F/G bearing reporter viruses will be verified for this specific ephrin ligand tropism in cell infection and/or syncytia formation experiments and comparisons made to wildtype VSV or VSV-ABLV G and also alternatively to rCedV, and rCedV NiV/HeV chimeras. We have generated murine mAbs to GhV F and G (10C4-LY and 10D5-LY to GhV G; and 5B7-LY and 7C11-LY to GhV F) and these will be used in neutralization tests. The VSV-GhV-GFP assays will be conducted by FRNTs and VSV-GhV-Luc in luciferase assays.

Evaluating neutralization potential of vaccine platforms from other research projects. Our rCeV chimeras (rCedV-NiV-B-GFP and rCedV-HeV-GFP) will allow this Core to conduct large numbers of neutralization tests by FRNT, to the many ML-derived prototype henipavirus vaccine antigen constructs, mAbs, and nanobinders, that will be generated by the Center's RPs. In Center years 1-3 the focus will be on HeV and NiV prototypes, and in years 4-5 the Core is expected to shift to GhV test case. The FRNT assay is rapid (36hrs), high throughput, and performed in 96-well plates to establish typical 7-point dose response virus neutralization data and performed at BSL-2. Typically, immune sera, mAbs, or other entry inhibitors are analyzed by serially dilution and mixed with virus (rCedV-NiV-B-GFP or rCedV-HeV-GFP) for 2hrs then samples used in triplicate to infect confluent Vero 76 cells in black-walled clear bottom 96-well plates and incubated 24hrs, and then plates are fixed and imaged and fluorescent foci quantitated with CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) is determined by 50% reduction in fluorescent foci versus untreated controls.

Anticipated Results, Potential Pitfalls and Alternative Strategies. We anticipate the reporter viruses will replicate to titers fit for purpose. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. If we encounter unexpected limitations in rescuing VSV-based or rCedV-based GhV F/G bearing reporter viruses; we will 1) test different ratios of helper plasmids and antigenome plasmids; 2) perform rescue using different cell lines; 3) construct alternate versions of the GhV F and G genes within the antigenome plasmids, such as truncating the GhV G protein which has been shown to enhance GhV mediated F/G membrane fusion (PMID: 31470664). Alternatively, fusion of the GhV F and G ectodomains with the transmembrane and cytoplasmic tail domains of the corresponding CedV F and G elements may facilitate virus particle formation and membrane fusion activity.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, immunology assays, virology assays, necropsies, and histopathological analysis.

The Core D Leader and Co-Leaders will work closely with the Project Leaders and key staff of RP1 and RP2 to develop vaccination, treatment and sampling regimens, study protocols, and animal protocols that utilize countermeasures identified in RP1 and RP2. The Core Leader and Co-Leaders will write and obtain UTMB IACUC approval for all animal protocols performed in Core D. The animal models and clinical pathology, immunology, virology, and histopathology assays employed for Aim 3 are detailed below. All raw data as well as graphed or tabulated data generated by Core D will be handled and analyzed in coordination with the Core B.

Animal models: Core D will employ established animal models for HeV, LASV, RVFV, and SFTSV. While animal models also exist for SOSV and JUNV we will optimize these models to fill gaps and provide new tools as detailed in Aim 4 below. Mice, guinea pigs, hamsters, ferrets, and cats have been used as small animal models of HeV infection (PMID: 22476556). This project will employ Syrian golden hamsters as the small animal model of HeV disease because they are more suitable than other small animals for screening large numbers of vaccines and mAbs. Using hamsters will allow us to select optimal vaccine and mAb formulations and regimens prior to efficacy testing in NHPs. In 2010 Drs. Geisbert and Broder developed the first highly lethal NHP model for henipavirus infection (Nipah virus) using African green monkeys (AGM) (PMCID: PMC2872660.). They subsequently developed a lethal AGM model for HeV infection (PMCID: PMC2937751.). Henipavirus NHP models mirror human disease with animals showing severe respiratory pathology, neurological disease, and generalized vasculitis. For LASV, Core D will use established lethal outbred guinea pig models that cover several lineages of LASV (PMCID: PMC1892204; PMCID: PMC4401668; PMCID: PMC5032844.; PMCID: PMC6763766; PMCID: PMC10052409) as guinea pigs are the only immunocompetent small animal model for Lassa fever. Core D will also use established cynomolgus monkey models that cover several genetically distinct lineages of LASV (PMCID: PMC2681932. ; PMCID: PMC3104370. ; PMCID: PMC6934204.)

Animal vaccinations: All animals will be obtained from commercial vendors and allowed to acclimate prior to vaccination (~ 3-7 days for mice, hamsters, and guinea pigs and ~ 14 days for ferrets and NHPs). Mice, hamsters, and guinea pigs will be vaccinated by intramuscular (i.m.) or intraperitoneal (i.p.) injection of the vaccines provided by RP1. Ferrets and NHPs will be vaccinated by i.m. injection of the vaccines provided by RP1. All animals will be vaccinated in ABSL-2. Animals will be monitored daily for any reaction or adverse events caused by the vaccination. Blood will be collected for plasma, serum, and PBMC isolations before vaccination and at times after vaccination for assessment of the host immune response.

Animal challenges: Hamsters, ferrets, and NHPs will be challenged with infectious HeV or SOSV as previously described (PMCID: PMC4971471. ; PMCID: PMC8527959.; PMCID: PMC7199785). After appropriate acclimation, hamsters and ferrets will be exposed to HeV or SOSV by intranasal (i.n.) or i.p. administration of up to 1x10^6 PFU of virus. NHPs will be challenged with HeV by the intratracheal (i.t.) and/or i.n. routes using up to 1 x 10^6 PFU of virus. Mice, guinea pigs, and ferrets will be exposed with up to 5,000 PFU of arenaviruses and phleboviruses by i.p. injection (PMCID: PMC7593599. ; PMCID: PMCID: PMC5032844. PMCID: PMC4843420.; PMCID: PMC8781158). NHPs will be exposed with up to 5.000 PFU of LASV by i.m. injection (PMCID: PMC5719877; PMCID: PMC6934204), Junin virus by intravenous (i.v.) injection (PMCID: PMC7980402) while marmosets will be exposed to up to 1x10^7 PFU of RVFV as previously described (PMCID: PMC3302397). For all animals, blood will be collected with or without anticoagulants for plasma and serum isolations, respectively, at times before paramyxovirus, arenavirus, or phlebovirus challenge, on the day of challenge, and at various times after challenge as detailed in RP1 and RP2. Animals will be monitored for clinical signs daily. We will apply a scoring sheet approved by the UTMB IACUC depicted in the Vertebrate Animals section to assist in determining the time of euthanasia depending on clinical signs (e.g., respiratory distress, anorexia, activity, appearance, neurologic signs, etc.). Animals will be euthanized according to the 2013 AVMA Guidelines on Euthanasia. Any surviving animals will be euthanized on day 35-42 days after paramyxovirus, arenavirus, or phlebovirus challenge.

Clinical pathology assays: *Hematology on EDTA whole blood*: For NHP studies, we will measure the following parameters at time points before and after HeV, LASV, or JUNV challenge: total white blood cell counts (WBC), percent lymphocytes (LY), percent monocytes (MO), percent neutrophils (NEU), percent eosinophils (EOS), percent basophils (BAS), total lymphocyte counts (LY#), total monocyte counts (MO#), total neutrophil counts (NEU#), total eosinophil counts (EOS #), total basophil counts (BAS #), red blood cell counts (RBC), total hemoglobin (Hgb), hematocrit values (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV), and Plateletcrit (PCT). Values will be obtained from the Abaxis VetScan HM5 following manufacturer's directions.

Clinical chemistry on serum: For NHP studies, the following parameters will be measured at time points before and after HeV, LASV, and JUNV challenge: Albumin (ALB), amylase (AMY), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), glucose (GLU), total protein (TP), blood urea nitrogen (BUN), and creatinine (CRE), Creactive protein (CRP), Calcium (Ca2+), and uric acid (UA). Values will be obtained from the Abaxis Piccolo chemistry analyzer following manufacturer's directions.

Viral load assays: *PCR Assay:* To assess viral loads in whole blood and tissues, RNA will be extracted using a virus RNA isolation kit (Qiagen, Valencia, CA) followed by TaqMan PCR for the viral genome being studied. Qiagen buffers have been safety tested for inactivation of paramyxoviruses, arenaviruses, and phleboviruses. PCR assays employed for HeV, SOSV, LASV, JUNV, RVFV, and SFTSV have been previously described

(PMCID: PMC7870971; PMCID: PMC7980402. ; PMID: 36521633; PMCID: PMC5719877; PMCID: PMC5627234).

Plaque Assay: The plaque assays employed for paramyxoviruses, arenaviruses, and phleboviruses have previously been described (PMCID: PMC7870971; PMCID: PMC7980402. ; PMCID: PMC5719877; PMCID: PMC1395455). Briefly, 10-fold dilutions (10-1 to 10-6) of serum, plasma, or whole blood samples are prepared in cell maintenance medium (DMEM/10%FBS/gentamicin) and adsorbed to 70-90% confluent monolayers of Vero E6 cells in 6-well plates, adding 0.2 mL/well. The plates are incubated at 37°C, 5% CO2 for 45 min, with periodical rocking every 10-15 min. The incubation period is followed by addition of 2 mL 1% agarose-containing medium (2X EBME/Hepes/10% FBS/gentamicin) to each well. After the agarose has solidified, the plates are returned to the C02 incubator and maintained for 2-14 days until plaques are visualized. At this time, 2 mL PBS containing 5% neutral red and 5% FBS is added to each well, the plates returned to the CO2 incubator and held overnight. The following day, the liquid stain overlay is removed from each well and plaques are counted.

Bead-based multiplex assays: Plasma inflammatory mediators, thrombosis markers, and fibrinolysis analytes were measured by flow cytometry using Biolegend LegendPlex assays and a FACS Canto-II cytometer (Becton Dickson). Samples will be processed in duplicate for each Nonhuman Primate Inflammation 13-plex (1:4 dilution), Human Thrombosis (1:100), and Human Fibrinolysis (1:40,000) panel as previously described (PMCID: PMC7790436; PMCID: PMC8406165; PMCID: PMC9225728).

Gross pathology and histopathology: A necropsy will be performed on all NHPs. We will examine the external surfaces of the body, all orifices, and the thoracic and peritoneal cavities and their contents. Core D will prepare a written report of the gross pathology findings of each NHP. The following NHP tissues will be collected at necropsy, fixed in 10% neutral-buffered formalin, embedded in paraffin, and stained by H&E: liver, spleen, lung, kidney, adrenal gland, pancreas, axillary lymph node, inguinal lymph node, mesenteric lymph node, duodenum, ileum, colon, brain (frontal cortex, cerebellum, brain stem), cervical spinal cord, urinary bladder, ovary or testis, prostate or uterus, and eye. A microscopic evaluation will be performed on H&E slides of all tissues collected from all NHPs by an ACVP board certified veterinary pathologist. Reports of the histologic findings of each tissue from each animal will be provided to RP1 Leaders. In consultation with the RP1 Leaders, Core D may also perform immunohistochemical and/or special stains such as PTAH for fibrin as previously described (PMCID: PMC5719877; PMCID: PMC5719877; PMCID: PMC7980402.).

Quality systems management of all records and data collected from animal studies: Although the animal studies conducted by Core D will not be required to be performed under GLP compliance, UTMB will provide Quality Assurance that will achieve data quality and integrity when conducting animal experiments. Quality refers to whether the study was conducted in accordance with the protocol, standard operating procedures, and applicable standards of research. Integrity refers to assurance that the raw data and documentation are consistent with reported results. All animal study personnel will be trained by the UTMB Office of Regulated Nonclinical Studies (ORNcS). on Good Documentation Practices (GDP). All animal study data generated by Core D will be provided to Core B as detailed in the Core B Research Strategy.

Aim 4: Develop and/or optimize animal models for Sosuga virus and Junin virus.

For HeV and LASV Core D will employ animal models that have already been developed and are well established. However, for SOSV, JUNV, RVFV, SFTSV while animal models exist there are gaps that need to be addressed. Recently, it was shown that i.n. or i.p. infection of hamsters with SOSV resulted in viremia and mild clinical disease (PMID: 36521633) but there is no lethal model for SOSV. Core B will further explore the hamster model for SOSV by 1) using higher challenge doses of SOSV which is often required to produce lethal disease in other paramyxovirus hamster models (PMCID: PMC8527959; PMCID: PMC3147900) or 2) serially passage of tissues in hamsters. Core D will also assess the pathogenic potential of ferrets for SOSV as they are often used as animal models for paramyxoviruses. For JUNV, serial adaptation is often required to produce lethal disease in outbred guinea pigs. We recently showed that for Ebolaviruses while serial adaptation is required to produce lethality in mice, hamsters, and guinea pigs, infection of ferrets with *Zaire ebolavirus*, *Sudan ebolavirus*, or *Bundibugyo ebolavirus* resulted in uniform lethality with wild type viruses and did not require adaptation (PMCID: PMC4957446.). Therefore, Core D will assess the pathogenic potential of JUNV in ferrets.

BIBLIOGRAPHY AND REFERENCES CITED

ANIMAL MODELS AND VACCINE EVALUATION SCIENTIFIC CORE (CORE D)

1. SPECIFIC AIMS

The main objective of the Animal Models and Vaccine Evaluation Scientific Core is to test and characterize the host response and efficacy of the vaccines and monoclonal antibody (mAb) based treatments identified in RP1 and RP2 against paramyxoviruses, arenaviruses, and phleboviruses under BSL-3/ABSL-3 and BSL-4/ABSL-4 containment. The Core will also develop select reporter viruses and conduct cell-based assays to characterize vaccine-induced neutralizing antibody responses and mAbs.

The Specific Aims of the Animal Models and Vaccine Evaluation Scientific Core are to:

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses.

Aim 2: Provide technical expertise and conduct *in vitro* assays.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, immunology assays, virology assays, necropsies, and histopathological analysis.

Aim 4: Develop and/or optimize animal models for Sosuga virus and Junin virus.

The Aims will be achieved by the following specific functions to be carried out by the Animal Models and Vaccine Evaluation Scientific Core Leader, Co-Core Leaders, and personnel:

1. Maintain records of well characterized BSL-3 and BSL-4 Hendra virus (HeV), Sosuga virus (SOSV), Lassa virus (LASV), Junin virus (JUNV), Rift valley fever virus (RVFV), and severe fever with thrombocytopenia syndrome virus (SFTSV) seed stocks including background history, passage history, genomic information, periodic monitoring of titer, mycoplasma testing, endotoxin testing, and sterility testing.
2. Perform *in vitro* antiviral activity assays. Including high-throughput BSL-2 based neutralization testing of henipavirus vaccine responses and mAbs.
3. Assist Center Investigators in designing animal studies, prepare animal protocols, and obtain IACUC approval of BSL-3 and BSL-4 animal studies.
4. Perform animal challenges using well characterized HeV, SOSV, LASV, JUNV, RVFV, and SFTSV seed stocks for RP1 and RP2.
5. Perform vaccination and treatment of animals with vaccines and treatments provided by RP1 and RP2.
6. Provide samples from animals collected during the vaccination portion of studies to RP1 for immunological analysis.
7. Perform all duties associated with animal challenge studies including monitoring of animals, collection of samples, and necropsies.
8. Perform clinical pathology assays including hematology and clinical chemistry assays.
9. Perform virology assays on blood and/or tissue samples collected from infected animals including viral load by plaque assay and RT-PCR.
10. Perform gross pathology analysis on all animals at necropsy.
11. Perform histopathologic analysis on tissues from animals collected at necropsy.
12. Develop and/or optimize animal models for SOSV and JUNV to fill gaps; make these models available to Center Research Projects and Scientific Cores as well as external ReVAMPP Centers
13. Provide all data generated in Core D to Data Management Core B.

RESEARCH STRATEGY

A. SIGNIFICANCE

Among viruses that cause disease in humans viral zoonoses within the family Paramyxoviridae and order Bunyavirales contain pathogens that cause significant lethality. Importantly, within the Paramyxovirdae the zoonotic henipavirus Hendra virus (HeV) is on the WHO Priority Pathogens list. Notably, henipaviruses stand out for their impressive lethality with case fatality rates averaging about 75%. The Arenaviridae and Phelboviridae families contain viruses that cause severe hemorrhagic disease in humans. These viruses include the arenaviruses Lassa virus (LASV) and Junin virus (JUNV) and the phlebovirus Rift Valley fever virus (RVFV). All of these viruses are also included among the World Health Organization's (WHO) List of Priority Pathogens and pose threats to global public health due to the absence of approved vaccines or therapeutics. There is a clear unmet need for medical countermeasures to combat the threat of natural outbreaks, epidemics, or deliberate release. The Machine Learning-(ML)-Enabled Design of Prototype Pathogen Vaccines and Antibodies Center is a well-integrated consortium conducting a set of discovery and translational research programs composed of an Administrative Core, a Data Management Core, X Scientific Cores, and X Research Projects (RPs). The synergistic activities of the X Center partners will be focused on developing novel vaccines, treatments, and tools to support pandemic preparedness. Federal law requires that the paramyxoviruses, arenaviruses, and phleboviruses used in the X Center be handled in approved Biosafety Level (BSL)-3 and BSL-4 containment laboratories. Core D provides approved BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities and a trained and highly experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2. The services provided by Core D will include 1) a secure repository of well characterized seed stocks of BSL-3 and BSL-4 paramyxoviruses, arenaviruses, and phleboviruses 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5); development and/or optimization of animal models for strains or variants of Sosuga virus (SOSV), JUNV, RVFV, and severe fever with thrombocytopenia syndrome virus (SFTSV) to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies.

All X of the research projects within the X Center focus on developing vaccines or monoclonal antibody (mAb) based therapies against paramyxoviruses, arenaviruses, and phleboviruses. All X of these research projects require that countermeasures be evaluated in animals against infectious paramyxoviruses, arenaviruses, and phleboviruses. As HeV, LASV, JUNV, and RVFV are CDC and/or USDA select agents Federal regulations require that these viruses be handled in Biosafety Level (BSL)-3 or BSL-4 containment. Core D provides approved BSL-3/ABSL-3 and BSL-4/BSL-4 facilities and a trained and experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2.

B. INNOVATION

Scientific Core D will be co-located within the Galveston National Laboratory (GNL) and Robert Shope Laboratory at the University of Texas Medical Branch (UTMB) at Galveston. The GNL is a high and maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda and stands ready to assist local, state and national public health efforts in the event of a public health emergency. The facility is unique in that it is one of two operational BSL-4 facilities in the United States that is located on an academic campus. This unique location benefits the X Center in several ways. Regarding physical resources the GNL is a state-of-the-art facility with many specialized assets including BSL-4 imaging, telemetry, and aerobiology equipment. In addition, being located on an academic campus Drs. Geisbert (Core D, Project Leader (PL), Cross, Freiberg, and Ikegami are faculty members in the Departments of Microbiology and Immunology and Pathology. This academic environment is a major strength, that fosters creativity and collaboration with other UTMB investigators which will be beneficial to the overall success of Core D. Dr. Broder, is faculty member of the Department of Microbiology and Immunology at Uniformed Services University (USU). Drs. Broder and Geisbert (PL) have a long-standing emerging virus research collaboration which has included the development and characterization the first nonhuman primate (NHP) models for HeV and Nipah virus (NiV) that will be employed by the Center's RPs. Another strength within the Core is Drs. Geisbert, Broder, Cross, and Veesler (Center Co-PI) currently collaborate and lead active henipavirus projects that are focused on translational countermeasure development which includes more traditional vaccine and mAb therapeutics approaches, and the shared knowledge base and experience in these areas will greatly facilitate the Core's abilities in supporting the Center's research goals in applying advanced machine learning-enabled

designs of effective prototype pathogen vaccines and antibodies. Along with animal model development and animal modeling expertise, many of the unique virological tools including recombinantly generated RNA viruses, reporter gene-encoding viruses, and other pseudotype virus technologies, that will be employed by the Core, have been developed from UTMB-USU collaboration.

C. APPROACH

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses.

Paramyxovirus, arenavirus, and phlebovirus seed stocks. UTMB will provide fully equipped BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities that are required to work with HeV, SOSV, LASV, JUNV, RVFV, and SFTSV. UTMB will also provide and maintain all required approvals to work these viruses. The BSL-3 and BSL-4 paramyxovirus, arenavirus, and phlebovirus seed stocks employed for RP1 and RP2 have been or will be titered by conventional standard plaque assay and qRT-PCR as detailed in Aim 2 below. The virus strain identity of each seed stock has been or will be confirmed by sequence analysis. In addition, RNA has been or will be deep sequenced to verify the complete viral genome of each virus seed stock used for animal challenge studies. We have or will test each seed stock for mycoplasma contamination. Endotoxin testing has been or will be performed on paramyxovirus, arenavirus, and phlebovirus stocks using the Endosafe PTS system (Charles River Laboratories) following the manufacturer's instructions. The Endosafe PTS system is a cartridge-based testing system utilizing FDA-licensed cartridges (0.1 EU/ml, 0.05 EU/ml, 0.01 EU/ml, 0.05 EU/ml) for ultrasensitive testing. Seed stocks will also be evaluated for morphological identity by transmission electron microscopy (TEM). Virus particle counts will be determined using a Virus Counter and confirmed by conventional TEM methods if needed. The bacteriological and mycological sterility of the product will be determined by selection of aliquots of the dispensed stock, inoculation of a sterile 500 ml bottle of Dulbecco's high glucose MEM with 10% FBS and then after 3 days subcultured to FTM and TBS media. paramyxovirus, arenavirus, and phlebovirus seed stocks will be inventoried in the UTMB BSL-4 and BSL-4 virus stock databases and secured in dedicated controlled access alarmed minus 80°C freezers in the GNL or Robert Shope Laboratory. Paramyxovirus, arenavirus, and phlebovirus seed stocks are monitored periodically for any changes in virus titer.

Plan for staffing, managing, and prioritizing Core use. The Core Leader and Co-Leaders have a cadre of staff that are trained and highly experienced in all BSL-3 and BSL-4 procedures and assays needed to conduct the proposed work. In addition to the Core Leader and Co-Leaders this staff includes an ACVP board certified veterinary pathologist, two BSL-4 contributing scientists, a BSL-4 Technical Director, a BSL-4 trained postdoctoral fellow, six BSL-4 experienced technical staff, a BSL-4 trained histotechnologist, and a BSL-4 trained graduate student. We do not anticipate any BSL-3 or BSL-4 scheduling problems regarding studies proposed in RP1 or RP2. However, we have developed a mitigation strategy if unforeseen events cause any prioritization of resources. A committee consisting of the Core D Leader, the PI of the Center, and the members of the external Scientific Advisory Committee (SAC) will review the status of RP1 and RP2 and any circumstances causing a prioritization of work. The Committee will also hold a meeting or teleconference (Zoom, Teams) with the Project Leaders and key staff of RP1 and RP2 to gather additional information. After careful review of all available information the Committee will then make a recommendation on prioritization to the Center PI. The X Center PI will then consult with NIAID/NIH program staff and make the final determination on prioritization.

Aim 2: Provide technical expertise and conduct *in vitro* assays.

In vitro antiviral activity assays: Core D will perform conventional virus neutralization assays for RP1 and RP2 to determine the ability of sera/plasma from immunized animals from RP1 and antibodies from RP2 to inhibit the replication of paramyxoviruses, arenaviruses, and phleboviruses *in vitro* using infectious viruses in the UTMB BSL-3 and BSL-4 laboratories. In brief, cultures of Vero, Vero E6, SW13, or other cell types will be infected with a range of m.o.i. of paramyxoviruses, arenaviruses, and bunyaviruses and treated with various concentrations of the provided sera/plasma or antibodies. Cells and/or culture fluids will be harvested at various time points after infection and infectious virus enumerated by conventional plaque assay as detailed below.

Develop chimeric reporter-gene encoding viruses and virus neutralization assays for BSL-2 use.
We have previously used reverse genetics to generate several types of replication competent VSV-GFP reporter viruses including those encoding Australian bat lyssavirus (ABLV) G glycoproteins (VSV-ABLV G-GFP) (PMID: 23849788 and PMID: 24576301), as well as employed reverse genetics to rescue authentic ABLV and various reporter gene-encoding viruses (PMID: 32407866 and PMID: 30875748). In addition, we have generated a reverse genetics platform for generating recombinant Cedar virus (rCedV) and rCedV chimeras whereby the

fusion (F) and attachment (G) glycoprotein genes of rCedV were replaced with those of NiV-Bangladesh (NiV-B) or HeV, generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV), both with and without reporter genes (PMID: 35617431, PMID: 31548390, PMID: 29587789, PMID: 37243163). The rCedV chimeras are a replication competent authentic henipavirus platform that is a high-throughput surrogate neutralization assay that is rapid, cost-effective and can be utilized outside high containment. Similar approaches will be applied here to generate new reporter viruses for Ghana bat virus (GhV). We will design, construct and rescue both VSV and rCedV chimeras that encode the envelope glycoproteins (fusion (F) and attachment (G) glycoproteins) of GhV. Two reporter-gene encoding versions will be attempted for VSV-GhV: one encoding GFP (VSV-GhV-GFP) and the other encoding the *Photinus pyralis* (firefly) luciferase gene (VSV-GhV-Luc). We will also construct and rescue GFP and Luc reporter gene encoding versions? of rCedV chimeras encoding the GhV F and G genes (rCedV-GhV-GFP and rCedV-GhV-Luc) (**Fig. 1**). The rescued viruses will be characterized using cell-based infection tropism and growth kinetics assays.

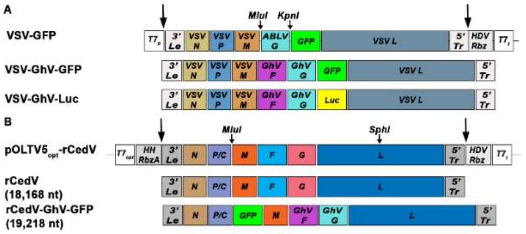

Figure 1. Schematic of chimeric reporter viruses. (**A**)The VSV-ABLV G-GFP plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and KpnI to construct VSV-GhV-GFP and VSV-GhV-Luc. (**B**) pOLTV5$_{opt}$-rCedV plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and SphI used to construct rCedV-GhV-GFP.

The rCedV (NiV-B and HeV) chimeric virus platform serves as a robust surrogate neutralization assay system for quantifying antibody neutralization potency of anti-NiV and anti-HeV antisera and mAbs [Amaya et al, Viruses 2023 see comment box]. The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G specific mAbs against rCedV-NiV-B-GFP and rCedV-HeV-GFP are highly correlated with those obtained using authentic NiV-B and HeV when tested in parallel by plaque reduction neutralization test (PRNT). Pearson correlation of rCedV chimeras BSL-2 PRNT to the NiV-B or HeV BSL-4 PRNT is shown in **Fig. 2a**. A high-throughput quantitative assay based on reduction of GFP foci (fluorescent reduction neutralization test (FRNT)) was also developed. Correlation analysis with mAb neutralization values by PRNT vs FRNT (**Fig. 2b)**, Pearson's r ≥ 0.9 and p-value < 0.001).

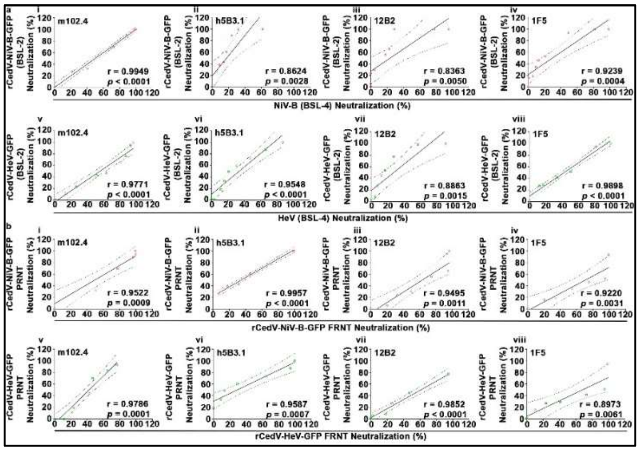

Figure 2. Panel a: Pearson correlation for BSL-2 PRNT vs BSL-4 PRNT (rCedV-NiV-B-GFP versus NiV-B (**i-iv**) and rCedV-HeV-GFP versus HeV (**v-viii**)). Panel b: Pearson correlation for PRNT vs FRNT ((rCedV-NiV-B-GFP (**i-iv**) and rCedV-HeV-GFP (**v-viii**)).

Rescue and characterize reporter viruses encoding GhV envelope glycoproteins.

Generation of a VSV encoding GhV envelope glycoproteins.

We will construct reporter gene encoding versions of a replication competent VSV encoding the GhV F and G glycoproteins (GFP and Luc) (**Fig. 1A)**. A large gene cassette comprising VSV untranslated intergenic regions flanking the GhV F and G coding sequences will be synthesized. The GhV F and G coding sequences will be based on the GhV isolate Eid_hel/GH-M74a/GHA/2009 (GenBank: NC_025256.1). The GenBank accession number for GhV F is YP_009091837.1 and that for the GhV G protein is YP_009091838.1. Unique restriction enzyme sites, MluI and KpnI will facilitate the insertion of the GhV F and G gene cassette into the VSV backbone plasmid. The VSV-GhV-Luc will be similarly made. Cloning procedures are performed in *E. coli* Stbl2 and all plasmids verified by sequencing. Rescue will be performed in BSR-T7/5 cells, transfected helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-GhV-GFP or VSV-GhV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus is performed using HEK293T cells by inoculation with the supernatant from step 1. When GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above and the virus stocks stored at -80°C. Larger viral stocks are prepared in T-150 flasks in HEK293T cells, and virus supernatants are purified by buffered sucrose cushion centrifugation and virus pellets resuspended in 10% sucrose/TNE buffer, and small volume single use aliquots stored at -80°C. All virus stocks are deep sequenced. Virus stocks are tittered by serial dilution on HEK293T cells by plaque assay (PMID: 32833351).

Generation of rCedV encoding GhV envelope glycoproteins. rCedV-GhV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the GhV F and G coding sequences. The CedV non-coding intergenic regions are retained as is adherence to the genomic "rule of six".

Unique restriction sites, MluI and SphI will facilitate the insertion of the GhV F and G gene cassette into the genome clone (pOLTV5$_{opt}$-rCedV) generating rCedV-GhV-GFP (**Fig. XB**). All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-GhV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (henipavirus CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. At maximal syncytia and GFP (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C (PMCID: PMC8631057, PMCID: PMC5869790, PMCID: PMC1563797).

Characterization of the recombinant reporter viruses in cell-based infection assays: The replication kinetics of the VSV-based and rCedV-based GhV F/G encoding viruses will be assayed by Vero cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in several 24hr intervals and tittered. VSV-GhV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G (PMID: 23849788). Similarly, rCedV-GhV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Unlike NiV and HeV, GhV G engages only the ephrin-B2 ligand to trigger membrane fusion (PMID: 24296468). The GhV F/G bearing reporter viruses will be verified for this specific ephrin ligand tropism in cell infection and/or syncytia formation experiments and comparisons made to wildtype VSV or VSV-ABLV G and also alternatively to rCedV, and rCedV NiV/HeV chimeras. We have generated murine mAbs to GhV F and G (10C4-LY and 10D5-LY to GhV G; and 5B7-LY and 7C11-LY to GhV F) and these will be used in neutralization tests. The VSV-GhV-GFP assays will be conducted by FRNTs and VSV-GhV-Luc in luciferase assays.

Evaluating neutralization potential of vaccine platforms from other research projects. Our rCeV chimeras (rCedV-NiV-B-GFP and rCedV-HeV-GFP) will allow this Core to conduct large numbers of neutralization tests by FRNT, to the many ML-derived prototype henipavirus vaccine antigen constructs, mAbs, and nanobinders, that will be generated by the Center's RPs. In Center years 1-3 the focus will be on HeV and NiV prototypes, and in years 4-5 the Core is expected to shift to GhV test case. The FRNT assay is rapid (36hrs), high throughput, and performed in 96-well plates to establish typical 7-point dose response virus neutralization data and performed at BSL-2. Typically, immune sera, mAbs, or other entry inhibitors are analyzed by serially dilution and mixed with virus (rCedV-NiV-B-GFP or rCedV-HeV-GFP) for 2hrs then samples used in triplicate to infect confluent Vero 76 cells in black-walled clear bottom 96-well plates and incubated 24hrs, and then plates are fixed and imaged and fluorescent foci quantitated with CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) is determined by 50% reduction in fluorescent foci versus untreated controls.

Anticipated Results, Potential Pitfalls and Alternative Strategies. We anticipate the reporter viruses will replicate to titers fit for purpose. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. If we encounter unexpected limitations in rescuing VSV-based or rCedV-based GhV F/G bearing reporter viruses; we will 1) test different ratios of helper plasmids and antigenome plasmids; 2) perform rescue using different cell lines; 3) construct alternate versions of the GhV F and G genes within the antigenome plasmids, such as truncating the GhV G protein which has been shown to enhance GhV mediated F/G membrane fusion (PMID: 31470664). Alternatively, fusion of the GhV F and G ectodomains with the transmembrane and cytoplasmic tail domains of the corresponding CedV F and G elements may facilitate virus particle formation and membrane fusion activity.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, immunology assays, virology assays, necropsies, and histopathological analysis.

The Core D Leader and Co-Leaders will work closely with the Project Leaders and key staff of RP1 and RP2 to develop vaccination, treatment and sampling regimens, study protocols, and animal protocols that utilize countermeasures identified in RP1 and RP2. The Core Leader and Co-Leaders will write and obtain UTMB IACUC approval for all animal protocols performed in Core D. The animal models and clinical pathology, immunology, virology, and histopathology assays employed for Aim 3 are detailed below. All raw data as well as graphed or tabulated data generated by Core D will be handled and analyzed in coordination with the Core B.

Animal models: Core D will employ established animal models for HeV, LASV, RVFV, and SFTSV. While animal models also exist for SOSV and JUNV we will optimize these models to fill gaps and provide new tools as detailed in Aim 4 below. Mice, guinea pigs, hamsters, ferrets, and cats have been used as small animal models of HeV infection (PMID: 22476556). This project will employ Syrian golden hamsters as the small animal model of HeV disease because they are more suitable than other small animals for screening large numbers of vaccines and mAbs. Using hamsters will allow us to select optimal vaccine and mAb formulations and regimens prior to efficacy testing in NHPs. In 2010 Drs. Geisbert and Broder developed the first highly lethal NHP model for henipavirus infection (Nipah virus) using African green monkeys (AGM) (PMCID: PMC2872660.). They subsequently developed a lethal AGM model for HeV infection (PMCID: PMC2937751.). Henipavirus NHP models mirror human disease with animals showing severe respiratory pathology, neurological disease, and generalized vasculitis. For LASV, Core D will use established lethal outbred guinea pig models that cover several lineages of LASV (PMCID: PMC1892204; PMCID: PMC4401668; PMCID: PMC5032844.; PMCID: PMC6763766; PMCID: PMC10052409) as guinea pigs are the only immunocompetent small animal model for Lassa fever. Core D will also use established cynomolgus monkey models that cover several genetically distinct lineages of LASV (PMCID: PMC2681932. ; PMCID: PMC3104370. ; PMCID: PMC6934204.)

Animal vaccinations: All animals will be obtained from commercial vendors and allowed to acclimate prior to vaccination (~ 3-7 days for mice, hamsters, and guinea pigs and ~ 14 days for ferrets and NHPs). Mice, hamsters, and guinea pigs will be vaccinated by intramuscular (i.m.) or intraperitoneal (i.p.) injection of the vaccines provided by RP1. Ferrets and NHPs will be vaccinated by i.m. injection of the vaccines provided by RP1. All animals will be vaccinated in ABSL-2. Animals will be monitored daily for any reaction or adverse events caused by the vaccination. Blood will be collected for plasma, serum, and PBMC isolations before vaccination and at times after vaccination for assessment of the host immune response.

Animal challenges: Hamsters, ferrets, and NHPs will be challenged with infectious HeV or SOSV as previously described (PMCID: PMC4971471. ; PMCID: PMC8527959.; PMCID: PMC7199785). After appropriate acclimation, hamsters and ferrets will be exposed to HeV or SOSV by intranasal (i.n.) or i.p. administration of up to 1x10^6 PFU of virus. NHPs will be challenged with HeV by the intratracheal (i.t.) and/or i.n. routes using up to 1 x 10^6 PFU of virus. Mice, guinea pigs, and ferrets will be exposed with up to 5,000 PFU of arenaviruses and phleboviruses by i.p. injection (PMCID: PMC7593599. ; PMCID: PMCID: PMC5032844. PMCID: PMC4843420.; PMCID: PMC8781158). NHPs will be exposed with up to 5.000 PFU of LASV by i.m. injection (PMCID: PMC5719877; PMCID: PMC6934204), Junin virus by intravenous (i.v.) injection (PMCID: PMC7980402) while marmosets will be exposed to up to 1x10^7 PFU of RVFV as previously described (PMCID: PMC3302397). For all animals, blood will be collected with or without anticoagulants for plasma and serum isolations, respectively, at times before paramyxovirus, arenavirus, or phlebovirus challenge, on the day of challenge, and at various times after challenge as detailed in RP1 and RP2. Animals will be monitored for clinical signs daily. We will apply a scoring sheet approved by the UTMB IACUC depicted in the Vertebrate Animals section to assist in determining the time of euthanasia depending on clinical signs (e.g., respiratory distress, anorexia, activity, appearance, neurologic signs, etc.). Animals will be euthanized according to the 2013 AVMA Guidelines on Euthanasia. Any surviving animals will be euthanized on day 35-42 days after paramyxovirus, arenavirus, or phlebovirus challenge.

Clinical pathology assays: *Hematology on EDTA whole blood*: For NHP studies, we will measure the following parameters at time points before and after HeV, LASV, or JUNV challenge: total white blood cell counts (WBC), percent lymphocytes (LY), percent monocytes (MO), percent neutrophils (NEU), percent eosinophils (EOS), percent basophils (BAS), total lymphocyte counts (LY#), total monocyte counts (MO#), total neutrophil counts (NEU#), total eosinophil counts (EOS #), total basophil counts (BAS #), red blood cell counts (RBC), total hemoglobin (Hgb), hematocrit values (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV), and Plateletcrit (PCT). Values will be obtained from the Abaxis VetScan HM5 following manufacturer's directions.

Clinical chemistry on serum: For NHP studies, the following parameters will be measured at time points before and after HeV, LASV, and JUNV challenge: Albumin (ALB), amylase (AMY), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), glucose (GLU), total protein (TP), blood urea nitrogen (BUN), and creatinine (CRE), Creactive protein (CRP), Calcium (Ca2+), and uric acid (UA). Values will be obtained from the Abaxis Piccolo chemistry analyzer following manufacturer's directions.

Viral load assays: *PCR Assay:* To assess viral loads in whole blood and tissues, RNA will be extracted using a virus RNA isolation kit (Qiagen, Valencia, CA) followed by TaqMan PCR for the viral genome being studied. Qiagen buffers have been safety tested for inactivation of paramyxoviruses, arenaviruses, and phleboviruses. ~~The~~ PCR assays employed for HeV, SOSV, LASV, JUNV, RVFV, and SFTSV have been previously described

(PMCID: PMC7870971; PMCID: PMC7980402. ; PMID: 36521633; PMCID: PMC5719877; PMCID: PMC5627234).

Plaque Assay: The plaque assays employed for paramyxoviruses, arenaviruses, and phleboviruses have previously been described (PMCID: PMC7870971; PMCID: PMC7980402. ; PMCID: PMC5719877; PMCID: PMC1395455). Briefly, 10-fold dilutions (10-1 to 10-6) of serum, plasma, or whole blood samples are prepared in cell maintenance medium (DMEM/10%FBS/gentamicin) and adsorbed to 70-90% confluent monolayers of Vero E6 cells in 6-well plates, adding 0.2 mL/well. The plates are incubated at 37°C, 5% CO2 for 45 min, with periodical rocking every 10-15 min. The incubation period is followed by addition of 2 mL 1% agarose-containing medium (2X EBME/Hepes/10% FBS/gentamicin) to each well. After the agarose has solidified, the plates are returned to the C02 incubator and maintained for 2-14 days until plaques are visualized. At this time, 2 mL PBS containing 5% neutral red and 5% FBS is added to each well, the plates returned to the CO2 incubator and held overnight. The following day, the liquid stain overlay is removed from each well and plaques are counted.

Bead-based multiplex assays: Plasma inflammatory mediators, thrombosis markers, and fibrinolysis analytes were measured by flow cytometry using Biolegend LegendPlex assays and a FACS Canto-II cytometer (Becton Dickson). Samples will be processed in duplicate for each Nonhuman Primate Inflammation 13-plex (1:4 dilution), Human Thrombosis (1:100), and Human Fibrinolysis (1:40,000) panel as previously described (PMCID: PMC7790436; PMCID: PMC8406165; PMCID: PMC9225728).

Gross pathology and histopathology: A necropsy will be performed on all NHPs. We will examine the external surfaces of the body, all orifices, and the thoracic and peritoneal cavities and their contents. Core D will prepare a written report of the gross pathology findings of each NHP. The following NHP tissues will be collected at necropsy, fixed in 10% neutral-buffered formalin, embedded in paraffin, and stained by H&E: liver, spleen, lung, kidney, adrenal gland, pancreas, axillary lymph node, inguinal lymph node, mesenteric lymph node, duodenum, ileum, colon, brain (frontal cortex, cerebellum, brain stem), cervical spinal cord, urinary bladder, ovary or testis, prostate or uterus, and eye. A microscopic evaluation will be performed on H&E slides of all tissues collected from all NHPs by an ACVP board certified veterinary pathologist. ~~A written r~~Reports of the histologic findings of each tissue from each animal will be provided to ~~the Project Leader of~~ RP1 Leaders. In consultation with the ~~Project Leader of~~ RP1 Leaders, Core D may also perform immunohistochemical and/or special stains such as PTAH for fibrin as previously described (PMCID: PMC5719877; PMCID: PMC5719877; PMCID: PMC7980402.).

Quality systems management of all records and data collected from animal studies: Although the animal studies conducted by Core D will not be required to be performed under GLP compliance, UTMB will provide Quality Assurance that will achieve data quality and integrity when conducting animal experiments. Quality refers to whether the study was conducted in accordance with the protocol, standard operating procedures, and applicable standards of research. Integrity refers to assurance that the raw data and documentation are consistent with reported results. All animal study personnel will be trained by the UTMB Office of Regulated Nonclinical Studies (ORNcS). on Good Documentation Practices (GDP). All animal study data generated by Core D will be provided to Core B as detailed in the Core B Research Strategy.

Aim 4: Develop and/or optimize animal models for Sosuga virus and Junin virus.

For HeV and LASV Core D will employ animal models that have already been developed and are well established. However, for SOSV, JUNV, RVFV, SFTSV while animal models exist there are gaps that need to be addressed. Recently, it was shown that i.n. or i.p. infection of hamsters with SOSV resulted in viremia and mild clinical disease (PMID: 36521633) but there is no lethal model for SOSV. Core B will further explore the hamster model for SOSV by 1) using higher challenge doses of SOSV which is often required to produce lethal disease in other paramyxovirus hamster models (PMCID: PMC8527959; PMCID: PMC3147900) or 2) serially passage of tissues in hamsters. Core D will also assess the pathogenic potential of ferrets for SOSV as they are often used as animal models for paramyxoviruses. For JUNV, serial adaptation is often required to produce lethal disease in outbred guinea pigs. We recently showed that for Ebolaviruses while serial adaptation is required to produce lethality in mice, hamsters, and guinea pigs, infection of ferrets with *Zaire ebolavirus*, *Sudan ebolavirus*, or *Bundibugyo ebolavirus* resulted in uniform lethality with wild type viruses and did not require adaptation (PMCID: PMC4957446.). Therefore, Core D will assess the pathogenic potential of JUNV in ferrets.

BIBLIOGRAPHY AND REFERENCES CITED

ANIMAL MODELS AND VACCINE EVALUATION SCIENTIFIC CORE (CORE D)

1. SPECIFIC AIMS

The main objective of the Animal Models and Vaccine Evaluation Scientific Core is to test and characterize the host response and efficacy of the vaccines and monoclonal antibody (mAb) based treatments identified in RP1 and RP2 against paramyxoviruses, arenaviruses, and phleboviruses under BSL-3/ABSL-3 and BSL-4/ABSL-4 containment. The Core will also develop select reporter viruses and conduct cell-based assays to characterize vaccine-induced neutralizing antibody responses and mAbs.

The Specific Aims of the Animal Models and Vaccine Evaluation Scientific Core are to:

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses.

Aim 2: Provide technical expertise and conduct *in vitro* assays in BSL-3 and BSL-4 containment.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, virology assays, and necropsies in BSL-3 and BSL-4 containment.

Aim 4: Develop and/or optimize animal models for Sosuga virus, Junin virus Rift valley fever virus, and severe fever with thrombocytopenia syndrome virus.

The Aims will be achieved by the following specific functions to be carried out by the Animal Models and Vaccine Evaluation Scientific Core Leader, Co-Core Leaders, and personnel:

1. Maintain records of well characterized BSL-3 and BSL-4 Hendra virus (HeV), Sosuga virus (SOSV), Lassa virus (LASV), Junin virus (JUNV), Rift valley fever virus (RVFV), and severe fever with thrombocytopenia syndrome virus (SFTSV) seed stocks including background history, passage history, genomic information, periodic monitoring of titer, mycoplasma testing, endotoxin testing, and sterility testing.
2. Perform *in vitro* antiviral activity assays. Including high-throughput BSL-2 based neutralization testing of henipavirus vaccine responses and mAbs.
3. Assist Center Investigators in designing animal studies, prepare animal protocols, and obtain IACUC approval of BSL-3 and BSL-4 animal studies.
4. Perform animal challenges using well characterized HeV, SOSV, LASV, JUNV, RVFV, and SFTSV seed stocks for RP1 and RP2.
5. Perform vaccination and treatment of animals with vaccines and treatments provided by RP1 and RP2.
6. Perform all duties associated with animal challenge studies including monitoring of animals, collection of samples, and necropsies.
7. Perform clinical pathology assays including hematology and clinical chemistry assays.
8. Perform virology assays on blood and/or tissue samples collected from infected animals including viral load by plaque assay and RT-PCR.
9. Perform gross pathology analysis on all animals at necropsy.
10. Perform histopathologic analysis on tissues from animals collected at necropsy.
11. Develop and/or optimize animal models for SOSV, JUNV, RFVF, and SFTSV to fill gaps where no models exist; make these models available to Center Research Projects and Scientific Cores as well as external ReVAMPP Centers
12. Provide all data generated in Core D to Data Management Core B.

RESEARCH STRATEGY

A. SIGNIFICANCE

Among viruses that cause disease in humans viral zoonoses within the family Paramyxoviridae and order Bunyavirales contain pathogens that cause significant lethality. Importantly, within the Paramyxovirdae the zoonotic henipavirus Hendra virus (HeV) is on the WHO Priority Pathogens list. Notably, henipaviruses stand out for their impressive lethality with case fatality rates averaging about 75%. The Arenaviridae and Phelboviridae families contain viruses that cause severe hemorrhagic disease in humans. These viruses include the arenaviruses Lassa virus (LASV) and Junin virus (JUNV) and the phlebovirus Rift Valley fever virus (RVFV). All of these viruses are also included among the World Health Organization's (WHO) List of Priority Pathogens and pose threats to global public health due to the absence of approved vaccines or therapeutics. There is a clear unmet need for medical countermeasures to combat the threat of natural outbreaks, epidemics, or deliberate release. The Machine Learning-(ML)-Enabled Design of Prototype Pathogen Vaccines and Antibodies Center is a well-integrated consortium conducting a set of discovery and translational research programs composed of an Administrative Core, a Data Management Core, X Scientific Cores, and X Research Projects (RPs). The synergistic activities of the X Center partners will be focused on developing novel vaccines, treatments, and tools to support pandemic preparedness. Federal law requires that the paramyxoviruses, arenaviruses, and phleboviruses used in the X Center be handled in approved Biosafety Level (BSL)-3 and BSL-4 containment laboratories. Core D provides approved BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities and a trained and highly experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2. The services provided by Core D will include 1) a secure repository of well characterized seed stocks of BSL-3 and BSL-4 paramyxoviruses, arenaviruses, and phleboviruses 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5); development and/or optimization of animal models for strains or variants of Sosuga virus (SOSV), JUNV, RVFV, and severe fever with thrombocytopenia syndrome virus (SFTSV) to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies.

All X of the research projects within the X Center focus on developing vaccines or monoclonal antibody (mAb) based therapies against paramyxoviruses, arenaviruses, and phleboviruses. All X of these research projects require that countermeasures be evaluated in animals against infectious paramyxoviruses, arenaviruses, and phleboviruses. As HeV, LASV, JUNV, and RVFV are CDC and/or USDA select agents Federal regulations require that these viruses be handled in Biosafety Level (BSL)-3 or BSL-4 containment. Core D provides approved BSL-3/ABSL-3 and BSL-4/BSL-4 facilities and a trained and experienced team of BSL-3 and BSL-4 investigators and staff to perform studies that support RP1 and RP2.

B. INNOVATION

Scientific Core D will be co-located within the Galveston National Laboratory (GNL) and Robert Shope Laboratory at the University of Texas Medical Branch (UTMB) at Galveston. The GNL is a high and maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda and stands ready to assist local, state and national public health efforts in the event of a public health emergency. The facility is unique in that it is one of two operational BSL-4 facilities in the United States that is located on an academic campus. This unique location benefits the X Center in several ways. Regarding physical resources the GNL is a state-of-the-art facility with many specialized assets including BSL-4 imaging, telemetry, and aerobiology equipment. In addition, being located on an academic campus Drs. Geisbert (Core D, Project Leader (PL), Cross, Freiberg, and Ikegami are faculty members in the Departments of Microbiology and Immunology and Pathology. This academic environment is a major strength, that fosters creativity and collaboration with other UTMB investigators which will be beneficial to the overall success of Core D. Dr. Broder, is faculty member of the Department of Microbiology and Immunology at Uniformed Services University (USU). Drs. Broder and Geisbert (PL) have a long-standing emerging virus research collaboration which has included the development and characterization the first nonhuman primate (NHP) models for HeV and Nipah virus (NiV) that will be employed by the Center's RPs. Another strength within the Core is Drs. Geisbert, Broder, Cross, and Veesler (Center Co-PI) currently collaborate and lead active henipavirus projects that are focused on translational countermeasure development which includes more traditional vaccine and mAb therapeutics approaches, and the shared knowledge base and experience in these areas will greatly facilitate the Core's abilities in supporting the Center's research goals in applying advanced machine learning-enabled

designs of effective prototype pathogen vaccines and antibodies. Along with animal model development and animal modeling expertise, many of the unique virological tools including recombinantly generated RNA viruses, reporter gene-encoding viruses, and other pseudotype virus technologies, that will be employed by the Core, have been developed from UTMB-USU collaboration.

C. APPROACH

Aim 1: Provide BSL-3 and BSL-4 resources including a secure repository of well characterized seed stocks of paramyxoviruses, arenaviruses, and phleboviruses.

Paramyxovirus, arenavirus, and phlebovirus seed stocks. UTMB will provide fully equipped BSL-3/ABSL-3 and BSL-4/ABSL-4 facilities that are required to work with HeV, SOSV, LASV, JUNV, RVFV, and SFTSV. UTMB will also provide and maintain all required approvals to work these viruses. The BSL-3 and BSL-4 paramyxovirus, arenavirus, and phlebovirus seed stocks employed for RP1 and RP2 have been or will be titered by conventional standard plaque assay and qRT-PCR as detailed in Aim 2 below. The virus strain identity of each seed stock has been or will be confirmed by sequence analysis. In addition, RNA has been or will be deep sequenced to verify the complete viral genome of each virus seed stock used for animal challenge studies. We have or will test each seed stock for mycoplasma contamination. Endotoxin testing has been or will be performed on paramyxovirus, arenavirus, and phlebovirus stocks using the Endosafe PTS system (Charles River Laboratories) following the manufacturer's instructions. The Endosafe PTS system is a cartridge-based testing system utilizing FDA-licensed cartridges (0.1 EU/ml, 0.05 EU/ml, 0.01 EU/ml, 0.05 EU/ml) for ultrasensitive testing. Seed stocks will also be evaluated for morphological identity by transmission electron microscopy. The bacteriological and mycological sterility of the product will be determined by selection of aliquots of the dispensed stock, inoculation of a sterile 500 ml bottle of Dulbecco's high glucose MEM with 10% FBS and then after 3 days subcultured to FTM and TBS media. paramyxovirus, arenavirus, and phlebovirus seed stocks will be inventoried in the UTMB BSL-4 and BSL-4 virus stock databases and secured in dedicated controlled access alarmed minus 80°C freezers in the GNL or Robert Shope Laboratory. Paramyxovirus, arenavirus, and phlebovirus seed stocks are monitored periodically for any changes in virus titer.

Plan for staffing, managing, and prioritizing Core use. The Core Leader and Co-Leaders have a cadre of staff that are trained and highly experienced in all BSL-3 and BSL-4 procedures and assays needed to conduct the proposed work. In addition to the Core Leader and Co-Leaders this staff includes an ACVP board certified veterinary pathologist, two BSL-4 contributing scientists, a BSL-4 Technical Director, a BSL-4 trained postdoctoral fellow, six BSL-4 experienced technical staff, a BSL-4 trained histotechnologist, and a BSL-4 trained graduate student. We do not anticipate any BSL-3 or BSL-4 scheduling problems regarding studies proposed in RP1 or RP2. However, we have developed a mitigation strategy if unforeseen events cause any prioritization of resources. A committee consisting of the Core D Leader, the PI of the Center, and the members of the external Scientific Advisory Committee (SAC) will review the status of RP1 and RP2 and any circumstances causing a prioritization of work. The Committee will also hold a meeting or teleconference (Zoom, Teams) with the Project Leaders and key staff of RP1 and RP2 to gather additional information. After careful review of all available information the Committee will then make a recommendation on prioritization to the Center PI. The X Center PI will then consult with NIAID/NIH program staff and make the final determination on prioritization.

Aim 2: Provide technical expertise and conduct *in vitro* assays.

In vitro antiviral activity assays: Core D will perform conventional virus neutralization assays for RP1 and RP2 to determine the ability of sera/plasma from immunized animals from RP1 and antibodies from RP2 to inhibit the replication of paramyxoviruses, arenaviruses, and phleboviruses *in vitro* using infectious viruses in the UTMB BSL-3 and BSL-4 laboratories. In brief, cultures of Vero, Vero E6, SW13, or other cell types will be infected with a range of m.o.i. of paramyxoviruses, arenaviruses, and bunyaviruses and treated with various concentrations of the provided sera/plasma or antibodies. Cells and/or culture fluids will be harvested at various time points after infection and infectious virus enumerated by conventional plaque assay as detailed below.

Develop chimeric reporter-gene encoding viruses and virus neutralization assays for BSL-2 use.
We have previously used reverse genetics to generate several types of replication competent VSV-GFP reporter viruses including those encoding Australian bat lyssavirus (ABLV) G glycoproteins (VSV-ABLV G-GFP) (PMID: 23849788 and PMID: 24576301), as well as employed reverse genetics to rescue authentic ABLV and various reporter gene-encoding viruses (PMID: 32407866 and PMID: 30875748). In addition, we have generated a reverse genetics platform for generating recombinant Cedar virus (rCedV) and rCedV chimeras whereby the fusion (F) and attachment (G) glycoprotein genes of rCedV were replaced with those of NiV-Bangladesh (NiV-

B) or HeV, generating replication competent chimeric viruses (rCedV-NiV-B and rCedV-HeV), both with and without reporter genes (PMID: 35617431, PMID: 31548390, PMID: 29587789, PMID: 37243163). The rCedV chimeras are a replication competent authentic henipavirus platform that is a high-throughput surrogate neutralization assay that is rapid, cost-effective and can be utilized outside high containment. Similar approaches will be applied here to generate new reporter viruses for Ghana bat virus (GhV). We will design, construct and rescue both VSV and rCedV chimeras that encode the envelope glycoproteins (fusion (F) and attachment (G) glycoproteins) of GhV. Two reporter-gene encoding versions will be attempted for VSV-GhV: one encoding GFP (VSV-GhV-GFP) and the other encoding the *Photinus pyralis* (firefly) luciferase gene (VSV-GhV-Luc). We will also construct and rescue GFP and Luc reporter gene encoding versions? of rCedV chimeras encoding the GhV F and G genes (rCedV-GhV-GFP and rCedV-GhV-Luc) (**Fig. 1**). The rescued viruses will be characterized using cell-based infection tropism and growth kinetics assays.

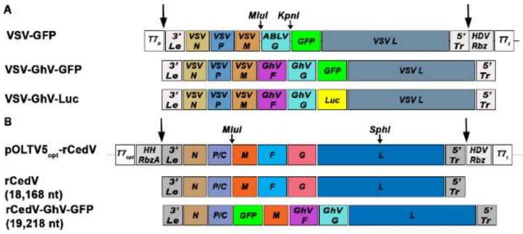

Figure 1. Schematic of chimeric reporter viruses. (**A**)The VSV-ABLV G-GFP plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and KpnI to construct VSV-GhV-GFP and VSV-GhV-Luc. (**B**) pOLTV5$_{opt}$-rCedV plasmid. Arrows indicate regions of self-cleavage. Unique sites MluI and SphI used to construct rCedV-GhV-GFP.

The rCedV (NiV-B and HeV) chimeric virus platform serves as a robust surrogate neutralization assay system for quantifying antibody neutralization potency of anti-NiV and anti-HeV antisera and mAbs [Amaya et al, Viruses 2023 see comment box]. The neutralizing potencies of several well-characterized cross-reactive NiV/HeV F and G specific mAbs against rCedV-NiV-B-GFP and rCedV-HeV-GFP are highly correlated with those obtained using authentic NiV-B and HeV when tested in parallel by plaque reduction neutralization test (PRNT). Pearson correlation of rCedV chimeras BSL-2 PRNT to the NiV-B or HeV BSL-4 PRNT is shown in **Fig. 2a**. A high-throughput quantitative assay based on reduction of GFP foci (fluorescent reduction neutralization test (FRNT)) was also developed. Correlation analysis with mAb neutralization values by PRNT vs FRNT (**Fig. 2b)**, Pearson's r ≥ 0.9 and p-value < 0.001).

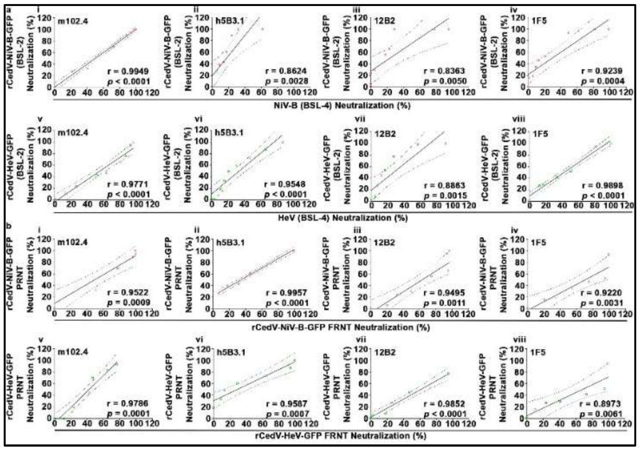

Figure 2. Panel a: Pearson correlation for BSL-2 PRNT vs BSL-4 PRNT (rCedV-NiV-B-GFP versus NiV-B (**i-iv**) and rCedV-HeV-GFP versus HeV (**v-viii**)). Panel b: Pearson correlation for PRNT vs FRNT ((rCedV-NiV-B-GFP (**i-iv**) and rCedV-HeV-GFP (**v-viii**)).

Rescue and characterize reporter viruses encoding GhV envelope glycoproteins.

<u>Generation of a VSV encoding GhV envelope glycoproteins.</u> We will construct reporter gene encoding versions of a replication competent VSV encoding the GhV F and G glycoproteins (GFP and Luc) (**Fig. 1A)**. A large gene cassette comprising VSV untranslated intergenic regions flanking the GhV F and G coding sequences will be synthesized. The GhV F and G coding sequences will be based on the GhV isolate Eid_hel/GH-M74a/GHA/2009 (GenBank: NC_025256.1). The GenBank accession number for GhV F is YP_009091837.1 and that for the GhV G protein is YP_009091838.1. Unique restriction enzyme sites, MluI and KpnI will facilitate the insertion of the GhV F and G gene cassette into the VSV backbone plasmid. The VSV-GhV-Luc will be similarly made. Cloning procedures are performed in *E. coli* Stbl2 and all plasmids verified by sequencing. Rescue will be performed in BSR-T7/5 cells, transfected helper plasmids, VSV-N, VSV-P, and VSV-L and the VSV-GhV-GFP or VSV-GhV-Luc antigenome plasmids. After 48hrs the cells and supernatants are collected and subjected to freeze-thawing and clarified by centrifugation. Amplification of rescued virus is performed using HEK293T cells by inoculation with the supernatant from step 1. When GFP fluorescence and/or cytopathic effects (CPE) is observed, the cells and supernatants are harvested as above and the virus stocks stored at -80°C. Larger viral stocks are prepared in T-150 flasks in HEK293T cells, and virus supernatants are purified by buffered sucrose cushion centrifugation and virus pellets resuspended in 10% sucrose/TNE buffer, and small volume single use aliquots stored at -80°C. All virus stocks are deep sequenced. Virus stocks are tittered by serial dilution on HEK293T cells by plaque assay (PMID: 32833351).

<u>Generation of rCedV encoding GhV envelope glycoproteins.</u> rCedV-GhV-GFP will be produced by synthesizing large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the GhV F and G coding sequences. The CedV non-coding intergenic regions are retained as is adherence to the genomic "rule of six". Unique restriction sites, MluI and SphI will facilitate the insertion of the GhV F and G gene cassette into the

genome clone (pOLTV5$_{opt}$-rCedV) generating rCedV-GhV-GFP (**Fig. XB**). All cloning is performed in *E. coli* Stbl2 cells and plasmids verified by sequencing. Rescue is by transfection of BSR-T7/5 cells (12-well plates) with CedV helper plasmids, pCMV-CedV-N, pCMV-CedV-P, pCMV-CedV-L and pOLTV5$_{opt}$-rCedV-GhV-GFP antigenome plasmid. Typically, after 4 days, cells will be observed for GFP expression and syncytia (henipavirus CPE). BSR-T7/5 cells and supernatants from successful rescue wells are collected and passaged onto naïve Vero 76 cells (T-75 flasks) to prepare a virus stock. At maximal syncytia and GFP (~2–3 days), supernatants are collected and clarified and stored as single-use aliquots at -80°C. Virus stocks will be characterized and deep sequenced. Working virus stocks are prepared by culture amplification and titered by plaque assay and stored as single use aliquots at -80°C (PMCID: PMC8631057, PMCID: PMC5869790, PMCID: PMC1563797).

Characterization of the recombinant reporter viruses in cell-based infection assays: The replication kinetics of the VSV-based and rCedV-based GhV F/G encoding viruses will be assayed by Vero cell culture infection using MOIs of 0.01, 0.1 and 1, and progeny virus recovered in several 24hr intervals and tittered. VSV-GhV F/G virus replication can be compared to expected virus production of wildtype VSV or VSV-ABLV G (PMID: 23849788). Similarly, rCedV-GhV-GFP virus replication can be compared to rCedV or rCedV NiV/HeV chimeras. Unlike NiV and HeV, GhV G engages only the ephrin-B2 ligand to trigger membrane fusion (PMID: 24296468). The GhV F/G bearing reporter viruses will be verified for this specific ephrin ligand tropism in cell infection and/or syncytia formation experiments and comparisons made to wildtype VSV or VSV-ABLV G and also alternatively to rCedV, and rCedV NiV/HeV chimeras. We have generated murine mAbs to GhV F and G (10C4-LY and 10D5-LY to GhV G; and 5B7-LY and 7C11-LY to GhV F) and these will be used in neutralization tests. The VSV-GhV-GFP assays will be conducted by FRNTs and VSV-GhV-Luc in luciferase assays.

Evaluating neutralization potential of vaccine platforms from other research projects. Our rCeV chimeras (rCedV-NiV-B-GFP and rCedV-HeV-GFP) will allow this Core to conduct large numbers of neutralization tests by FRNT, to the many ML-derived prototype henipavirus vaccine antigen constructs, mAbs, and nanobinders, that will be generated by the Center's RPs. In Center years 1-3 the focus will be on HeV and NiV prototypes, and in years 4-5 the Core is expected to shift to GhV test case. The FRNT assay is rapid (36hrs), high throughput, and performed in 96-well plates to establish typical 7-point dose response virus neutralization data and performed at BSL-2. Typically, immune sera, mAbs, or other entry inhibitors are analyzed by serially dilution and mixed with virus (rCedV-NiV-B-GFP or rCedV-HeV-GFP) for 2hrs then samples used in triplicate to infect confluent Vero 76 cells in black-walled clear bottom 96-well plates and incubated 24hrs, and then plates are fixed and imaged and fluorescent foci quantitated with CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) is determined by 50% reduction in fluorescent foci versus untreated controls.

Anticipated Results, Potential Pitfalls and Alternative Strategies. We anticipate the reporter viruses will replicate to titers fit for purpose. Neutralization assays will typically be conducted as 3 independent repeats and the data generated from these assays will be analyzed and graphed using GraphPad Prism 9. If we encounter unexpected limitations in rescuing VSV-based or rCedV-based GhV F/G bearing reporter viruses; we will 1) test different ratios of helper plasmids and antigenome plasmids; 2) perform rescue using different cell lines; 3) construct alternate versions of the GhV F and G genes within the antigenome plasmids, such as truncating the GhV G protein which has been shown to enhance GhV mediated F/G membrane fusion (PMID: 31470664). Alternatively, fusion of the GhV F and G ectodomains with the transmembrane and cytoplasmic tail domains of the corresponding CedV F and G elements may facilitate virus particle formation and membrane fusion activity.

Aim 3: Provide technical expertise and conduct animal challenges, vaccinations, treatments, clinical pathology assays, virology assays, and necropsies in BSL-3 and BSL-4 containment.

The Core D Leader and Co-Leaders will work closely with the Project Leaders and key staff of RP1 and RP2 to develop vaccination, treatment and sampling regimens, study protocols, and animal protocols that utilize countermeasures identified in RP1 and RP2. The Core Leader and Co-Leaders will write and obtain UTMB IACUC approval for all animal protocols performed in Core D. The animal models and clinical pathology, immunology, virology, and histopathology assays employed for Aim 3 are detailed below. All raw data as well as graphed or tabulated data generated by Core D will be handled and analyzed in coordination with the Core B.

Animal models: Core D will employ established animal models for HeV and LASV. While animal models also exist for SOSV, JUNV, RVFV, and SFTSV we will optimize these models to fill gaps and provide new tools as detailed in Aim 4 below. Mice, guinea pigs, hamsters, ferrets, and cats have been used as small animal models of HeV infection (refs). This project will employ Syrian golden hamsters as the small animal model of HeV disease because they are more suitable than other small animals for screening large numbers of vaccines and mAbs. Using hamsters will allow us to select optimal vaccine and mAb formulations and regimens prior to efficacy testing in NHPs. In 2010 Drs. Geisbert and Broder developed the first highly lethal NHP model for henipavirus infection (Nipah virus) using African green monkeys (AGM) (ref). They subsequently developed a lethal AGM model for HeV infection (ref). Henipavirus NHP models mirror human disease with animals showing severe respiratory pathology, neurological disease, and generalized vasculitis. For LASV, Core D will use established lethal outbred guinea pig models that cover several lineages of LASV (refs) as guinea pigs are the only immunocompetent small animal model for Lassa fever. Core D will also use established cynomolgus monkey models that cover several genetically distinct lineages of LASV (refs).

Animal vaccinations: All animals will be obtained from commercial vendors and allowed to acclimate prior to vaccination (~ 3-7 days for mice, hamsters, and guinea pigs and ~ 14 days for ferrets and NHPs). Mice, hamsters, and guinea pigs will be vaccinated by intramuscular (i.m.) or intraperitoneal (i.p.) injection of the vaccines provided by RP1. Ferrets and NHPs will be vaccinated by i.m. injection of the vaccines provided by RP1. All animals will be vaccinated in ABSL-2. Animals will be monitored daily for any reaction or adverse events caused by the vaccination. Blood will be collected for plasma, serum, and PBMC isolations before vaccination and at times after vaccination for assessment of the host immune response.

Animal challenges: Hamsters, ferrets, and NHPs will be challenged with infectious HeV or SOSV as previously described (refs). After appropriate acclimation, hamsters and ferrets will be exposed to HeV or SOSV by intranasal (i.n.) or i.p. administration of up to 1x10^6 PFU of virus. NHPs will be challenged with HeV by the intratracheal (i.t.) and/or i.n. routes using up to 1 x 10^6 PFU of virus. Mice, guinea pigs, and ferrets will be exposed to arenaviruses and phleboviruses by i.p. injection. NHPs will be exposed to LASV by i.m. injection and Junin virus by intravenous (i.v.) injection as previously described (refs). For all animals, blood will be collected with or without anticoagulants for plasma and serum isolations, respectively, at times before paramyxovirus, arenavirus, or phlebovirus challenge, on the day of challenge, and at various times after challenge as detailed in RP1 and RP2. Animals will be monitored for clinical signs daily. We will apply a scoring sheet approved by the UTMB IACUC depicted in the Vertebrate Animals section to assist in determining the time of euthanasia depending on clinical signs (e.g., respiratory distress, anorexia, activity, appearance, neurologic signs, etc.). Animals will be euthanized according to the 2013 AVMA Guidelines on Euthanasia. Any surviving animals will be euthanized on day 35-42 days after paramyxovirus, arenavirus, or phlebovirus challenge.

Clinical pathology assays: *Hematology on EDTA whole blood*: For NHP studies, we will measure the following parameters at time points before and after HeV, LASV, or JUNV challenge: total white blood cell counts (WBC), percent lymphocytes (LY), percent monocytes (MO), percent neutrophils (NEU), percent eosinophils (EOS), percent basophils (BAS), total lymphocyte counts (LY#), total monocyte counts (MO#), total neutrophil counts (NEU#), total eosinophil counts (EOS #), total basophil counts (BAS #), red blood cell counts (RBC), total hemoglobin (Hgb), hematocrit values (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), and mean platelet volume (MPV), and Plateletcrit (PCT). Values will be obtained from the Abaxis VetScan HM5 following manufacturer's directions.

Clinical chemistry on serum: For NHP studies, the following parameters will be measured at time points before and after HeV, LASV, and JUNV challenge: Albumin (ALB), amylase (AMY), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), glucose (GLU), total protein (TP), blood urea nitrogen (BUN), and creatinine (CRE), Creactive protein (CRP), Calcium (Ca2+), and uric acid (UA). Values will be obtained from the Abaxis Piccolo chemistry analyzer following manufacturer's directions.

Viral load assays: *PCR Assay:* To assess viral loads in whole blood and tissues, RNA will be extracted using a virus RNA isolation kit (Qiagen, Valencia, CA) followed by TaqMan PCR for the viral genome being studied. Qiagen buffers have been safety tested for inactivation of paramyxoviruses, arenaviruses, and phleboviruses. ~~The~~ PCR assays employed for HeV, SOSV, LASV, JUNV, RVFV, and SFTSV have been previously described (refs).

Plaque Assay: The plaque assays employed for paramyxoviruses, arenaviruses, and phleboviruses have previously been described (refs). Briefly, 10-fold dilutions (10-1 to 10-6) of serum, plasma, or whole blood samples are prepared in cell maintenance medium (DMEM/10%FBS/gentamicin) and adsorbed to 70-90% confluent monolayers of Vero E6 cells in 6-well plates, adding 0.2 mL/well. The plates are incubated at 37°C, 5% CO2 for 45 min, with periodical rocking every 10-15 min. The incubation period is followed by addition of 2 mL 1% agarose-containing medium (2X EBME/Hepes/10% FBS/gentamicin) to each well. After the agarose has solidified, the plates are returned to the C02 incubator and maintained for 2-14 days until plaques are visualized. At this time, 2 mL PBS containing 5% neutral red and 5% FBS is added to each well, the plates returned to the CO2 incubator and held overnight. The following day, the liquid stain overlay is removed from each well and plaques are counted.

Gross pathology and histopathology: A necropsy will be performed on all NHPs. We will examine the external surfaces of the body, all orifices, and the thoracic and peritoneal cavities and their contents. Core D will prepare a written report of the gross pathology findings of each NHP. The following NHP tissues will be collected at necropsy, fixed in 10% neutral-buffered formalin, embedded in paraffin, and stained by H&E: liver, spleen, lung, kidney, adrenal gland, pancreas, axillary lymph node, inguinal lymph node, mesenteric lymph node, duodenum, ileum, colon, brain (frontal cortex, cerebellum, brain stem), cervical spinal cord, urinary bladder, ovary or testis, prostate or uterus, and eye. A microscopic evaluation will be performed on H&E slides of all tissues collected from all NHPs by an ACVP board certified veterinary pathologist. ~~A written r~~Reports of the histologic findings of each tissue from each animal will be provided to ~~the Project Leader of~~ RP1 Leaders. In consultation with the ~~Project Leader of~~ RP1 Leaders, Core D may also perform immunohistochemical and/or special stains such as PTAH for fibrin as previously described (refs).

Quality systems management of all records and data collected from animal studies: Although the animal studies conducted by Core D will not be required to be performed under GLP compliance, UTMB will provide Quality Assurance that will achieve data quality and integrity when conducting animal experiments. Quality refers to whether the study was conducted in accordance with the protocol, standard operating procedures, and applicable standards of research. Integrity refers to assurance that the raw data and documentation are consistent with reported results. All animal study personnel will be trained by the UTMB Office of Regulated Nonclinical Studies (ORNcS). on Good Documentation Practices (GDP). All animal study data generated by Core D will be provided to Core B as detailed in the Core B Research Strategy.

Aim 4: Develop and/or optimize animal models for Sosuga virus, Junin virus Rift valley fever virus, and severe fever with thrombocytopenia syndrome virus.

For HeV and LASV Core D will employ animal models that have already been developed and are well established. However, for SOSV, JUNV, RVFV, SFTSV while animal models exist there are gaps that need to be addressed. Recently, it was shown that i.n. or i.p. infection of hamsters with SOSV resulted in viremia and mild clinical disease (ref) but there is no lethal model for SOSV. Core B will further explore the hamster model for SOSV by 1) using higher challenge doses of SOSV which is often required to produce lethal disease in other paramyxovirus hamster models (refs) or 2) serially passage of tissues in hamsters. Core D will also assess the pathogenic potential of ferrets for SOSV as they are often used as animal models for paramyxoviruses. For JUNV, serial adaptation is often required to produce lethal disease in outbred guinea pigs. We recently showed that for Ebolaviruses while serial adaptation is required to produce lethality in mice, hamsters, and guinea pigs, infection of ferrets with *Zaire ebolavirus*, *Sudan ebolavirus*, or *Bundibugyo ebolavirus* resulted in uniform lethality with wild type viruses and did not require adaptation (ref). Therefore, Core D will assess the pathogenic potential of JUNV in ferrets.

RVFV and SFTSV text needed here.

BIBLIOGRAPHY AND REFERENCES CITED

From: Broder, Christopher on behalf of Broder, Christopher <christopher.broder@usuhs.edu>
To: Geisbert, Thomas W.
Cc: Cross, Robert W.
Subject: Re: UWA Core Aim
Date: Friday, June 2, 2023 8:24:03 AM
Attachments: UWashington-Core D-Research Strategy-02Jun23.docx

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this looks done.

you are going to format this right?

On Thu, Jun 1, 2023 at 5:45 PM Geisbert, Thomas W. <twgeisbe@utmb.edu> wrote:

> This is looking good. I split out the Aims page (first document) as it is separate and we then have 6 pages for the Core text. I inserted the references. I think once you get those in that will save some space. Also, I went back through the documents from Alex Freiberg and they are not proposing any animal model development so I fixed that.
>
> ---
>
> **From:** Broder, Christopher <christopher.broder@usuhs.edu>
> **Sent:** Thursday, June 1, 2023 1:48 PM
> **To:** Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
> **Cc:** Cross, Robert W. <rwcross@UTMB.EDU>
> **Subject:** Re: UWA Core Aim
>
>
>
> guys
> i think the core is 6 pages plus a Specific Aims page (total 7)
> if im wrong then cut out fig 2
>
> its getting close. last section needed. insert your refs now, and
> it will get space.
> there are a lot of bits that can be wordsmithed out in the animal methods.
>
> if you make a near final formatted version with section added for RVFV and SFTSV
> send back and i will edit it down to fit
>
> On Thu, May 25, 2023 at 2:50 PM Broder, Christopher <christopher.broder@usuhs.edu> wrote:
>
> > i know,
> > best to have too much and cut to start off
> >
> > i am working back on Overall 12 pages
> >
> > On Thu, May 25, 2023 at 2:18 PM Geisbert, Thomas W. <twgeisbe@utmb.edu> wrote:

> Thanks Chris! We are going to have a lot of stuff to cram into 6 pages. I will start working on this but may do this over the weekend.
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> **From:** Broder, Christopher <christopher.broder@usuhs.edu>
> **Sent:** Thursday, May 25, 2023 1:01 PM
> **To:** Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Cross, Robert W. <rwcross@UTMB.EDU>
> **Subject:** UWA Core Aim
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> Tom, Bob
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> here is revised Aim for us, for the 6 page Core D for UWA

CENTER OVERVIEW: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction

Emerging and periodically reemerging pathogenic enveloped RNA viruses are ~~a~~ continuous ~~infectious disease and potentially~~ pandemic threats to global public health. Among the many groups of these types of pathogens are viral zoonoses within the family **Paramyxoviridae** and order ***Bunyavirales*** where humans can acquire infections directly from animal reservoir hosts such as rodents or bats~~,~~ through an intermediate amplifying host such as livestock, or from arthropod vectors such as mosquitos and ticks (Fields virology). More often than not, humans represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur, and should RNA viral pathogens mutate and adapt to more efficient replication and shedding in the human host, the epidemic potential of these viruses could manifest (PMID: 23911335 -Nipah / others?). The viral order *Bunyavirales* is composed of several viral families known to contain multiple high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic fever diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and are also identified as NIAID Category A pathogens due to their ease of dissemination and/or abilities to transmit person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special actions for public health preparedness. Also, within the WHO Priority Pathogens list is Nipah virus (NiV) and henipaviral disease which includes Hendra virus (HeV) which are zoonotic henipaviruses (HNVs) in the family **Paramyxoviridae**. NiV and HeV have long been considered the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease (PMID: 16357858). The genus now includes nine additional reported HNVs but only HeV and NiV are known to be associated with severe and often fatal henipaviral disease in humans and a number of animal species (PMID: 32991264). Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illnesses in humans [PMID: 35921459].

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Taken together, these viral threats to global public health are heightened due to the absence of approved vaccines or therapeutics. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for vaccine and monoclonal antibody (mAb) countermeasures in developing prototype candidates (**Table 1**) against a selection of these viruses. In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Table 1. PABVAX selected prototype and test case viral agents.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old world), Machupo (New world)	Lujo (Old world), Chapare (New world)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

The *Bunyavirales*.

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The henipavirus paramyxoviruses. The recognized, highly pathogenic HNVs, NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality (Murray, PMID: 7701348). NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts (Chua, PMID: 10827955). NiV and HeV

can infect at least 18 animal species across 6 orders of mammals, and among these they will cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans (PMID: 32991264; PMID: 22476556). The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission (PMID: 23911335). Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and South East Asia and Australia. Their broad host tropism and ability of these viruses to infect livestock offers an amplification opportunity and increased risk of subsequent human infections. The henipaviruses are single-stranded, negative sense, enveloped RNA viruses and their biology also lends themselves to be synthetically produced and agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents [Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; Department of Health and Human Services (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087]. because they could be engineered for mass dissemination and possess the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the WHO R&D Blueprint list of priority pathogens with epidemic potential (Sweileh, W. M. PMC5299748).

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb, postexposure treatments have been demonstrated to be effective therapies against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP) (PMID: 22013123, PMID: 24964990, PMID: 27484128, PMID: 31686101). The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia (PMID: 32027842). In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs and several vaccine platforms have clear potential for use in people (PMC8782152). The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies (PMC8782152). Such preventive vaccines would have utility for lab workers, first responders or individuals at high risk exposure and in mitigating the spread of an outbreak; whereas in the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent (Middleton, 2014 PMID: 24572697). It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection (Geisbert, 2012;Geisbert, 2010). The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished) (PMID: 33809833). The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons; 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Also, another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including the rescue of recombinant LayV (rLayV), explore animal model development, construct additional LayV and AngV reporter virus tools, and then translate and test the MNP

vaccine ‘plug-and-play’ approach, and also evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME

A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center’s RPs and Core leaders currently have active and productive collaborative research programs in related areas, some spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

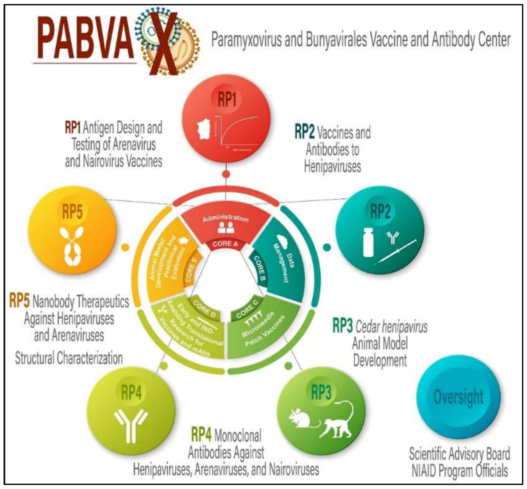

Figure 1: Organizational chart of the Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center-(PABVAX). **(connecting arrows are needed between elements and the SAB)**

The organizational structure of the PABVAX is diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **Arenaviridae** and **Nairoviridae** viruses, and **RP2** will target the prototype **henipaviruses** (HNVs). Both RP1 and RP2 will also be developing new prototype virus tools (recombinantly generated prototype viruses) along with existing tools, to be used in the analysis of vaccine responses as well as in the evaluation of new mAbs and nabs from the other RPs. A third RP will be engaged in the development of a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant Cedar henipavirus (rCedV) in vivo and a bioluminescence methodology that can longitudinally trace the dynamics and anatomical progression of luciferase encoding virus infection in individual animals (**RP3**). There will also be two RPs that will target the development of human mAbs and nanobodies to the PABVAX select viral prototypes. **RP4** will isolate human mAbs derived from NiV infected survivors with a specific focus on the F glycoprotein targeted mAbs, and additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses along with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will be engaged in manufacture and quality control & testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform.
The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from

the UPitt and IBBR/UMD (**Core C**) that has tested a dissolvable MNP-based vaccine technology that is ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine approach (PMID: 32924651). This vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable MNPs that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants (PMID: 19864632) to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as facilitate the improved vaccine storage and distribution due to their thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, a Scientific **Core C**: (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ) adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (Paramyxoviridae). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity in vitro and polyfunctional T-cell responses by in vitro stimulation of isolated cells with antigenic peptides, intracellular cytokine staining (ICS) and flow cytometry) responses.

Discover effective antibody-based prophylactics and therapies.
~~PREP can be critical in the absence of available vaccines or cases in which vaccination is not possible.~~
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Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah [1], Hendra [2], Ebola [3, 4], Marburg [5] and Lassa [6]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc*.), a feature to be exploited in **Core D**. In addition to therapeutic applications, mAbs with Fc mutations to dramatically extend serum half-life [18][19, 20] offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g.* rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

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Develop new tools and animal models to facilitate the translation of effective countermeasures.
Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the A~~A~~nimal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation S~~s~~cientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including ~~……~~ animal model development and the use of animal models for the study of viral pathogenesis and assessing the protective ~~therapeutic~~ efficacy of antivirals and vaccines as well as studies to define mechanisms and/or correlates of protection. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation; including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera

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or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they can be adapted to greater high-throughput platforms, are often less laborious, and they can make use of other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput florescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic Nipah and Hendra virus vaccine induced immune sera, mAbs (**RP4**) and nbs (**RP5**) and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either the HeV or NiV-B envelope glycoproteins) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals, ideally suited to explore nab based therapeutics to CNS resident henipavirus (PMID: 35821488).

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. ~~Data generated to support of possible future regulatory submissions to the Food and Drug Administration (FDA) must be collected in accordance with quality systems, like good laboratory practice (GLP) regulations, to be useful and reliable, and to facilitate FDA review to ensure safety and efficacy.~~ The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the henipaviruses (Paramyxoviridae) and the Bunyavirales (Arenaviridae and Nairoviridae) that will bolster vaccine endurance to storage and transportation; and also improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology and mAb and vaccine translational drug development and includes Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Henipaviruses: Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals and are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of Cedar virus (CedV), Gamak virus, Daeryong virus, and Langya virus (LayV) [xxx] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV) [xxx]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV,

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Lee, S.H.; Kim, K.; Viruses 2021, 13, doi:10.3390/v13102020.

Zhang, X.A.; Li, H.; Jiang, N Engl J Med 2022, 387, 470-472, doi:10.1056/NEJMc2202705.

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

are old world pteropid fruit bats. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province). The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**.

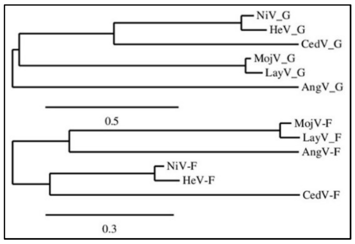

Figure 2. Phylogenetic analysis of select HNV G and F glycoproteins. Phylogenetic trees were constructed for the HNV F and G glycoproteins using the PhyML program's maximum likelihood method. The trees were generated by aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal henipaviral disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters (Geisbert 2012), and African green monkeys (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P), and the P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists (xx). The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2 (XXX) and CedV is now recognized as a BSL-2 restricted agent [CDC manual]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D, and E**).

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Laing, E.D.; Navaratnarajah, C.K.; Proc Natl Acad Sci U S A 2019, doi:10.1073/pnas.1911773116.

Commented [CB8]: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. HHS Publication No. (CDC) 300859. US Government Printing Office: Washington, 2020; pp. 248-250.

Arenaviridae* and *Nairoviridae:

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs; such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**), (aarenavirus and nairovirus~~renaviridae and nairoviridae~~ projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine the active and passive immunization approaches to further improved the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

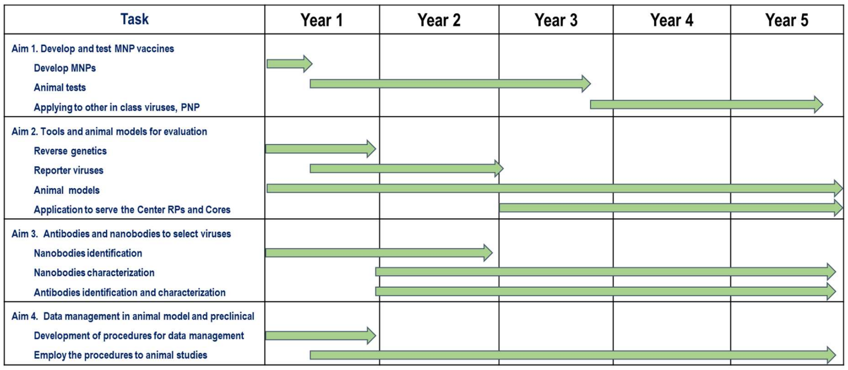
An additional and outstanding advantage of the PABVAX Center is the **Animal M~~m~~odel D~~d~~evelopment and P~~p~~reclinical E~~e~~valuation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL. (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for all the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.**
2. **Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.**
3. **Develop antibodies and nanobodies to selected prototype viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.



Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. This is in addition to each PR's individual Milestone plan. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (Details are within the Administration **Core A**). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommend new directions as appropriate and provide Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

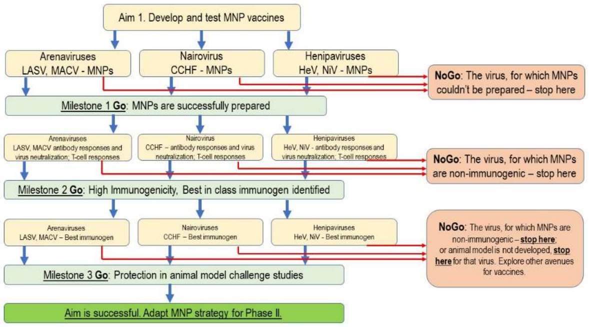


Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will be whether proper formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be made based on immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction and virus-neutralization and polyfunctional T-cell responses by in vitro stimulation of isolated cells). Go/no go decisions will be made based on the outcomes of these first tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as success. Comparisons will also be made, such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of best-in-class immunogens, or best combination immunogens which will also be explored, will be performed in order to down-select vaccine choice. The third milestone will be the determination of whether MNP vaccination affords protection in animal model challenge experiments. In the case of the NiV and HeV prototypes the animal models chosen (ferret and the African green monkey (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales* there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASA and there are guinea pig models for some but not all of those lineages. For MACV there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys are now each greater than 35K. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of the AGM for the arenaviruses and possibly also for CCHF as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.

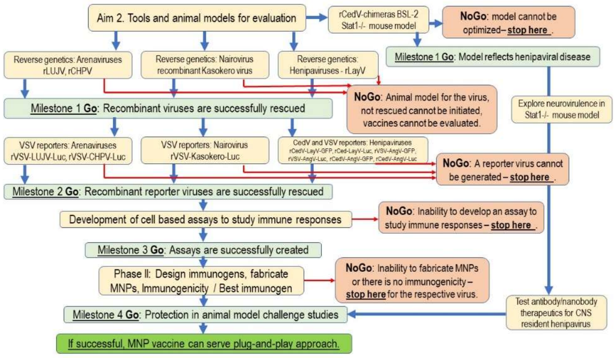


Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center overall objectives.

The PABVAX second overall objective is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined in vivo (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, a a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**) the milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new in vivo bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease in vivo safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**) it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and RP3 will collaborate with RP5 in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

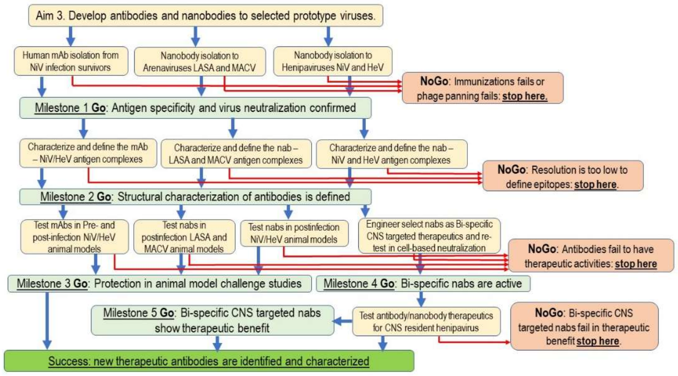


Figure 6. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

New human mAbs and nanobodies (nbs) as tools and potential therapeutics will be developed by RP4 and RP5 in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); and transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on the mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs and in collaboration with **RP2** neutralization potencies will be rapidly assessed as well as evaluating synergistic cell-based mAb neutralization that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

Nanobodies, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nanobodies can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nanobodies targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), and also for therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus) and select animal models of LASV and MACV by **Core E**. Success will be measured by the identification of virus-neutralizing nbs, potential discovery of more broadly reactive nbs, particularly to the multiple LASA clades, the evidence of therapeutic benefit in animal models, and the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

INDUSTRY PARTNERSHIPS

Industry Partnership

Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine

expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX.

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SPECIAL FEATURES OF THE PABVAX CENTER ENVIRONMENT AND OTHER RESOURCES ??

Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

Galveston National Laboratory (GNL)

The GNL, is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics and therapeutics against them. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 370~~187~~ separate NHP studies employing a total of 2,772~~1,318~~ animals and 10 different BSL-4 viruses including arenaviruses, nairoviruses, and henipaviruses ~~Ebola and Marburg viruses and also Nipah and Hendra~~ have been successfully performed at the GNL. These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder and Zeitlin. The GNL has

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a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited NHP~~nonhuman primate~~ facility and a staff of full-time veterinarians. In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained ACVP board-certified veterinary pathologist. As noted above another special feature is the location of WRCEVA virus collection within the GNL.

Core E (Animal model development and preclinical evaluation)

Core E operations will be conducted at the GNL, and in conjunction with **Core A** also includes the UTMB Institutional Office of Regulated Nonclinical Studies (ONcRS). The ONcRS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies with good documentation, ~~to meet regulatory requirements by assuring regulatory compliance,~~ quality assurance, and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a “well documented” quality system program. ~~Quality system and regulatory science processes for NHP studies performed at UTMB will be implemented and executed in conjunction with the ORNcS.~~ Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at the US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses and the henipaviruses and he has published over 250~~XXX~~ peer-reviewed articles in these areas. Dr. Geisbert’s research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI) was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

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Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs)

Mapp Biopharmaceutical has deep experience with antigens and monoclonal antibodies (mAbs) against these antigens. ~~’s product development team has grown significantly in the last two years in response to the development efforts on its anti-Ebola therapeutic, ZMapp™ which is being advanced to licensure as part of Project Bioshield. OTHER MAbs~~ Their early development and research group has expertise in immunology, infectious diseases, and production of antigens, antibodies and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies {Mulangu, 2019 #818} {Group, 2016 #649}; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1 {Politch, 2021 #897}. ~~developed multiple products, including a licensed biodefense biologic. As with Profectus, Mapp Bio’s expertise and experience will be a very important and valuable asset to the Center.~~

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Core A (Administrative Core)

Research Project teams?? Maybe not depending on space

The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills and his experience in translational research activities qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe’s (**RP4**) research focuses on the molecular basis for the development of effective B cell responses to viruses in humans and he has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of

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human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently henipaviruses NiV and HeV owing to the collaboration between the Crowe, Broder and Geisbert laboratories. Perhaps of greatest import, the SARS-CoV-2 mAbs generated by Dr. Crowe's laboratory were used in xxx,000 of people during the COVID-19 pandemic. RP4 will work closely with Cores D and E in advancing mAb prototypes. ~~The Core B of the Center will work together with RP2 and RP3 along with the Core C to develop the first cocktail human mAb immunotherapeutics, and facilitate the transition products suitable for IND filing and human testing including design, construction, assessment, and optimization of these antibody-based therapies.~~

As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

CENTER OVERVIEW: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction

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Emerging and periodically reemerging pathogenic enveloped RNA viruses are ~~a~~ continuous ~~infectious disease and potentially~~ pandemic threats to global public health. Among the many groups of these types of pathogens are viral zoonoses within the family **Paramyxoviridae** and order ***Bunyavirales*** where humans can acquire infections directly from animal reservoir hosts such as rodents or bats~~,~~ through an intermediate amplifying host such as livestock, or from arthropod vectors such as mosquitos and ticks (Fields virology). More often than not, humans represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur, and should RNA viral pathogens mutate and adapt to more efficient replication and shedding in the human host, the epidemic potential of these viruses could manifest (PMID: 23911335 -Nipah / others?). The viral order *Bunyavirales* is composed of several viral families known to contain multiple high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic fever diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and are also identified as NIAID Category A pathogens due to their ease of dissemination and/or abilities to transmit person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special actions for public health preparedness. Also, within the WHO Priority Pathogens list is Nipah virus (NiV) and henipaviral disease which includes Hendra virus (HeV) which are zoonotic henipaviruses (HNVs) in the family **Paramyxoviridae**. NiV and HeV have long been considered the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease (PMID: 16357858). The genus now includes nine additional reported HNVs but only HeV and NiV are known to be associated with severe and often fatal henipaviral disease in humans and a number of animal species (PMID: 32991264). Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illnesses in humans [PMID: 35921459].

Taken together, these viral threats to global public health are heightened due to the absence of approved vaccines or therapeutics. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for vaccine and monoclonal antibody (mAb) countermeasures in developing prototype candidates (**Table 1**) against a selection of these viruses. In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Table 1. PABVAX selected prototype and test case viral agents.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old world), Machupo (New world)	Lujo (Old world), Chapare (New world)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

The *Bunyavirales*.

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The henipavirus paramyxoviruses. The recognized, highly pathogenic HNVs, NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality (Murray, PMID: 7701348). NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts (Chua, PMID: 10827955). NiV and HeV

can infect at least 18 animal species across 6 orders of mammals, and among these they will cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans (PMID: 32991264; PMID: 22476556). The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission (PMID: 23911335). Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and South East Asia and Australia. Their broad host tropism and ability of these viruses to infect livestock offers an amplification opportunity and increased risk of subsequent human infections. The henipaviruses are single-stranded, negative sense, enveloped RNA viruses and their biology also lends themselves to be synthetically produced and agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents [Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; Department of Health and Human Services (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087]. because they could be engineered for mass dissemination and possess the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the WHO R&D Blueprint list of priority pathogens with epidemic potential (Sweileh, W. M. PMC5299748).

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb, postexposure treatments have been demonstrated to be effective therapies against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP) (PMID: 22013123, PMID: 24964990, PMID: 27484128, PMID: 31686101). The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia (PMID: 32027842). In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs and several vaccine platforms have clear potential for use in people (PMC8782152). The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies (PMC8782152). Such preventive vaccines would have utility for lab workers, first responders or individuals at high risk exposure and in mitigating the spread of an outbreak; whereas in the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent (Middleton, 2014 PMID: 24572697). It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection (Geisbert, 2012;Geisbert, 2010). The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished) (PMID: 33809833). The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons; 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Also, another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including the rescue of recombinant LayV (rLayV), explore animal model development, construct additional LayV and AngV reporter virus tools, and then translate and test the MNP

vaccine ‘plug-and-play’ approach, and also evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME

A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), and Mapp Biopharmaceutical. In addition, nearly all of the Center’s RPs and Core leaders currently have active and productive collaborative research programs in related areas, some spanning more than a decade. Further, the PABVAX Industrial Partners, Emergent Biosolutions and Moderna, bring unique and valuable vaccine development expertise to the team.

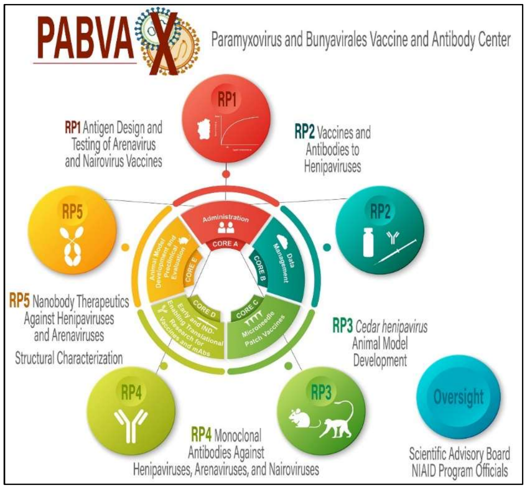

Figure 1: Organizational chart of the Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center- (PABVAX). **(connecting arrows are needed between elements and the SAB)**

The organizational structure of the PABVAX is diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **Arenaviridae** and **Nairoviridae** viruses, and **RP2** will target the prototype **henipaviruses** (HNVs). Both RP1 and RP2 will also be developing new prototype virus tools (recombinantly generated prototype viruses) along with existing tools, to be used in the analysis of vaccine responses as well as in the evaluation of new mAbs and nabs from the other RPs. A third RP will be engaged in the development of a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant Cedar henipavirus (rCedV) in vivo and a bioluminescence methodology that can longitudinally trace the dynamics and anatomical progression of luciferase encoding virus infection in individual animals (**RP3**). There will also be two RPs that will target the development of human mAbs and nanobodies to the PABVAX select viral prototypes. **RP4** will isolate human mAbs derived from NiV infected survivors with a specific focus on the F glycoprotein targeted mAbs, and additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses along with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will be engaged in manufacture and quality control & testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**).

With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:

1) Establish an effective and rapidly adaptable vaccination technology.

2) Discover effective antibody-based therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

An adaptable plug-and-play vaccine platform.
The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from

the UPitt and IBBR/UMD (**Core C**) that has tested a dissolvable MNP-based vaccine technology that is ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine approach (PMID: 32924651). This vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable MNPs that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants (PMID: 19864632) to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as facilitate the improved vaccine storage and distribution due to their thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, a Scientific **Core C**: (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ) adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (Paramyxoviridae). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity in vitro and polyfunctional T-cell responses by in vitro stimulation of isolated cells with antigenic peptides, intracellular cytokine staining (ICS) and flow cytometry) responses.

Discover effective antibody-based prophylactics and therapies.
~~PREP can be critical in the absence of available vaccines or cases in which vaccination is not possible.~~
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Monoclonal antibodies (mAbs) are one of the most successful classes of drugs with the proven ability to address a variety of human health needs including oncology, inflammatory diseases, and infectious diseases. Recent studies have demonstrated therapeutic efficacy of mAbs against some of the most lethal viruses including Nipah [1], Hendra [2], Ebola [3, 4], Marburg [5] and Lassa [6]. MAbs also offer a stunning degree of customization based on the importance of mechanisms of action (such as viral neutralization, ADCC, ADCP, *etc*.), a feature to be exploited in **Core D**. In addition to therapeutic applications, mAbs with Fc mutations to dramatically extend serum half-life [18][19, 20] offer an important tool for prophylactic use for indications in which vaccination is not possible: 1) for diseases where no vaccine is available; 2) in immunocompromised patients; and 3) in populations where the time required by a vaccine for protective immunity to be established by the host is not practical (*e.g.* rapid response personnel). Members of PABVAX have extensive experience with the discovery, characterization and translational activities necessary to advance mAbs to the clinic. The manufacturing platforms, formulation methods, and safety profiles of mAbs are well-established. Thus, mAbs offer a low-risk technology platform for prophylactics and therapeutics targeting the viral threats to be addressed by PABVAX.

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Develop new tools and animal models to facilitate the translation of effective countermeasures.
Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal model development and preclinical evaluation scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including…….. animal model development and use of animal models for the study of viral pathogenesis and assessing the therapeutic efficacy of antivirals and vaccines. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation; including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and

alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they can be adapted to greater high-throughput platforms, are often less laborious, and they can make use of other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput florescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic Nipah and Hendra virus vaccine induced immune sera, mAbs (**RP4**) and nbs (**RP5**) and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either the HeV or NiV-B envelope glycoproteins) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals, ideally suited to explore nab based therapeutics to CNS resident henipavirus (PMID: 35821488).

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. Data generated to support ~~of~~ possible future regulatory submissions to the Food and Drug Administration (FDA) must be collected in accordance with quality systems, like good laboratory practice (GLP) regulations, to be useful and reliable, and to facilitate FDA review to ensure safety and efficacy. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the henipaviruses (Paramyxoviridae) and the Bunyavirales (Arenaviridae and Nairoviridae) that will bolster vaccine endurance to storage and transportation; and also improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology and mAb and vaccine translational drug development and includes Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Henipaviruses: Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals and are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of Cedar virus (CedV), Gamak virus, Daeryong virus, and Langya virus (LayV) [xxx] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV) [xxx]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV, are old world pteropid fruit bats. Whereas the six other HNVs are, or are likely, of rodent origins, including the

Commented [CB4]: Marsh, G.A.; PLoS pathogens 2012, 8, e1002836, doi:10.1371/journal.ppat.1002836.

Lee, S.H.; Kim, K.; Viruses 2021, 13, doi:10.3390/v13102020.

Zhang, X.A.; Li, H.; Jiang, N Engl J Med 2022, 387, 470-472, doi:10.1056/NEJMc2202705.

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province). The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**.

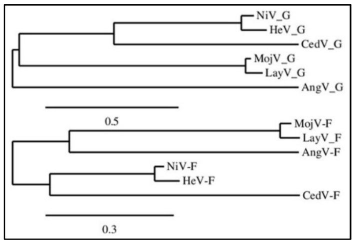

Figure 2. Phylogenetic analysis of select HNV G and F glycoproteins. Phylogenetic trees were constructed for the HNV F and G glycoproteins using the PhyML program's maximum likelihood method. The trees were generated by aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal henipaviral disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease including guinea pigs, ferrets, hamsters (Geisbert 2012), and African green monkeys (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P), and the P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists (xx). The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2 (XXX) and CedV is now recognized as a BSL-2 restricted agent [CDC manual]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D, and E**).

Commented [CB6]: Marsh, G.A.; Wang, L.F. Curr Opin Virol 2012, 2, 242-247, doi:10.1016/j.coviro.2012.03.006.

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Laing, E.D.; Navaratnarajah, C.K.; Proc Natl Acad Sci U S A 2019, doi:10.1073/pnas.1911773116.

Commented [CB8]: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. HHS Publication No. (CDC) 300859. US Government Printing Office: Washington, 2020; pp. 248-250.

Arenaviridae* and *Nairoviridae:

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs; such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**), (arenaviridae and nairoviridae projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine the active and passive immunization approaches to further improved the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

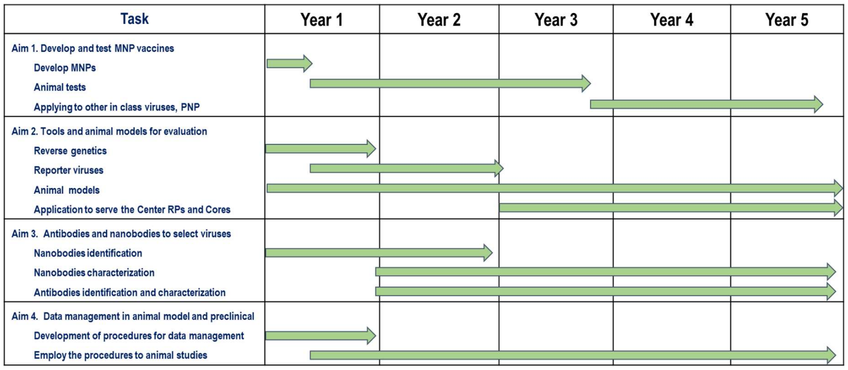
An additional and outstanding advantage of the PABVAX Center is the **Animal model development and preclinical evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL. (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for all the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.**
2. **Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.**
3. **Develop antibodies and nanobodies to selected prototype viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.



Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. This is in addition to each PR's individual Milestone plan. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (Details are within the Administration **Core A**). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommend new directions as appropriate and provide Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

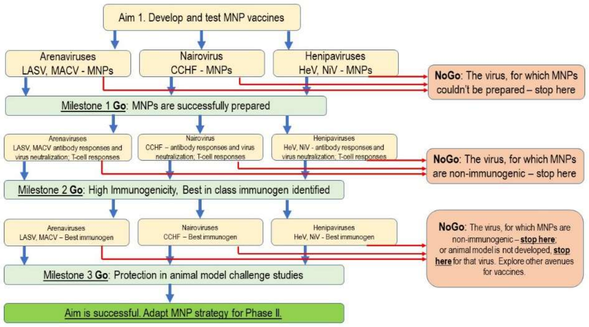


Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will be whether proper formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be made based on immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction and virus-neutralization and polyfunctional T-cell responses by in vitro stimulation of isolated cells). Go/no go decisions will be made based on the outcomes of these first tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as success. Comparisons will also be made, such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of best-in-class immunogens, or best combination immunogens which will also be explored, will be performed in order to down-select vaccine choice. The third milestone will be the determination of whether MNP vaccination affords protection in animal model challenge experiments. In the case of the NiV and HeV prototypes the animal models chosen (ferret and the African green monkey (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales* there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASA and there are guinea pig models for some but not all of those lineages. For MACV there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys are now each greater than 35K. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of the AGM for the arenaviruses and possibly also for CCHF as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.

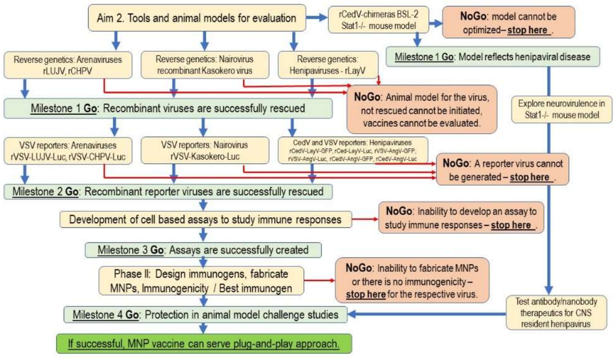


Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center overall objectives.

The PABVAX second overall objective is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined in vivo (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, a a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**) the milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new in vivo bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease in vivo safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**) it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and RP3 will collaborate with RP5 in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

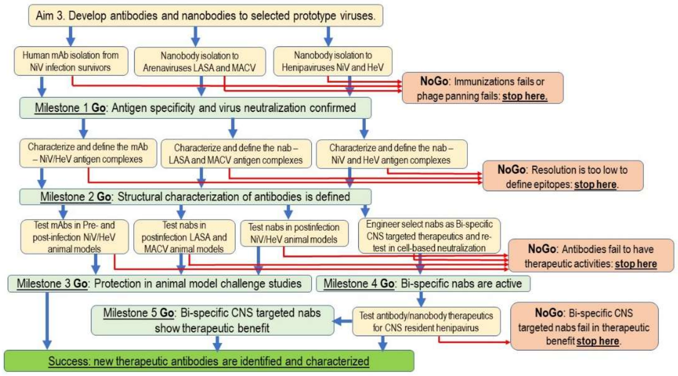


Figure 6. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

New human mAbs and nanobodies (nbs) as tools and potential therapeutics will be developed by RP4 and RP5 in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); and transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on the mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs and in collaboration with **RP2** neutralization potencies will be rapidly assessed as well as evaluating synergistic cell-based mAb neutralization that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

Nanobodies, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nanobodies can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nanobodies targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), and also for therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus) and select animal models of LASV and MACV by **Core E**. Success will be measured by the identification of virus-neutralizing nbs, potential discovery of more broadly reactive nbs, particularly to the multiple LASA clades, the evidence of therapeutic benefit in animal models, and the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

INDUSTRY PARTNERSHIPS

Industry Partnership

Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine

expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.

PABVAX has partnered with Emergent Biosolutions (Rockville, MD) and Moderna (Cambridge, MA). Emergent and Moderna have extensive experience developing medical countermeasures that protect against public health threats, including development and approval of multiple vaccine and antibody products for infectious diseases. They can contribute regulatory, manufacturing, and commercial experience for the prototypes and make an ideal partner for PABVAX.

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SPECIAL FEATURES OF THE PABVAX CENTER ENVIRONMENT AND OTHER RESOURCES ??

Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

Galveston National Laboratory (GNL)

The GNL, is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics and therapeutics against them. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 187 separate NHP studies employing a total of 1,318 animals and 10 different BSL-4 viruses including Ebola and Marburg viruses and also Nipah and Hendra have been successfully performed at the GNL. These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited nonhuman primate facility and a staff of full-time veterinarians. In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained board-certified veterinary pathologist. As noted above another special feature is the location of WRCEVA virus collection within the GNL.

Core E (Animal model development and preclinical evaluation)

Core E operations will be conducted at the GNL, and also includes the UTMB Institutional Office of Regulated Nonclinical Studies (ONcRS). The ONcRS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies to meet regulatory requirements by assuring regulatory compliance, quality assurance and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a “well documented” quality system program. Quality system and regulatory science processes for NHP studies performed at UTMB will be implemented and executed in conjunction with the ORNcS. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses and the henipaviruses and he has published over XXX peer-reviewed articles in these areas. Dr. Geisbert’s research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI) was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs)

Mapp Biopharmaceutical has deep experience with antigens and monoclonal antibodies (mAbs) against these antigens. ~~’s product development team has grown significantly in the last two years in response to the development efforts on its anti-Ebola therapeutic, ZMapp™ which is being advanced to licensure as part of Project Bioshield. OTHER MAbs~~ Their early development and research group has expertise in immunology, infectious diseases, and production of antigens, antibodies and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has advanced multiple mAb discoveries to the clinic, including: ZMapp, a 3 mAb cocktail for Ebola Zaire virus, tested in two Phase 3 studies {Mulangu, 2019 #818} {Group, 2016 #649}; MBP091, a pan-*Marburgvirus* therapeutic that has completed Phase 1 safety testing; MBP134, a pan-*Ebolavirus* therapeutic that has completed Phase 1; and MB66, a vaginal microbicide film containing anti-HIV and anti-HSV mAbs that has completed Phase 1 {Politch, 2021 #897}. ~~developed multiple products, including a licensed biodefense biologic. As with Profectus, Mapp Bio’s expertise and experience will be a very important and valuable asset to the Center.~~

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Core A (Administrative Core)

Research Project teams?? Maybe not depending on space

The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills and his experience in translational research activities qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe’s **(RP4)** research focuses on the molecular basis for the development of effective B cell responses to viruses in humans and he has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of

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human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently henipaviruses NiV and HeV owing to the collaboration between the Crowe, Broder and Geisbert laboratories. Perhaps of greatest import, the SARS-CoV-2 mAbs generated by Dr. Crowe's laboratory were used in xxx,000 of people during the COVID-19 pandemic. RP4 will work closely with Cores D and E in advancing mAb prototypes. ~~The Core B of the Center will work together with RP2 and RP3 along with the Core C to develop the first cocktail human mAb immunotherapeutics, and facilitate the transition products suitable for IND filing and human testing including design, construction, assessment, and optimization of these antibody-based therapies.~~

As per NIAID's expectations described in the RFA, PABVAX intends to provide lead candidates for comparative studies using NIAID's preclinical services or other mechanisms. The Center PIs, the Scientific Advisory Committee and NIAID staff will select prototype candidates at the appropriate development stage for these studies. Members of PABVAX have over 10 years of experience working with NIAID's preclinical services for *in vitro* anti-viral testing, *in vivo* animal model testing (RSV, filoviruses), and IND-enabling GLP studies. Additionally, the team has experience collaborating with DoD and BARDA to advance candidate countermeasures to the clinic.

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BIBLIOGRAPHY AND REFERENCES CITED

CENTER OVERVIEW: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)

RESEARCH STRATEGY

Introduction

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Emerging and periodically reemerging pathogenic enveloped RNA viruses are a continuous infectious disease and potentially pandemic threats to global public health. Among the many groups of these types of pathogens are viral zoonoses within the family **Paramyxoviridae** and order ***Bunyavirales*** where humans can acquire infections directly from animal reservoir hosts such as rodents or bats, through an intermediate amplifying host such as livestock, or from arthropod vectors such as mosquitos and ticks (Fields virology). More often than not, humans represent a dead-end host infection, but for some viral agents several rounds of human-to-human transmission can occur, and should RNA viral pathogens mutate and adapt to more efficient replication and shedding in the human host, the epidemic potential of these viruses could manifest (PMID: 23911335 -Nipah / others?). The viral order *Bunyavirales* is composed of several viral families known to contain multiple high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic fever diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and are also identified as NIAID Category A pathogens due to their ease of dissemination and/or abilities to transmit person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special actions for public health preparedness. Also, within the WHO Priority Pathogens list is Nipah virus (NiV) and henipaviral disease which includes Hendra virus (HeV) which are zoonotic henipaviruses (HNVs) in the family **Paramyxoviridae**. NiV and HeV have long been considered the prototype members of the genus *Henipavirus* and have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease (PMID: 16357858). The genus now includes nine additional reported HNVs but only HeV and NiV are known to be associated with severe and often fatal henipaviral disease in humans and a number of animal species (PMID: 32991264). Langya virus (LayV) is the most recently identified HNV and was associated with nonfatal febrile illnesses in humans [PMID: 35921459].

Taken together, these viral threats to global public health are heightened due to the absence of approved vaccines or therapeutics. *There is a clear unmet need for countermeasures such as vaccines and antibody-based therapeutics to address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these viral agents*. The PABVAX Center will focus on elucidating key principles for vaccine and monoclonal antibody (mAb) countermeasures in developing prototype candidates (**Table 1**) against a selection of these viruses. In the out years of the Center effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Table 1. PABVAX selected prototype and test case viral agents.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old world), Machupo (New world)	Lujo (Old world), Chapare (New world)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

The *Bunyavirales*.

Fill out

The henipavirus paramyxoviruses. The recognized, highly pathogenic HNVs, NiV and HeV are zoonotic agents distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV was recognized first, and emerged in Queensland, Australia in 1994 causing a severe and fatal respiratory disease among horses along with a human case fatality (Murray, PMID: 7701348). NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts (Chua, PMID: 10827955). NiV and HeV

can infect at least 18 animal species across 6 orders of mammals, and among these they will cause a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans (PMID: 32991264; PMID: 22476556). The NiV strain in Bangladesh (NiV-B) is of particular concern where outbreaks occur nearly on an annual occurrence and often with case fatality rates exceeding 90% with little evidence of subclinical infections, and well-documented person-to-person transmission (PMID: 23911335). Currently, there are no vaccines or therapeutics approved for human use. NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to public health and economically important livestock throughout South and South East Asia and Australia. Their broad host tropism and ability of these viruses to infect livestock offers an amplification opportunity and increased risk of subsequent human infections. The henipaviruses are single-stranded, negative sense, enveloped RNA viruses and their biology also lends themselves to be synthetically produced and agents of potential and deliberate misuse. NiV and HeV are BSL-4 pathogens and also classified as priority agents by US Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC) has recently proposed that NiV be added to the list of Tier 1 Select Agents [Possession, Use, and Transfer of Select Agents and Toxins; Biennial Review; Department of Health and Human Services (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087]. because they could be engineered for mass dissemination and possess the potential for high morbidity and mortality rates and major public health impacts. Recently, NiV was selected by the WHO as an epidemic threat needing urgent research and development (R&D) action and has been included in the WHO R&D Blueprint list of priority pathogens with epidemic potential (Sweileh, W. M. PMC5299748).

While there are no licensed countermeasures for the treatment of NiV and HeV infections, there has been notable progress over the 15 years in the development of vaccines and postexposure human or humanized monoclonal antibody (mAb) therapies. Among antiviral countermeasures likely suitable for use in people, to date, one human monoclonal antibody (mAb) and one humanized mAb, postexposure treatments have been demonstrated to be effective therapies against NiV and HeV infection in animal challenge studies including nonhuman primates (NHP) (PMID: 22013123, PMID: 24964990, PMID: 27484128, PMID: 31686101). The success of one particular human mAb (m102.4) in the NHP model was instrumental in both its application in several emergency use protocols in Australia and the United States as a result of high-risk virus exposure (18 individuals to date), and also in the conduct of the very first Phase I clinical trial of an anti-HeV/NiV therapy in collaboration with USU and Queensland Health in Australia (PMID: 32027842). In contrast, several preventative vaccines against NiV and HeV have been shown to be 100% effective in preventing infection and disease in animal models including NHPs and several vaccine platforms have clear potential for use in people (PMC8782152). The HNV G and F membrane glycoproteins are the major targets of neutralizing antibodies and the cornerstone of all vaccine strategies (PMC8782152). Such preventive vaccines would have utility for lab workers, first responders or individuals at high risk exposure and in mitigating the spread of an outbreak; whereas in the case of a biological attack or natural outbreak, a postexposure treatment would augment a pandemic preparedness posture. The most extensively studied HNV vaccine is a soluble form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including NHPs. The HeV-sG immunogen is also the basis of the horse vaccine against HeV (Equivac® HeV) marketed by Zoetis, Inc., which is the first commercialized vaccine to a BSL-4 agent (Middleton, 2014 PMID: 24572697). It is widely considered that the African green monkey (AGM) is the current “gold standard” animal model for NiV and HeV research as the disease pathogenesis appears to mirror that of human NiV/HeV infection (Geisbert, 2012;Geisbert, 2010). The evaluation of successful countermeasures for the prototype NiV and HeV and other related HNVs in the NHP model will likely be pivotal for IND applications and future licensure of select countermeasures for human use.

More recently, divergent and pathogenic zoonotic HNVs have been discovered, namely the related Mòjiāng virus and Langya virus (LayV), and in contrast to NiV/HeV, both appear to be rodent borne and do not appear to employ ephrin ligands as entry receptors (C. Broder and K. Xu unpublished) (PMID: 33809833). The PABVAX Center has chosen to initiate studies on the divergent LayV as the Phase II test case scenario. The choice is justifiable for several important reasons; 1) LayV is a known pathogenic zoonotic HNV; 2) there is a known virus genome sequence derived from a human sourced virus isolate; 3) we have already successfully engineered and expressed LayV sF (stabilized) and sG (tetramer) glycoproteins antigens. Also, another divergent bat-borne HNV, Angavokely virus (AngV), has recently been described. The Center will initiate studies on LayV in preparation for Phase II, including the rescue of recombinant LayV (rLayV), explore animal model development, construct additional LayV and AngV reporter virus tools, and then translate and test the MNP

vaccine ‘plug-and-play’ approach, and also evaluate new mAbs and nanobodies (nbs) to these new divergent and/or potential zoonotic HNVs.

PABVAX THEME

A strength of the PABVAX is that a majority of the Center Research Project (RP) and Core leaders have extensive experience in antiviral translational research and/or product development: Uniformed Services University (USU), University of Texas Medical Branch (UTMB), Vanderbilt Vaccine Center (VVC), University of Pittsburg (UPitt), Institute for Bioscience and Biotechnology Research (IBBR) at the University of Maryland (UMD), Mapp Biopharmaceutical. In addition, nearly all of the Center’s RPs and Core leaders currently have active and productive collaborative research programs in related areas, some spanning more than a decade.

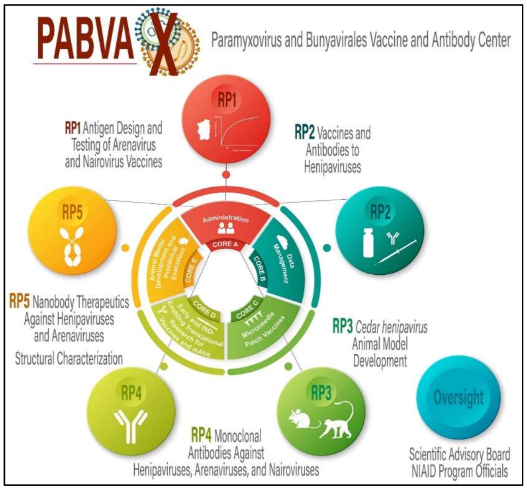

Figure 1: Organizational chart of the Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center-(PABVAX). **(connecting arrows are needed between elements and the SAB)**

The organizational structure of the PABVAX is diagramed in **Figure 1**. Two Research Projects (RPs) in the PABVAX Center will focus on developing and evaluating a new, thermostable, needle-free, adaptable, and widely deployable, dissolvable microneedle patch (MNP)-based skin immunization vaccine technology that will use recombinant viral protein subunit immunogens; **RP1** will target select prototype **Arenaviridae** and **Nairoviridae** viruses, and **RP2** will target the prototype **henipaviruses** (HNVs). Both RP1 and RP2 will also be developing new prototype virus tools (recombinantly generated prototype viruses) along with existing tools, to be used in the analysis of vaccine responses as well as in the evaluation of new mAbs and nabs from the other RPs. A third RP will be engaged in the development of a unique low-containment (BSL-2) mouse model of HNV infection that uses recombinant Cedar henipavirus (rCedV) in vivo and a bioluminescence methodology that can longitudinally trace the dynamics and anatomical progression of luciferase encoding virus infection in individual animals (**RP3**). There will also be two RPs that will target the development human mAbs and nanobodies to the PABVAX select viral prototypes. **RP4** will isolate human mAbs derived from NiV infected survivors with a specific focus on the F glycoprotein targeted mAbs, and additional human mAbs will be generated from humanized mice by viral prototype antigen immunization. Finally, **RP5** will develop nanobody-based therapeutics targeting henipaviruses and arenaviruses along with a focus on targeting the CNS environment in animal modeling experiments using the select virus prototype vaccine antigens and immunization of nanomice as well as human nanobody phage library approaches. Critical research support to all the RPs will come from three Scientific Cores that will be engaged in manufacture and quality control & testing of MNP vaccines (**Core C**); production of recombinant viral proteins and antibody refinements (**Core D**); and animal modeling of prototype virus infections along with preclinical testing and evaluation of vaccines and antibody-based therapeutics (**Core E**).

<u>With a focus on the Paramyxoviridae and Bunyavirales; the overall Objectives of the PABVAX are 3-fold:</u>

1) Establish an effective and adaptable vaccination technology.

2) Discover effective antibody-based therapies.

3) Develop new tools and animal models to facilitate the translation of effective countermeasures.

<u>An adaptable plug-and-play vaccine platform.</u>
The PABVAX Center will take advantage of a successful piloted research project using recombinant soluble Hendra virus G glycoprotein (HeV-sG) that several lead investigators recently co-developed with scientists from the UPitt and IBBR/UMD (**Core C**) that has tested a dissolvable MNP-based vaccine technology that is ideally suited for the development of a rapid and inherently safe, recombinant protein-based subunit vaccine approach

(PMID: 32924651). This vaccine technology effectively harnesses the highly immunoresponsive cutaneous microenvironments using dissolvable MNPs that incorporates recombinant viral glycoprotein antigens and polyphosphazene (PPZ) adjuvants (PMID: 19864632) to elicit robust, strong, and long-lasting protective immunity against the target pathogen. PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs. PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, forming nanoassemblies with virus particle dimensions (60-100 nm). The PPZ macromolecules serve as an immunoadjuvant that provides immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. Dissolvable MNPs will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as facilitate the improved vaccine storage and distribution due to their thermostability. This broadly adaptable and widely deployable vaccination approach is enabled by a synergistic combination of a plug-and-play approach with a clinically feasible immunoadjuvant and skin-targeted vaccine delivery platform. Here, a Scientific **Core C**: (Microneedle Patch Vaccines Core) will produce polyphosphazene (PPZ) adjuvanted MNP vaccines with select recombinant vaccine antigens (prototype viral targets) in support of **RP1** (*Bunyavirales*) and **RP2** (Paramyxoviridae). **Core C** will batch release PPZ MNP vaccines on well-defined quantitative criteria of their validated physicochemical and immunological properties. The RPs focused on vaccine development will explore a variety of viral immunogens and combinations. Down selection of vaccine formulations and components will be made based on first tier immune response data derived from mouse immunization studies with a focus on assaying antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity in vitro and polyfunctional T-cell responses by in vitro stimulation of isolated cells with antigenic peptides, intracellular cytokine staining (ICS) and flow cytometry) responses.

Discover effective antibody-based therapies.
PREP can be critical in the absence of available vaccines or cases in which vaccination is not possible.
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Develop new tools and animal models to facilitate the translation of effective countermeasures.
Virological tools and animal models are essential in assessing the effectiveness and protective correlates of antiviral vaccines and therapeutic countermeasures. The investigators within the Animal model development and preclinical evaluation scientific **Core E**, at the GNL, UTMB, have the expertise in a wide variety of high-containment and select agent research areas including…….. animal model development and use of animal models for the study of viral pathogenesis and assessing the therapeutic efficacy of antivirals and vaccines. In addition, **Core E** has the facilities for cell-based assays for authentic pathogenic virus use and manipulation; including the establishment and use of reverse genetics for rescue of pathogenic viruses including novel reporter-gene encoding versions which will be performed within **RP1**. Such virus tools are essential for the characterization of vaccine responses such as neutralizing antibody induction in animal subjects, as well as validation of antisera or antiviral antibody neutralization using authentic viruses. Other reporter virus tools and alternative animal models that can be utilized at low-containment (BSL-2) are also useful in antiviral development strategies because they can be adapted to greater high-throughput platforms, are often less laborious, and they can make use of other innovative technologies. **RP2** will make use of recently generated rCedV chimeric viruses that allow for rapid high-throughput florescence-based virus neutralization assays at BSL-2 ideally suited to analyze otherwise BSL-4 restricted authentic Nipah and Hendra virus vaccine induced immune sera, mAbs (**RP4**) and nbs (**RP5**) and antibody neutralization synergy. **RP3** will make use of other versions of rCedV and chimeras (those bearing either the HeV or NiV-B envelope glycoproteins) in the development of a BSL-2 animal model that uses in vivo bioluminescence to visualize the anatomical location and progression of virus in live animals, ideally suited to explore nab based therapeutics to CNS resident henipavirus (PMID: 35821488).

The overall objectives of the PABVAX Center will be integrated into quality system data management processes (**Core B**) that will be employed in both the preparation of vaccines and downstream antibody-based therapeutics by the Center's highly experienced commercial partners, and in the conduct of animal studies by the Animal model development and preclinical evaluation **Core E** at the GNL, UTMB. Data generated to support of possible future regulatory submissions to the Food and Drug Administration (FDA) must be collected in accordance with quality systems, like good laboratory practice (GLP) regulations, to be useful and reliable, and to facilitate FDA review to ensure safety and efficacy. The UTMB and GNL is a national resource and benchmark provider for the conduct of high containment animal studies and quality system data management; often partnering with the FDA in academic development and training programs for GLP in high containment environments. We envision several deliverables emanating from the proposed studies of the Center, including significantly improved subunit vaccine formulations against several prototype viruses within the henipaviruses (Paramyxoviridae) and the Bunyavirales (Arenaviridae and Nairoviridae) that will bolster vaccine endurance to storage and transportation; and also improved human mAbs and/or nabs for the treatment of disease caused by the select prototype viruses.

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology and mAb and vaccine translational drug development and includes Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its RPs and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAb and nanobody prototypes to foster pandemic preparedness.

THE PABVAX CENTER PROTOTYPE VIRUSES

The prototype viruses chosen by the PABVAX have been made based on several criteria discussed below, and because they represent known threats to global public health that is exacerbated due to a lack of approved vaccines or therapeutics. There is a clear need for effective countermeasures in order to improve a global pandemic preparedness posture that can address the threat of natural outbreaks, epidemics, or bio-weaponization and deliberate release of these prototype virus species and/or related viruses within those groups.

Henipaviruses: Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia (NiV-M). NiV and HeV are both considered the **prototype species** of the genus *Henipavirus*, family *Paramyxoviridae*. The natural reservoir of the two pathogenic and prototype HNVs, NiV and HeV, are old world fruit bats (flying foxes) of the genus *Pteropus* (Order *Chiroptera*, suborder, *Megachiroptera*, Family *Pteropodidae*). NiV and HeV are zoonotic agents, and human infection with either virus causes a widespread vasculitis, that will often progress to a fatal encephalitis or pneumonia. People who recover from acute infection with either of these HNVs are also at risk of recrudescent infection and encephalitis (Luby SP, Broder CC. 2014. Paramyxoviruses: Henipaviruses, p 519-536. In Kaslow RA, Stanberry LR, Le Duc JW (ed), Viral Infections of Humans, Epidemiology and Control. Springer Science+Business Media, New York.; Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses, p 1070-1085. In Knipe DM, Howley PM (ed), Fields Virology, vol 1. Lippincott Williams & Wilkins, Philadelphia). HeV and NiV are BSL-4 restricted pathogens because of their high lethality and lack of approved vaccines or antivirals and are transboundary agents of significant disease threats to livestock and people in Australia, and South and Southeast Asia, respectively. The genus *Henipavirus* now includes nine other reported HNVs; these include the four viral isolates of Cedar virus (CedV), Gamak virus, Daeryong virus, and Langya virus (LayV) [xxx] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV) [xxx]. Similar to NiV and HeV, the natural reservoirs of CedV, along with the genomic data of GhV and AngV, are old world pteropid fruit bats. Whereas the six other HNVs are, or are likely, of rodent origins, including the human isolate LayV from East China (Shandong and Henan provinces), which is most closely related to Mòjiāng virus that is also of apparent rodent origin from Southeast China (Yunnan province). The phylogenetic relationship of selected HNVs by F and G glycoprotein comparisons is shown in **Figure 2**.

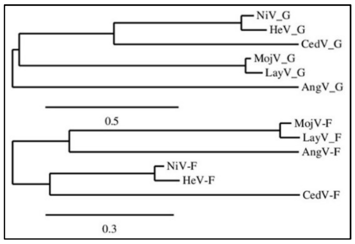

Figure 2. Phylogenetic analysis of select HNV G and F glycoproteins. Phylogenetic trees were constructed for the HNV F and G glycoproteins using the PhyML program's maximum likelihood method. The trees were generated by aligning the protein sequences with the MUSCLE algorithm. The genetic distance between the sequences is represented by the bar located under each tree, providing information about the level of divergence among them.

Investigators in the Center **PR2** and **RP5** have already initiated new studies on LayV, having engineered stabilized sG and sF glycoprotein antigens in collaboration with Genovac, Inc. (part of **Core D**) and identified cross-reactive mAbs to both F and G previously generated to Mòjiāng virus. Whereas HeV and NiV cause severe and often fatal henipaviral disease, LayV was associated with nonfatal febrile illnesses in humans. In contrast, CedV is the only HNV isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and

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Lee, S.H.; Kim, K.; Viruses 2021, 13, doi:10.3390/v13102020.

Zhang, X.A.; Li, H.; Jiang, N Engl J Med 2022, 387, 470-472, doi:10.1056/NEJMc2202705.

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Drexler, J.F.; Corman, Nature communications 2012, 3, 796, doi:10.1038/ncomms1796.

Wu, Z.; Yang, L.; Emerging infectious diseases 2014, 20, 1064-1066, doi:10.3201/eid2006.131022.

Vanmechelen, B.; Meurs, S.; Virus Evol 2022, 8, veac061.

Madera, S.; Kistler, A.; J Virol 2022, 96, e0092122, doi:10.1128/jvi.00921-22.

disease including guinea pigs, ferrets, hamsters (Geisbert 2012), and African green monkeys (Geisbert and Broder, unpublished). An important distinction between CedV and other HNVs lies within the P gene which encodes the phosphoprotein (P), and the P gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists (xx). The CedV P gene lacks both RNA editing and does not encode V or W. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections. All other recognized or proposed HNVs have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is the key factor underlying its nonpathogenic nature. These data permitted the rescue and characterization of rCedV by reverse genetics at BSL-2 (XXX) and CedV is now recognized as a BSL-2 restricted agent [CDC manual]. Because the HNV, LayV, is known to be a human pathogenic and zoonotic HNV, is genetically divergent from NiV and HeV, and also of apparent alternate origin (rodent), together with some preliminary data on the sF and sG LayV glycoproteins, the PABVAX Center will explore LayV as part of the Center's Phase II research plan (**RP2** and **PR5** with **Cores C, D, and E**).

Commented [CB5]: Marsh, G.A.; Wang, L.F. Curr Opin Virol 2012, 2, 242-247, doi:10.1016/j.coviro.2012.03.006.

Commented [CB6]: Laing, E.D.; Amaya, M.; Virology journal 2018, 15, 56, doi:10.1186/s12985-018-0964-0.

Laing, E.D.; Navaratnarajah, C.K.; Proc Natl Acad Sci U S A 2019, doi:10.1073/pnas.1911773116.

Commented [CB7]: Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition. HHS Publication No. (CDC) 300859. US Government Printing Office: Washington, 2020; pp. 248-250.

Arenaviridae* and *Nairoviridae:

EXPECTED SYNERGIES PROVIDED BY THE CENTER

The 5 RPs and 3 scientific Cores, together with the Administration and Data Cores, that comprise the **Paramyxoviridae and Bunyavirales Vaccines and Antibodies – PABVAX** Center have been specifically tailored to be synergistic (**Figure 1**). All five RPs will work collaboratively and synergistically with the scientific Cores in support of developing and testing pre- and postexposure treatments for the selected virus prototypes. This includes the production of vaccine immunogens for both the manufacture of the vaccine patches as well as for the conduct of assays to assess vaccine immune responses, isolation of mAbs and nbs, and in select cases the structural characterization of the important viral vaccine immunogens and interaction with antibodies. Additionally, individual RPs will conduct a variety of experimental lines of investigation in synergistic collaboration with other PRs; such as the conduct of virus neutralization analyses of vaccine-elicited antibody responses and virus specific mAbs and nbs (henipavirus projects: **RP2, RP3, RP4, RP5**), (arenaviridae and nairoviridae projects: **RP1, RP4, RP5**). Of unique importance to the goals of the PABVAX is that the countermeasures anticipated to be developed from the RPs may provide a future opportunity to combine the active and passive immunization approaches to further improved the therapeutic value in an inherently synergistic manner. The RPs will work closely together in a harmonious and cooperative manner with each group providing their own countermeasure and expertise so as not to duplicate effort. This opportunity would be difficult if not impossible if each project were pursued independently. This collaborative effort among all RPs and Cores will provide strong synergy among the groups and PABVAX participants, many of which have established collaborative and synergistic research activities. For example, the early development and testing of particular antiviral mAbs to some of the prototype viruses under investigation is already an established collaboration between PABVAX partners at Vanderbilt's VVC and USU and UTMB together with Mapp Biopharmaceutical. Also, the pilot MNP vaccine platform project was a collaboration between USU, UPitt, UMD-IBBR, and UTMB.

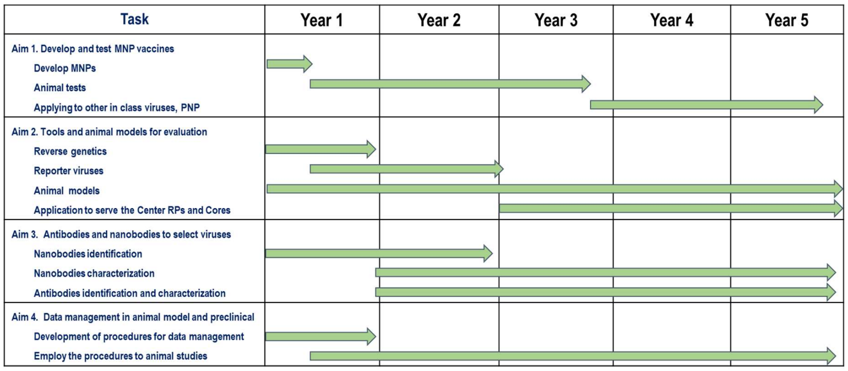
An additional and outstanding advantage of the PABVAX Center is the **Animal model development and preclinical evaluation Core E** at the GNL, UTMB. Here, BSL-4 containment experiments on the select prototype viruses can be carried out. The strength of this Center partner is synergistically enhanced by the relationship of **Core E** with the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA) also located within the GNL. (https://www.niaid.nih.gov/research/wrceva). Through the WRCEVA collection there is ease of access to many of the virus isolates that will be used in the conduct of the Center's proposed studies. All BSL-4 animal work done by **Core E** in support of the RPs and other Cores will utilize the exact same virus stocks in challenge doses, and challenge routes. In addition, all BSL-4 assays for all the Center RPs will be performed by the same BSL-4 staff under the same experimental conditions. This approach will allow for better comparison among countermeasures within the Center and will ensure consistency among results.

PABVAX MILESTONES AND TIMELINES

There are four overarching aims of the PABVAX Center:

1. **Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.**
2. **Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.**
3. **Develop antibodies and nanobodies to selected prototype viruses.**
4. **Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures.**

The projected timeline for the PABVAX to accomplish the Center objectives is diagramed in **Figure 3**.



Embedded within the overall PABVAX Aims are a cohort of specific Milestones that are laid out to gauge Center accomplishments and progress towards achieving the Center objectives. This is in addition to each PR's individual Milestone plan. The oversight and evaluation of progress will be in concert with quarterly reports to the PABVAX Scientific Advisory Board (SAB) as well as an annual meeting with key Center researchers and leaders with SAB members (Details are within the Administration **Core A**). The SAB will meet yearly typically in coordination with the Center annual meeting, to review progress, the adherence to milestones and timelines, the continued relevance of each RP and Core to the Center's overall objectives, discuss each project and progress, and advise on directions for the coming year, both for the overall PABVAX Center and for individual RPs. The SAB will also be a key factor and advisory panel in facilitating go/no-go decision making on specific RP activities, recommend new directions as appropriate and provide Center Leadership and NIAID staff a comprehensive written evaluation of the group's activities and the panel's recommendations following the annual meeting.

Overall Objectives

Aim 1. Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae.

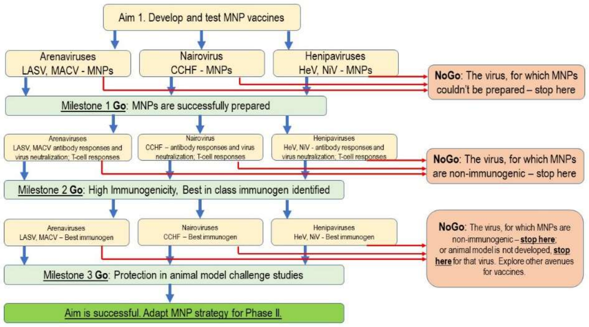


Figure 4. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

The first milestone will be whether proper formulation of recombinant antigens with adjuvant in the context of MNP fabrication can be made. The determination of MNP vaccine potential success will first be made based on immune response data derived from mouse immunization studies conducted in **Core C**. Multi-parameter humoral and cellular immune responses will be assessed (antigen specific antibody induction and virus-neutralization and polyfunctional T-cell responses by in vitro stimulation of isolated cells). Go/no go decisions will be made based on the outcomes of these first tier experiments as indicated. If robust humoral and cellular responses are evident this will be graded as success. Comparisons will also be made, such as the levels of virus-neutralizing antibody generated. Viral antigens producing poor immune responses will be terminated and an attempt in selection of best-in-class immunogens, or best combination immunogens which will also be explored, will be performed in order to down-select vaccine choice. The third milestone will be the determination of whether MNP vaccination affords protection in animal model challenge experiments. In the case of the NiV and HeV prototypes the animal models chosen (ferret and the African green monkey (AGM)) are extremely well-developed, and outcomes at this stage should be readily assessable. However, for the Center's chosen viral prototypes within the *Bunyavirales* there will be additional animal model refinements conducted. For example, there are seven genetically distinct lineages of LASA and there are guinea pig models for some but not all of those lineages. For MACV there are 8 clades with similar challenges, and for CCHF there are 6 clades. An additional confounding issue for both of these very important groups of viruses is that rhesus macaques are nearly unavailable and cynomolgus monkeys are now each greater than 35K. Thus, as proposed by **RP1**, together with **Core E**, a critical research avenue will be further developing and evaluating animal model systems, including new models, within the PABVAX program, including further development of the AGM for the arenaviruses and possibly also for CCHF as a risk mitigation strategy. Thus, individual go/no-go decisions will inevitably be more challenging during the first Phase I of the PABVAX.

Aim 2. Develop new tools and animal models for the conduct of cell-based and in vivo therapeutics evaluation.

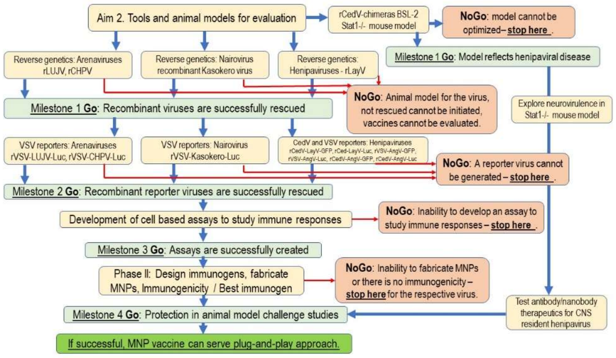


Figure 5. Milestones and criteria for progress for **Aim 2** of the PABVAX Center overall objectives.

The PABVAX second overall objective is primarily focused on the development of new viruses and tools for downstream initiation and testing of the Center's vaccine program and antiviral antibody testing and evaluation. These materials and viruses will be key in the execution of Phase II of the program. **RP1** will generate recombinant infectious clones (r = recombinant viruses) and reporter-gene versions, as well as alternate reporter virus tools (VSV-based) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined in vivo (animal model development) within **Core E**. In **RP2**, the most recently discovered pathogenic henipavirus, LayV, will be rescued by reverse genetics (rLayV) in collaboration with **Core E**, and additional LayV and AngV reporter virus tools also generated with alternate reporter virus platforms (VSV and/or rCedV based). New virus tools will be characterized in cell-based assays. Only AngV alternate reporter virus tools are proposed as the complete genomic data is incomplete. rLayV pathogenesis and animal model development will conducted by **Core E**. If these objectives are successful, a a large battery of new reagents and tools will be available to share with the ReVAMPP network, as well as serving as key components for initiation and execution of Phase II of the PABVAX Center program.

The translation and testing the MNP vaccine plug-and-play approach will be initiated in the Phase II prototype viruses (**Table 1**) the milestones here will be similar to those outlined in **Figure 4**. The new virus tools will also be used to evaluate new mAbs and nanobodies generated by **Core D** and **RP5** to the new divergent and/or potential zoonotic HNVs (LayV and AngV). Also embedded in this overall aim is a new in vivo bioluminescence-based immune deficient mouse model (newly developed for Lyssavirus infection by the PIs of **PR2** and **RP3**) that can longitudinally trace the dynamics and anatomical progression of rCedV-luciferase virus (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, the model can sustain replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. This new unique model can be used to study henipaviral disease in vivo safely and expediently at BSL-2 containment. If the model can be optimized (**Figure 5**) it will be deployed to test a critically important therapeutic need, the use of antivirals against CNS resident HNV infection, and RP3 will collaborate with RP5 in the testing of engineered nabs specific for the prototype HNVs NiV and HeV.

Aim 3. Develop antibodies and nanobodies to selected prototype viruses.

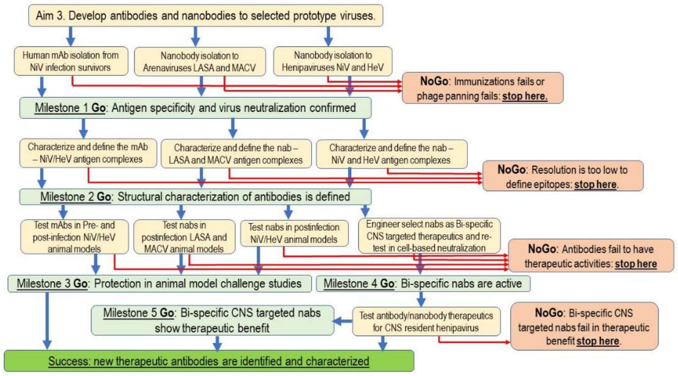


Figure 6. Milestones and criteria for progress for **Aim 1** of the PABVAX Center's overall objectives.

New human mAbs and nanobodies (nbs) as tools and potential therapeutics will be developed by RP4 and RP5 in collaboration with **RP2** and **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); and transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses. Human mAbs are expected to be valuable therapeutics as countermeasures to a variety of viral infections and disease in humans. Here, **RP4** will isolate panels of naturally occurring human mAbs that bind cross-reactively to both the NiV and HeV prototype HNVs and neutralize both viruses. This effort will focus especially on the mAbs to the HNV fusion (F) proteins derived from natural survivors of NiV infection, which to date has been an elusive goal. Previously, the **RP4** PI (Crowe) isolated multiple human mAbs to the G protein with several that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic (PREP) and therapeutic molecules for humans. **RP4** will isolate new F mAbs and in collaboration with **RP2** neutralization potencies will be rapidly assessed as well as evaluating synergistic cell-based mAb neutralization that will then be translated and evaluated in animal models as both PREP and post-infection treatments by **Core E**. These new human mAbs will build on the findings to date generated by several of the collaborating Center investigators, and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of prototype HNVs in preparation for clinical studies.

Nanobodies, derived from the heavy chain-only antibodies of camelid animals, offer a promising new direction in antibody-based antiviral countermeasures. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes, which could translate into a more cross-reactive feature between virus strains or clades. Importantly, nanobodies can efficiently penetrate tissues and cross the blood-brain barrier (BBB), making them particularly effective in treating CNS-resident viral infections. Here, the prototype viruses from the Center's viral groups to which there are no approved therapeutics and are also recognized to cause CNS pathogenesis will be targeted by **RP5**. To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody phage-displayed libraries that closely mimic the natural human antibody heavy chain variable domain. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. **RP5** will also engineer a combination of nanobodies targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved BBB penetration. Specific nanobodies and engineered nanobodies will be evaluated in cell-based virus neutralization tests by **RP1** (LASV and MACV) and **RP2** (NiV and HeV), and also for therapeutic activity in the rCedV-NiV-B chimera mouse model in **RP3** (Henipavirus) and select animal models of LASV and MACV by **Core E**. Success will be measured by the identification of virus-neutralizing nbs, potential discovery of more broadly reactive nbs, particularly to the multiple LASA clades, the evidence of therapeutic benefit in animal models, and the successful antiviral activity of nabs or engineered bi-specific nabs in treating CNS resident virus infections.

INDUSTRY PARTNERSHIPS

Industry Partnership

Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine

expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required.

SPECIAL FEATURES OF THE PABVAX CENTER ENVIRONMENT AND OTHER RESOURCES ??

Our proposal combines the expertise of several established groups including investigators in academia and in private industry. The Center is supported by outstanding physical resources and an experienced and skilled group of researchers and support staff.

Galveston National Laboratory (GNL)

The GNL, is a NIH-funded, maximum-containment facility that serves as a national resource in support of NIH's biodefense and emerging infectious diseases research agenda. The GNL is a state-of-the-art facility uniquely designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism for the purpose of developing vaccines, diagnostics and therapeutics against them. The 7-story GNL offers over 12,000 square feet of BSL-4/ABSL-4 space. Additionally, there are BSL-4 aerobiology facilities. The GNL BSL 4/ABSL-4 laboratories were inspected by the CDC and were approved for operations and handling of Select Agents and became operational in September of 2010. The first NHP studies were performed in the new GNL BSL-4 laboratories in April of 2011. To date, 187 separate NHP studies employing a total of 1,318 animals and 10 different BSL-4 viruses including Ebola and Marburg viruses and also Nipah and Hendra have been successfully performed at the GNL. These NHP studies include a number of collaborative efforts between Drs. Geisbert, Cross, Crowe, Broder and Zeitlin. The GNL has a fully Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited nonhuman primate facility and a staff of full-time veterinarians. In addition, **Core E** is staffed with an experienced team of GNL researchers and technicians several of which have more than 25 years of BSL-4/ABSL-4 experience. This team also includes a BSL-4/ABSL-4 trained board-certified veterinary pathologist. As noted above another special feature is the location of WRCEVA virus collection within the GNL.

Core E (Animal model development and preclinical evaluation)

Core E operations will be conducted at the GNL, and also includes the UTMB Institutional Office of Regulated Nonclinical Studies (ONcRS). The ONcRS's primary responsibility is to work with academic researchers who are doing product-oriented research and to help them design and conduct their studies to meet regulatory requirements by assuring regulatory compliance, quality assurance and data management. NIH has requested that all NHP studies conducted in the GNL BSL-4 laboratory at UTMB that are on a product development path be conducted under a "well documented" quality system program. Quality system and regulatory science processes for NHP studies performed at UTMB will be implemented and executed in conjunction with the ORNcS. Dr. Geisbert (Overall Center PI and Core E Director) has over 34 years of BSL-4/ABSL-4 experience at US Army Medical Research Institute of Infectious Diseases (USAMRIID), the GNL and other BSL-4 facilities. His research interests have focused on hemorrhagic fever viruses and the henipaviruses and he has published over XXX peer-reviewed articles in these areas. Dr. Geisbert's research group, in collaboration with Dr. Broder (Center Co-PI and PR2 PI) was the first to develop a NHP model of NiV/HeV infection and disease and also to demonstrate complete protection of NHPs against both NiV and HeV using henipavirus vaccines and also human mAb therapy.

Core D (Early and IND-Enabling Translational Research for Vaccines and mAbs)

Mapp Biopharmaceutical's product development team has grown significantly in the last two years in response to the development efforts on its anti-Ebola therapeutic, ZMapp^{TM} which is being advanced to licensure as part of Project Bioshield. OTHER MAbs The early development and research group has expertise in immunology, infectious diseases, and production of antibodies and antibody fusion proteins in CHO systems. The advanced development group has experience in medium-size and large pharmaceutical companies and expertise in various aspects of mAb development and commercialization including chemistry, manufacturing & controls, analytical development, clinical and nonclinical development, regulatory affairs, quality assurance, and project management. The team has developed multiple products, including a licensed biodefense biologic. As with Profectus, Mapp Bio's expertise and experience will be a very important and valuable asset to the Center.

Bonnie Genovac

Core A (Administrative Core)

Research Project teams?? Maybe not depending on space

The investigators at USU, led by Dr. Broder have an extensive history in henipavirus research which he established in 1999, and he has published more than 75 articles, reviews and chapters in this area. His group has also made significant translational research accomplishments in the area of henipavirus countermeasure developments including Equivac® HeV (Zoetis, Inc.) for use in horses in Australia, and the human mAb (m102.4) having a Phase I clinical trial completed (2016) in Australia, in partnership with the Queensland Government and NIAID. Dr. Broder was the recipient of the Federal Laboratory Consortium for Technology Transfer, Excellence Award in 2013. His administrative experience and skills and his experience in translational research activities qualify him to serve as the director of the PABVAX Center and Administrative Core.

Dr. Crowe's research focuses on the molecular basis for the development of effective B cell responses to viruses in humans and he has published over 200 peer-reviewed research articles in this area. The Crowe laboratory is one of the leading groups in the development of innovative technologies for isolation and study of human mAbs. Dr. Crowe's group has isolated human mAbs that neutralize HIV, influenza, dengue, chikungunya, respiratory syncytial virus, human metapneumovirus, rotavirus, norovirus, and recently henipaviruses NiV and HeV owing to the collaboration between the Crowe, Broder and Geisbert laboratories. The Core B of the Center will work together with RP2 and RP3 along with the Core C to develop the first cocktail human mAb immunotherapeutics, and facilitate the transition products suitable for IND filing and human testing including design, construction, assessment, and optimization of these antibody-based therapies.

BIBLIOGRAPHY AND REFERENCES CITED

CENTER OVERVIEW: Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center – (PABVAX)

SPECIFIC AIMS

Emerging and reemerging pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans worldwide with associated high morbidity and mortality. A host of arenaviruses viruses, including Lassa, Machupo, Lujo, and Chapare (LASV, MACV, LUJV, CHPV) and the Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and are also included among the World Health Organization's (WHO) List of Priority Pathogens. Several of these viruses are specifically identified as NIAID Category A pathogens. Also, within the WHO Priority Pathogens list is Nipah virus (NiV) and henipaviral disease which includes Hendra virus (HeV) which are zoonotic henipaviruses (HNVs) in the **Paramyxoviridae**. These HNVs have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease, and are significant biothreats to humans and livestock in South and South East Asia and Australia. These threats to global public health are heightened due to the absence of approved vaccines or therapeutics. *There is a clear unmet need for countermeasures to address the threat of natural outbreaks, epidemics, or deliberate release*.

The PABVAX Center will focus on elucidating key principles for vaccine and monoclonal antibody (mAb) countermeasures in developing prototype candidates against a selection of these viruses. In the out years of the effort, PABVAX will exploit these learned key principles in a "plug and play" demonstration against related viruses.

Family	Prototype	Plug and Play Test Case
Arenaviruses	Lassa (Old world), Machupo (New world)	Lujo (Old world), Chapare (New world)
Nairoviruses	CCHF	Kasokero
Paramyxoviruses	Nipah, Hendra	Langya

The PABVAX team consists of highly credentialed experts in virology, structural biology, immunology and mAb and vaccine translational drug development and includes Moderna and Emergent Biosolutions as Industry Partners. The synergistic activities and research goals of this Center proposal through its Research Projects (RPs) and Scientific Cores, will develop, test, and translate, prototype vaccines using a novel vaccination platform (an adjuvanted dissolvable microneedle patch (MNP) vaccine), develop new virus and animal model tools, and develop and test mAbs and nanobodies to the virus prototypes to foster pandemic preparedness. The Center's translational objectives will be accomplished by the completion of the following Aims:

AIM 1: Develop and test MNP vaccines for the Bunyavirales and Paramyxoviridae. Subunit viral antigen(s) MNP vaccines will be explored. **RP1** will test vaccines for both Old world (LASV) and New world (MACV) prototype viruses. **RP2** will test vaccines against the prototype viruses NiV and HeV. Each RP will implement this 'plug-and-play' vaccine approach with test case virus antigens in Phase 2. All three Scientific Cores (MNP-**Core C**), (Antigens and Antibodies-**Core D**) and (Animal models and testing-**Core E**) will support the RPs.

AIM 2: Develop new tools and animal models for the conduct of cell-based and *in vivo* therapeutics evaluation. Reverse genetics and recombinant pseudoviruses will be developed for both prototype and test case viruses by **RP1** and **RP2**. New high-containment (**RP1, RP2,** and **Core E**) and novel low-containment (**RP3**) animal models will be developed to explore pathogenesis and facilitate therapeutics testing.

AIM 3: Develop antibodies and nanobodies to selected prototype viruses. New human mAbs and nanobodies (nbs) as tools and potential therapeutics will be developed in collaboration with **Core D**. NiV infection survivor volunteers and MNP vaccination of transgenic mice will be used to generate human mAbs (**RP2** and **RP4**); and transgenic nanomice and human nb phage-display approaches will generate nbs (**RP5**) to the selected prototype and test case viruses.

AIM 4: Employ quality system data management in animal model development and preclinical evaluation of candidate countermeasures. The Center will conduct a well-integrated, discovery and translational research program, and the goal of this Aim is to ensure the quality and integrity of collected data. Each RP and Core will employ procedures ensuring quality system data management in collaboration with Data Management-**Core B**. Further, quality system and regulatory science processes for animal studies (**Core E**) will be used under the guidance of a quality system team.

From: Geisbert, Thomas W. on behalf of Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
To: Larry Zeitlin; CHRISTOPHER BRODER; Dafna Abelson; Cross, Robert W.; Antony Dimitrov; Woolsey, Courtney B. (Micro)
Subject: Re: overall 12 page res plan
Date: Thursday, June 1, 2023 10:44:23 AM
Attachments: Overall_Research-Strategy_PABVAX-12pages-V1-05312023_LZ-tg.docx

OK I made some edits. It looks like Chris is asking for some input from Bob. Looping Courtney in on the Figure and any thoughts or changes there.

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Thursday, June 1, 2023 8:17 AM
To: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Dafna Abelson <dafna.abelson@mappbio.com>; Cross, Robert W. <rwcross@UTMB.EDU>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: overall 12 page res plan

Gang,

Some minor edits and text for the green highlighted sections that were in my wheelhouse…

Best
LZ

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Date: Thursday, June 1, 2023 at 8:40 AM
To: Larry Zeitlin <larry.zeitlin@mappbio.com>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Dafna Abelson <dafna.abelson@mappbio.com>, Cross, Robert W. <rwcross@UTMB.EDU>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: overall 12 page res plan

Yes, that is correct. Here is a draft of the Core E Research Strategy. I am going to try to tighten it up today.

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Sent: Thursday, June 1, 2023 7:38 AM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Dafna Abelson <dafna.abelson@mappbio.com>; Cross, Robert W. <rwcross@UTMB.EDU>; Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: overall 12 page res plan

Tom/Bob…am I correct in assuming you are including animals for therapy studies with the mAbs/nanobodies?

From: Larry Zeitlin <larry.zeitlin@mappbio.com>
Date: Wednesday, May 31, 2023 at 7:21 PM
To: Broder, Christopher <christopher.broder@usuhs.edu>, twgeisbe@UTMB.EDU <twgeisbe@utmb.edu>, Dafna Abelson <dafna.abelson@mappbio.com>, Cross, Robert W. <rwcross@utmb.edu>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: overall 12 page res plan

Thanks Chris – looks like a great start. If anyone works on this today, please shoot me the latest version when you are done. I'll work on it first thing tomorrow EST and send back to the group asap.

From: Broder, Christopher <christopher.broder@usuhs.edu>
Date: Wednesday, May 31, 2023 at 6:59 PM
To: twgeisbe@UTMB.EDU <twgeisbe@utmb.edu>, Larry Zeitlin <larry.zeitlin@mappbio.com>, Dafna Abelson <dafna.abelson@mappbio.com>, Cross, Robert W. <rwcross@utmb.edu>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: overall 12 page res plan

ok,
based on the RFA i made sections to address the requirements
some bits are from the CETR

The biggest thing this RFA wanted was the Milestones and go and no go
and timelines and diagrams.

needed items are green. need your magic guys especially industry and mabs and bunyas

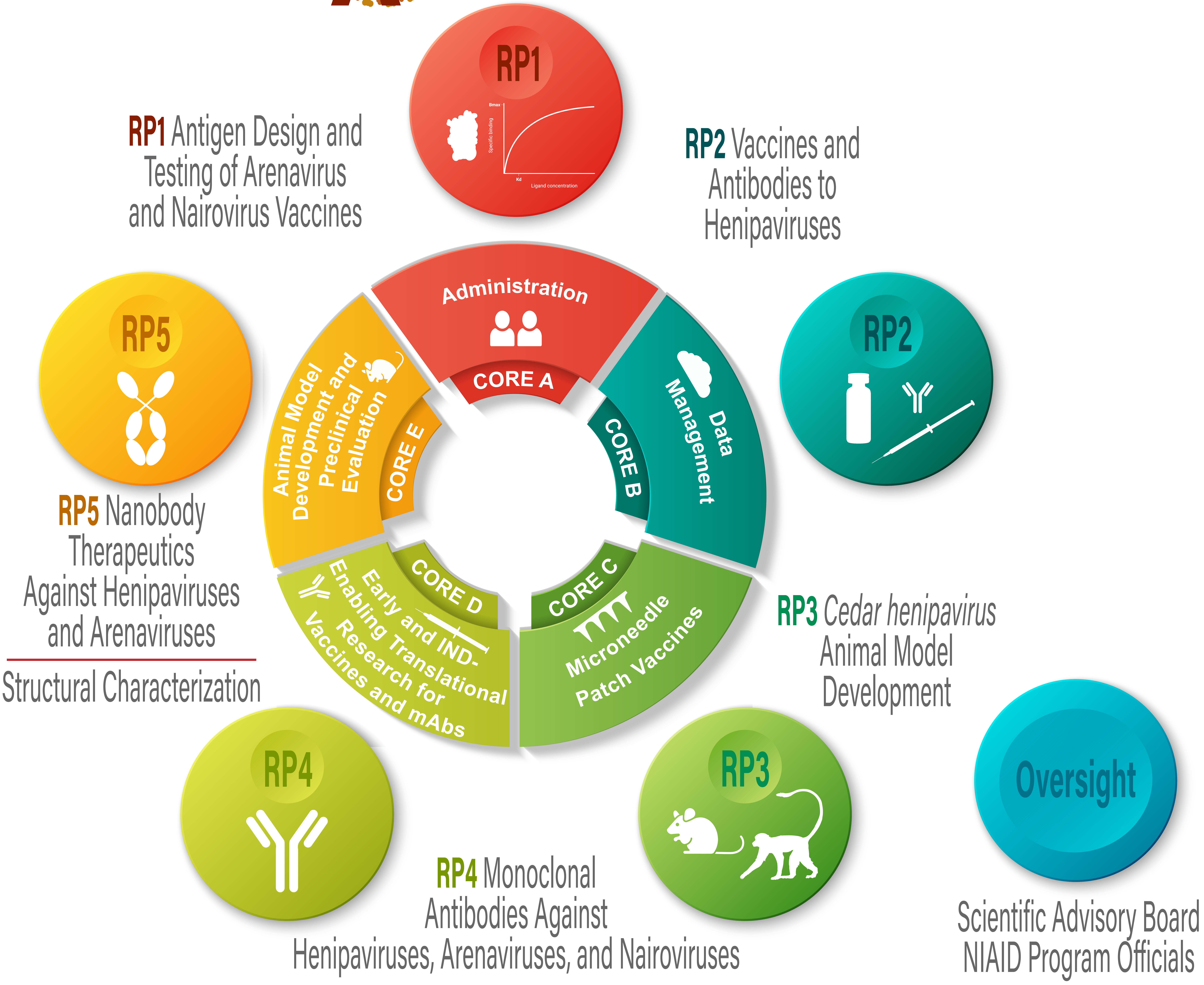
there are never to many refs needed, yellow, so best to
have Sherry and Sonya do this (add them from your library) like the admin ect,,

pending space, the other bits are yellow and optional depending on what you think

cb

PABVAX

Paramyxovirus and Bunyavirales Vaccine and Antibody Center



RP1

RP1 Antigen Design and Testing of Arenavirus and Nairovirus Vaccines

RP2

RP2 Vaccines and Antibodies to Henipaviruses

Administration
CORE A

Data Management
CORE B

Microneedle Patch Vaccines
CORE C

Early and IND-Enabling Translational Research for Vaccines and mAbs
CORE D

Animal Model Development and Preclinical Evaluation
CORE E

RP5

RP5 Nanobody Therapeutics Against Henipaviruses and Arenaviruses

Structural Characterization

RP3

RP3 *Cedar henipavirus* Animal Model Development

RP4

RP4 Monoclonal Antibodies Against Henipaviruses, Arenaviruses, and Nairoviruses

Oversight

Scientific Advisory Board
NIAID Program Officials

Core C - Microneedle Patch Vaccines (Falo, Andrianov, Korkmaz): ABSTRACT/PROJECT SUMMARY

The threat of emerging and re-emerging infectious diseases highlights the need for continued improvement of vaccination strategies to enable more effective, sustainable, and equitable global immunization campaigns. As a complementary strategy to the proposed work in the Research Projects (RPs) of this Center, the ***goal*** of Core C is to develop, characterize, and validate highly innovative, *plug-and-play* polyphosphazene (PPZ) adjuvanted dissolvable microneedle patch (MNP) platforms with RP-specific viral antigens to support the proposed research in the RPs of the Center. Development of efficacious, patient-friendly, broadly deployable, and rapidly adaptable PPZ MNP vaccination platforms and validation of their product attributes and immunological properties with the RP-specific antigens against prototype viruses will be of great significance to maximize pandemic preparedness against existing and novel human pathogenic viruses. The Core C of the Center will work seamlessly with Core D and RPs of the Center to bring together the transformative project components, ***1)*** *cutaneous immunization*, ***2)*** Polyphosphazene (*PPZ) adjuvant*, and ***3)*** *dissolvable MNPs*, with the rationally designed antigens that will be developed in RPs against high-priority pathogens to enable the achievement of the overall goals of the Center, as well as to support the translational development of a next-generation immunization strategy. The RP-specific *recombinant viral glycoprotein antigens* will drive the generation of virus-specific protective immune responses. *The PPZ macromolecules* will serve as an immunoadjuvant nanoassembly that will provide many immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. *Dissolvable MNPs* will enable targeting vaccine components to the highly immunoresponsive skin layers in a reproducible and self-administered manner for efficacious immunization, as well as facilitate the improved vaccine storage, distribution, and coverage due to their thermostability and cost-effectiveness. Together, PPZ MNP vaccines will effectively and safely harness the highly immunoresponsive cutaneous microenvironments to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The Core C will develop and batch release PPZ MNP vaccine platforms with RP-specific antigens based on well-defined quantitative release criteria of their validated physicochemical and immunological properties to enable the development of clinically feasible, highly protective vaccines against several prototype pathogens from multiple viral families in the RPs. Ultimately, these clinically translatable PPZ MNP vaccine platforms are readily adaptable to develop efficacious and broadly deployable vaccines against emerging and re-emerging viruses, including those with RP-specific viral antigens.

Core D will leverage the relationship between vaccines and antibodies to optimize the translational activities performed by the PABVAX Center. Vaccines and antibodies are inextricably linked:

1. mAbs can help define protective epitopes for a vaccine
2. mAbs can help establish correlates of protection for vaccines
3. Vaccine antigens can be used to discover mAbs
4. mAbs can provide an alternative to a vaccine in immunocompromised populations and in emergency situations where immediate protection is required (*i.e.* there isn't sufficient time to allow natural host immunity to develop from vaccination)

Developing mAb countermeasures and vaccines simultaneously will allow us to take advantage of natural synergies between the two types of interventions and more efficiently advance superior prototypes.

To meet the goals of the PABVAX Center, Core D has the following Specific Aims:

Aim 1*: Manufacture vaccine antigens and mAbs in support of the RPs and Cores*

Manufacturing will be performed by Mapp Biopharmaceutical and Genovac to support the efforts of the other Scientific Cores and the Research Projects. The largest scale manufacturing runs will be sufficient to support stability testing and NHP dosing. This will also include production of a mAb prototype for an intramuscular (IM) long-acting alternative to a vaccine to be evaluated in Core E (Animal Models).

Aim 2*: Stability, manufacturability testing, and early process development of subunit vaccines.*

Small scale manufacturing, stability testing and early-stage process development will be performed. As vaccines are regulated by CBER, all activities will be performed consistent with regulatory expectations for this branch of the FDA. Input from the Core's vaccine industrial partners (**Emergent Biosolutions** and **Moderna**) will be integrated into the translational development efforts.

Aim 3*: Identification of correlates of protection, lead optimization, stability and manufacturability testing of mAbs*.

Together with Dr. Gunn, engineered mAbs (altered N-glycans, Fc mutations for effector functions and extended half-life, multimeric formats and nanobodies from RP5) will be evaluated to correlate mechanisms of action with maximal efficacy. This work, in conjunction with stability and manufacturability assessment, will identify a lead prototype format to transition to early process development. The goal will be to identify a single prototype for long-term intramuscular prophylaxis as well as for intravenous post-exposure prophylaxis and therapy. As mAbs are regulated by CDER, performance of all activities will be as per regulatory expectations.

Project Summary

Among viruses that cause disease in humans viral zoonoses within the family Paramyxoviridae and order Bunyavirales contain pathogens that cause significant lethality. Importantly, the Arenaviridae and Nairoviridae families contain viruses that cause severe hemorrhagic disease in humans. These viruses include the arenaviruses Lassa, Machupo, Lujo, and Chapare, and the nairovirus Crimean-Congo hemorrhagic fever virus. All of these viruses are also included among the World Health Organization's (WHO) List of Priority Pathogens. Within the Paramyxovirdae the zoonotic henipaviruses Hendra virus and Nipah virus (NiV) are also on the WHO Priority Pathogens list. Notably, these henipaviruses stand out for their impressive lethality with case fatality rates averaging about 75% for NiV. Significantly, there is evidence of multiple rounds of person-to-person transmission of NiV. All of these RNA viruses pose threats to global public health due to the absence of approved vaccines or therapeutics. There is a clear unmet need for countermeasures to address the threat of natural outbreaks, epidemics, or deliberate release. The Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center (PABVAX) is a well-integrated consortium conducting a set of discovery and translational research programs composed of a Data Management Core, three Scientific Cores, and five Research Projects (RPs). The synergistic activities of PABVAX partners will be focused on developing novel vaccines, treatments, and tools to foster pandemic preparedness. Federal law requires that the arenaviruses, nairoviruses, and henipaviruses used in the PABVAX Center be handled in an approved Biosafety Level (BSL)-4 containment laboratory. Core E provides an approved BSL-4 facility and a trained and highly experienced team of BSL-4 investigators and staff to perform studies that support RP1, RP2, RP4, RP5, Core C, and Core D. Core E will perform "well-documented" animal efficacy studies based on a quality system approach that will be supported by Core A with all data generated managed by Core B. The services provided by Core E will include 1) a secure repository of well characterized seed stocks of BSL-4 arenaviruses, nairoviruses, and henipaviruses 2) *in vitro* antiviral activity assays; 3) procurement of UTMB IACUC approval of animal protocols; 4) procurement, housing, and husbandry of animals; 5); development and/or optimization of animal models for strains or variants of henipaviruses, arenaviruses, and nairoviruses to fill gaps where models are needed; 6) virus challenge, treatment, and collection of samples from animals; 7) technical expertise and equipment to conduct clinical pathological, immunological, and virological analysis of samples and to perform necropsies in BLS-4 containment; 8) histopathological analysis of tissues collected from animals infected with BSL-4 viruses; and 9) quality systems management of all records and data collected from animal studies.

Relevance

The Animal Model Development and Preclinical Evaluation (Core E) provides BSL-4 resources and expertise for RP1, RP2, RP4, RP5, Core C, and Core D. The goal of Core E is to work closely with Research Project and Scientific Core Leaders and staff, the Scientific Advisory Committee, and NIAID/NIH to advance the development of countermeasures against Paramyxoviridae and Bunyavlrales.

TITLE: Vaccines and antibodies to henipavirus
Funding announcement: RFA-AI-23-020
Funding Title: Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picronaviridae
PRINCIPAL INVESTIGATOR: Christopher Broder, Ph.D., Site Principal Investigator
Moushimi Amaya, Ph.D and Antony Dimitrov, Ph.D., Co-investigators
Prime PI: Thomas Geisbert, Ph.D., UTMB
Period of Performance: March 1, 2024 - February 28, 2029

Project Summary/Abstract

Pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. Among these are the two emerged zoonotic paramyxoviruses: the ‘prototype’ henipaviruses (HNVs); Nipah virus (NiV) and Hendra virus (HeV), discovered in the mid to late 1990s. The prototype HNVs have a uniquely broad tropism capable of infecting 18 species across 6 orders of mammals using the conserved ephrin-B2 and -B3 ligands as entry receptors. NiV and HeV infection often results in a systemic and fatal respiratory and/or neurological disease in multiple mammalian species including humans. NiV and henipaviral disease are also included in the WHO List of Priority Pathogens, and they are significant biothreats to humans and livestock in South and South East Asia and Australia. HNVs have two membrane glycoproteins: the attachment glycoprotein (G) and the fusion glycoprotein (F) that are the major targets of neutralizing antibodies and the focus of all vaccine and antiviral monoclonal antibody (mAb) strategies. The most extensively studied HNV vaccine is a soluble recombinant form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including nonhuman primates (NHPs). With no licensed vaccines or treatments available for human use, this project will use the prototype HNVs as a model to develop, test, and translate a novel vaccination platform (a thermostable, needle-free, adaptable, and widely deployable polyphosphazene (PPZ) adjuvanted dissolvable microneedle patch (MNP)). Preliminary data demonstrates potent immunogenicity of a PPZ-MNP-HeV-sG vaccine in mice, inducing robust antigen-specific HNV cross-neutralizing antibody and cellular immune responses. The MNP vaccines will be used to evaluate HeV-sG and compare stabilized soluble forms of HNV G and F (sG_{tet} and sF_{stb}) and combinations in challenge studies. A reverse genetics system has been developed to generate recombinant, nonpathogenic Cedar virus (rCedV) and rCedV chimeric virus tools by swapping the G and F glycoproteins with those of NiV/HeV as a rapid, surrogate, BSL-2 high-throughput neutralization platform to be used to rapidly evaluate HNV vaccine responses. Also, divergent and pathogenic zoonotic HNVs have been discovered including the related rodent borne Mòjiāng virus and Langya virus (LayV)), and the divergent bat-borne Angavokely virus (AngV). This project will develop and test the rapid, adaptable and readily deployable MNP vaccine platform together with new virological tools to facilitate the translational nature of this pandemic preparedness approach. Specifically, we will: 1) Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV vaccines; 2) Construct new reporter virus tools; 3) Evaluate vaccine-induced immune responses and HNV-specific mAbs and nbs; and 4) Validate the PPZ-MNP platform with another paramyxovirus antigen.

TITLE: Cedar henipavirus animal model

Funding announcement: RFA-AI-23-020

Funding Title: Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picronaviridae

PRINCIPAL INVESTIGATOR: Brian Schaefer, Ph.D., Site Principal Investigator

Prime PI: Thomas Geisbert, Ph.D., UTMB

Period of Performance: March 1, 2024 - February 28, 2029

Abstract

Henipaviruses are single-stranded, negative-sense enveloped RNA viruses of the paramyxovirus family. Two henipaviruses, Nipah virus (NiV) and Hendra virus (HeV), cause a systemic and often fatal respiratory and/or encephalitic disease in humans and ten other mammalian species. Importantly, NiV and HeV are significant biothreats to humans and economically important livestock in Australia and Southeast Asia. There are currently no vaccines or therapeutics approved for human use. Notably, development of countermeasures for NiV and HeV is hampered by the fact that these viruses require BSL-4 containment, meaning that very few research groups worldwide have access to the required biocontainment facilities to perform preclinical studies with these important human viral pathogens. To address this problem, we are developing a BSL-2 animal model that is based on Cedar virus (CedV), which is a non-pathogenic henipavirus. Specifically, we are employing recombinant Cedar viruses (rCedVs) in which the NiV and HeV fusion (F) and receptor-binding glycoprotein (G) are expressed in the rCedV genome, replacing CedV F and G. Additionally, we have also incorporated our recently developed *in vivo* bioluminescence methodology to longitudinally trace the dynamics and anatomical progression of rCedV-luciferase (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host innate immune response in mice, we have demonstrated sustained replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Importantly, whereas rCedV-luc does not establish stable expression in the brain, both of the chimeric viruses do. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in specific strains of mice. The rCedV-luc platform is thus an authentic henipavirus system that can be used to study henipavirus *in vivo* biology safely and expediently under BSL-2 containment. Our overall hypothesis is that rCedV-luc infection of mice lacking specific innate immune responses represents a BSL-2 platform for the study of henipavirus biology and pathogenesis, as well as for development and testing of anti-viral countermeasures. We will address this hypothesis through three Specific Aims: Aim 1: To optimize the use of immunodeficient mice and rCedV-NiV-luc/rCedV-HeV-luc chimeras as a robust BSL2 model of pathogenic henipavirus disease; Aim 2: To determine the mechanism of rCedV-NiV neurovirulence; Aim 3: To define the efficacy and mechanisms of mAb-based therapeutics for CNS-resident henipavirus infections. Aims involve synergistic collaborations with several other research projects and cores in this U19 program. Successful completion of these Aims will establish the rCedV-luc mouse model as a robust BSL-2 platform for the exploration of henipavirus pathogenesis and countermeasures.

The *Henipavirus* genus of RNA viruses in the family *Paramyxoviridae* contains diverse species, and this group of viruses is ideal for prototype pathogen immunity approaches. Henipaviruses are found naturally in bats in Australia, Asia, and Africa and have a wide host range. These zoonotic pathogens can cause severe illness and death in domestic animals and humans. Prophylactic and therapeutic options for Hendra and Nipah virus infections in man are limited. Anti-Hendra/Nipah human mAbs are expected to be valuable antiviral therapeutics as countermeasures to Hendra and Nipah virus disease in humans. Here, we will isolate panels of naturally occurring human monoclonal antibodies (mAbs) that bind cross-reactively to both Hendra and Nipah virus surface proteins and neutralize both viruses. This effort will focus especially on the mAbs to the fusion (F) proteins. In previous experiments, we have isolated some mAbs for the G protein that exhibit very high potency in neutralization assays, suggesting they have high potential as prophylactic and therapeutic molecules for humans. We propose here a series of aims that will discover new F mAb and contribute significantly to the development and characterization of human mAbs reactive to the F and G glycoproteins of Hendra and Nipah virus in preparation for clinical studies. The work will identify and fully characterize a panel of highly promising mAbs with the goal of identifying and selecting lead compounds and advancing their preclinical development. The work is organized in two major Specific Aims: Aim 1) Isolation of human mAbs from patients previously infected with henipavirus. In this Aim, human mAbs will be identified that recognize epitopes that are conserved across henipaviruses and neutralize those viruses at low concentration, with a focus on F-specific mAbs that neutralize virus and inhibit virus-cell fusion. Blood cells from individuals with natural prior exposure to Nipah virus will be used for study. These B cells will be interrogated with several well developed human mAb discovery platforms, including high-throughput single cell sorting and single cell RNAseq techniques, or converted to stable human hybridoma cell lines. B cell line supernatants or recombinant mAbs will be subjected to high-throughput screening to identify Abs that bind to Hendra and Nipah surface proteins and functionally inhibit virus replication. Aim 2: Develop Abs for the treatment of henipavirus infections. MAbs identified in Aim 1 will be tested for their broad recognition of conserved epitopes across all henipaviruses and for their ability to neutralize in culture. We also will investigate the role of Fc effector functions on antiviral activity, and the effect of long half-life Fc mutations that enable use of human mAbs for prophylaxis. Prioritized mAbs then will be tested for therapeutic efficacy in multiple animal models of infection including nonhuman primates. The leads will be selected, and CHO cell lines will be made by Mapp Biopharmaceutical for Ab production, in preparation for cGMP manufacture and IND planning. The work promises to yield a best-in-class mAb combination preparation for broad and potent activity against henipaviruses that can be used to treat or prevent human henipavirus infections.

SPECIFIC AIMS

The COVID-19 pandemic has highlighted the urgent need for rapid drug development and novel antiviral therapies to combat emerging pathogens. Nanobodies, derived from the heavy chain-only antibodies of camelid animals, offer a promising solution. These small (15 kDa) antigen-binding entities exhibit full antigen specificity and possess the unique ability to recognize otherwise inaccessible epitopes. Importantly, nanobodies can efficiently penetrate tissues and cross the blood-brain barrier, making them particularly effective in treating Central Nervous System (CNS) diseases caused by viral infections.

Henipaviruses (HNV) and arenaviruses (ARV) represent significant global public health risks, yet currently lack specific licensed treatments. To address this critical gap, prototype viruses from each viral group have been selected based on their impact. The research strategy developed for this project can be extended to encompass a broader range of viruses within these groups. Specifically, the study focuses on Nipah virus (NiV) and Hendra virus (HeV), both of which cause severe respiratory and CNS illnesses in humans and have alarmingly high mortality rates ranging from 40% to 90%. Additionally, Lassa virus (LASV) and Machupo virus (MACV), both belonging to the Arenavirus genus, are targeted due to their capacity to induce severe illness and fatalities. LASV is responsible for endemic Lassa fever in West Africa, while MACV is associated with Bolivian hemorrhagic fever.

The overarching objective of this study is to develop nanobody-based therapeutic approaches for combating HNV and ARV infections. The key targets for intervention are the viral entry machinery, specifically the glycoproteins G and F for HNV and the glycoprotein complex (GPC) for ARV. Employing a structure-based approach, the research aims to unravel the intricate mechanisms of viral neutralization, enhance nanobody engineering techniques, and design blood-brain barrier-penetrating nanobodies. The underlying hypothesis is that nanobodies can effectively counteract CNS diseases caused by these viruses.

To facilitate the development of nanobody-based therapies, three distinct nanobody development platforms will be employed. These include camelid animals, nanomice, and low-immunogenic human nanobody libraries that closely mimic the natural human antibody heavy chain variable domain. Antigen-specific enriched nanobody libraries will be deep-sequenced to ensure comprehensive coverage. Leveraging these platforms will enable rapid identification of nanobody leads with minimal optimization required for clinical use. The project will be conducted with following specific aims.

Aim 1: To identify cross-reactive nanobodies to HNVs. Given their high degree of glycoprotein (F and G) similarity, it feasible to identify cross-reactive neutralizing nanobodies against both HeV and NiV for broad protection. We will employ sequential immunization and antigen panning strategies to identify nanobodies that cross-recognize HeV and NiV. Furthermore, the neutralizing activity of the nanobodies will be evaluated by RP2.

Aim 2: Identification of nanobodies to ARVs. To overcome the challenges posed by the heavily glycosylated and conformationally labile surface glycoprotein (GPC) of Arenaviruses (ARVs), we will develop nanobodies as a therapy option. Nanobodies can potentially penetrate the GPC's glycan shield and neutralize the virus. We will assess individual antigen-specific nanobodies by evaluating their neutralization activity in RP1.

Aim 3: Structural characterization. To gain insight into nanobody epitopes and neutralization mechanisms, we will employ structural characterization techniques such as X-ray crystallography and Electron Microscopy Single Particle Analysis. This structural analysis will be instrumental in guiding the design and optimization of nanobody-based therapeutic interventions.

Aim 4: Enhancing efficacy and CNS bioavailability of nanobodies. We will engineer a combination of nanobodies targeting multiple epitopes using rational design. Additionally, we will enhance CNS bioavailability by engineering bi-specific nanobody therapy that utilizes transferrin receptor 1 (TfR1) for improved blood-brain barrier (BBB) penetration. Finally, we will compare the efficacy of the original nanobodies (from Aim 1 and 2) with the engineered nanobodies in either the rCedV-NiV-B chimera mouse model in RP3 (for HNVs) or animal models of ARV developed at UTMB in RP1.

Aim 5: Expanding to other members of the viral groups in Center Phase 2. Building upon the knowledge gained from Aims 1-4, we will extend our research to develop nanobody therapies targeting other members of HNV and ARV, such as *Mojiang virus, Langya virus, Lujo virus, or Chapare virus*. The research strategy developed in the earlier aims will be applied and tested for these additional viral targets.

By pursuing these specific aims, our objective is to advance the development of nanobody-based therapies for HNV and ARV infections. The ultimate goal is to contribute to the development of effective treatments against these viral pathogens and mitigate the global public health risks they pose.

Project Summary (OVERALL)

Emerging and reemerging pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. Among the many groups of these types of pathogens are viral zoonoses within the family **Paramyxoviridae** and order ***Bunyavirales***. The *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans worldwide with associated high morbidity and mortality. A host of arenaviruses viruses, including Lassa, Machupo, Lujo, and Chapare viruses and the Nairovirus-Crimean-Congo Hemorrhagic Fever Virus and are also included among the World Health Organization's (WHO) List of Priority Pathogens. The zoonotic henipaviruses (HNVs) including Nipah virus (NiV) and Hendra virus (HeV), in the **Paramyxoviridae** family are also on the WHO Priority Pathogens . ~~Also, within the Priority Pathogens list is Nipah virus and henipaviral disease which includes Hendra virus which are zoonotic henipaviruses (HNVs) in the family~~ ~~**Paramyxoviridae**.~~ These HNVs have a uniquely broad host tropism, causing an often fatal respiratory and/or neurological disease, and are significant biothreats to humans and livestock in South and South East Asia and Australia. All these RNA virus threats to global public health are heightened due to the absence of approved vaccines or therapeutics. There is a clear unmet need for countermeasures to address the threat of natural outbreaks, epidemics, or deliberate release. The Paramyxoviridae and Bunyavirales Vaccines and Antibodies (PABVAX) is a well-integrated Center conducting a set of discovery and translational research programs composed of a Data Management Core, 3 Scientific Cores, and 5 Research Projects (RPs). The synergistic activities of Center partners will be focused on: developing, testing, and translating, prototype subunit vaccines ~~using~~ employing a novel~~,~~ adjuvanted~~,~~ dissolvable, microneedle patch (MNP) vaccine platform; developing new virus and animal model tools; and developing and testing monoclonal antibody and nanobody prototypes to foster pandemic preparedness. The level of ~~certain~~ vaccines and antibody countermeasure advancements among the RPs is a major strength and advantage of this Center. The Center's overall objective is to elucidate key principles for vaccine and antibody-based countermeasures in developing prototype candidates against a selection of these viruses. In the out years of the effort, the Center will exploit these learned key principles in a "plug and play" ~~demonstration~~ approach against related viruses.

Commented [LZ1]: Clarify? Not sure what the intent of this sentence is…

Relevance (OVERALL)

Select zoonotic RNA viruses within the Paramyxoviridae and Bunyavirales ~~are a~~represent a continuous threat to global publich health ~~continuous infectious disease and potentially pandemic threats to global public health because of~~due to their ease of dissemination and/or ~~abilities to~~transmissibility ~~transmit~~ from person-to-person ~~,~~ and ~~produce~~ result in significant morbidity and mortality, ~~and require~~thus requiring special actions for public health preparedness. These viral threats to global public health are heightened due to the absence of approved vaccines or therapeutics. There is a clear unmet need for vaccines and antibody-based therapeutics to address these threats that can arise from natural outbreaks, epidemics, or bio-weaponization and deliberate release.

From:	Haller, Sherry on behalf of Haller, Sherry <shhaller@UTMB.EDU>
To:	Golovko, Georgiy
Cc:	Geisbert, Thomas W.; CHRISTOPHER BRODER
Subject:	RE: Data Mgmt Core Final Documents - UTMB/USUHS U19
Date:	Tuesday, May 30, 2023 2:55:57 PM
Attachments:	REVAMPP_Org_Chart_Core_Enhancement2.pdf F-U19 - UTMB_CORE C - (UPITT and UMD) - Abstract-Project Summary - 05-19-2023.docx Abstract-Summary.docx UTMB Core E-Project Summary and Relevance-25May23.docx Abstract-RP2-Broder-v2pdf.pdf Abstract-RP3-Schaefer-v2.pdf RP4 geisbert project Summary_Abstrast_30 lines.docx RP5-Specific Aim_U19-UTMB_USU_23-05-18.pdf Overall-PABVAX-Project Summary-and-Relevance-V1-05232023_DA_LZ.docx image001.png

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George,
An org chart is attached as well as abstracts/specific aims for the cores and research projects and overall for the center goals. (all but RP1).

Core C: Microneedle Patch Vaccines (UMD; Alexander Andrianov/UPitt; Louis Falo/Emrullah Korkmas)
Core D: Early and IND-Enabling Translational Research for Vaccines and mAbs (MappBio; Larry Zeitlin/Dafna Abelson- WSU; Bonnie Gunn-UTMB; Tom Geisbert)
Core E: Animal Model Development and Preclinical Evaluation (UTMB; Tom Geisbert/Courtney Woolsey)
RP1: Antigen Design and Testing of Arena virus and Nairovirus Vaccines (UTMB; Bob Cross- WSU; Bonnie Gunn)
RP2: Vaccines and Antibodies to Henipaviruses (USUHS; Chris Broder/Amaya Moushimi/Antony Dimitrov)
RP3: Cedar henipavirus Animal Model Development (USUHS; Brian Schaefer)
RP4: Monoclonal Antibodies Against Henipaviruses, Arenaviruses, and Nairoviruses (VUMC; Jim Crowe)
RP5: Nanovody Therapeutics Against Henipaviruses and Arenaviruses (OSU; Kai Xu/GSU; Jianliang Xu)

Let me know if this helps or you need anything else.

Best,
Sherry

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Emerging Infectious Diseases

From: Golovko, Georgiy <gegolovk@UTMB.EDU>
Sent: Tuesday, May 30, 2023 1:13 PM
To: Haller, Sherry <shhaller@UTMB.EDU>
Cc: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Re: Data Mgmt Core Final Documents - UTMB/USUHS U19

Hi Sherry,

The following documents are not needed for the data management core:

- Resource sharing plan - I'm providing the data management sharing plan instead for the final submission.
- Authentication of key biologicals - don't have any.

For an Abstract, I would need the organizational chart for the entire proposal to integrate what was asked for earlier.
Based on other U19, this is what my abstract will include:

- Aims " Aim 1 will..... In Aim 2 we will....". And include 1-2 sentences following each aim to elaborate a little.
- Overall goal
- **Mention of Cores and RPs to emphasize synergy**
- End with a statement about how the team of experts at UTMB has been assembled

I can skip the synergy part for now, add it based on the organizational chart if we have one, or pull from the methods from core and RPs.

Please advise.

Best,
George

From: Haller, Sherry <shhaller@UTMB.EDU>
Sent: Tuesday, May 30, 2023 12:22 PM
To: Golovko, Georgiy <gegolovk@UTMB.EDU>
Cc: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>
Subject: Data Mgmt Core Final Documents - UTMB/USUHS U19

Hello Dr. Golovko,
This is a reminder of the documents that we still need to get from you for Core B (Data Mgmt) on the UTMB/USUHS U19 application. Our administrative review is this coming Thursday, June 1, so we will need all of the items listed below in final form by **EOB tomorrow, Wednesday, May 31**.

- Abstract – 30 lines
- Resource sharing plan
- Authentication of key biologicals

For the final application, please provide the following by **EOB Monday June 5** *(hard deadline!).*

- Research Strategy + Bibliography

Please let me know if you have any questions about these or have any concerns about getting them back to us by the deadlines.
Thank you,

Sherry L. Haller, PhD
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OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

NAME: Schaefer, Brian Charles

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: Professor of Microbiology and Immunology, and Director (Interim), Emerging Infectious Diseases Graduate Program

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Massachusetts Institute of Technology	B.S.	1989	Biology
Harvard University	PhD	1995	Immunology
Harvard Univ/Dana Farber Cancer Institute	(Postdoc)	1995-1996	Tumor Virology
National Jewish Medical and Research Center	(Postdoc)	1996-2002	Immunology

A. Personal Statement

My group has historically focused on two major research programs. Our longest-standing program involves elucidation of mechanisms of signal transduction in immune cells and regulation of immune cell effector function, with a particular emphasis on the pathway that connects the T cell receptor (TCR) signaling to the transcription factor, NF-κB. This work has also stimulated our studies of the function of an understudied CARD protein, CARD19, which was originally (incorrectly) identified as a negative regulator of TCR signaling to NF-κB. These studies have resulted in numerous publications that define novel signaling mechanisms in the CARD11-BCL10-MALT1 signaling cascade and additional pathways. Our second major area of research is centered on immune responses in the central nervous system. Our initial work in this area involved elucidation of the role of the immune response initiated by traumatic brain injury, particularly NF-κB-mediated inflammatory responses. However, we have now shifted our emphasis to translational research on immune responses to neurotropic viruses, with an overall goal of developing immunotherapy strategies that are broadly effective against this dangerous class of human pathogens. This work is focused on lyssaviruses and henipaviruses, due to their neurotropic nature and propensity to cause severe human disease with a very high fatality rate. Our use of bioluminescence imaging to track viral replication has been a particularly important innovation for this work, as it allows us to collect highly granular data regarding viral spread and amplification from individual animals in a longitudinal manner. Specific current projects include a DoD-funded translational study in which we are defining the utility of a novel, mAb-based anti-lyssavirus immunotherapy against rabies strains circulating in Europe and Africa, and a study to develop novel henipavirus therapies (both are collaborations with Dr. Christopher Broder's group).

I am ideally suited to be PI of RP3: *Cedar henipavirus animal model*, as my group has established the only mouse model of Cedar virus infection (to our knowledge), and we now have several years of experience in the development of this very important BSL-2 animal model to investigate mechanisms of henipavirus pathogenesis, and to test efficacy of novel therapies. The following are notable ongoing projects:

HU00011920118 (**PI: Schaefer**), USU Center for Global Health Engagement
09/30/2019 – 09/29/2022 (NCE to 9/30/23)
"Efficacy testing of a novel human monoclonal antibody therapy for late-stage rabies/lyssavirus infection"

U19 AI142764-01 (PI:Broder; **Schaefer Co-I**), NIH/NIAID
03/01/2019 – 02/29/2024
"Advancement of Vaccines and Therapies for Henipaviruses"

R01 AI125552 (**PI: Schaefer**). NIH/NIAID
06/01/2017 – 05/31/2022 (NCE to 5/31/2024)
“T cell receptor-activated autophagy as a regulator of T cell effector responses”

HU00011921048 (**PI: Schaefer**), USU Multi-domain Operations Research (MDO)
02/28/2023 - 09/30/2025
“Defining the mechanism of enhancement of the pathogen-directed host adaptive immune response following anti-lyssavirus monoclonal antibody therapy”

B. Positions, Scientific Appointments, and Honors

Positions

2017 - current	Professor (with tenure) in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
2009 - 2017	Associate Professor (with tenure) in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
2002 - 2009	Assistant Professor in Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD.
1996 - 2002	Post-doctoral Research Associate, Howard Hughes Medical Research Institute, National Jewish Medical and Research Center, Denver, CO.
1995 - 1996	Research Fellow in Pathology, Harvard University, Dana-Farber Cancer Institute

Professional Memberships and Service

2011 - 2016	NIH CMIB (Cell and Molecular Immunology-B) study section for review of R01/R21 grants (Ad hoc member Feb & May 2011, Feb & May 2013, Feb 2014, Feb 2015, May 2016)
2008, 2021	ZRG1 CB-D 30 Confocal Microscopy Shared Instrumentation NIH Study Section (Ad hoc member)
2006 - current	American Society for Cell Biology (ASCB)
2006 - current	American Association of Immunologists (AAI)
2006 - 2007	ZRG1 IMM-L 29 L, DBBD Minority and Disability Predoctoral Fellowship NIH Study Section (Ad hoc member)
2005-2006	ZRG1 F07 Immunology Fellowship and AREA NIH Study Section (Ad hoc member)

Honors

2016	Uniformed Services University Henry Wu Award for excellence in basic science research
2005 - 2008	Dana Foundation Program in Brain and Immuno-Imaging
2004 - 2006	Kimmel Scholar - Sidney Kimmel Foundation for Cancer Research
2000 - 2002	Leukemia and Lymphoma Society Special Fellowship
1989 - 1992	Office of Naval Research Graduate Research Fellowship
1989	Phi Beta Kappa
(1989)	National Science Foundation Graduate Research Fellowship (Declined)

Patents

2007	US Patent 7,169,570: *Method to Identify Regulators of Cellular Activation Using Bcl10* – BC Schaefer, JW Kappler and P Marrack

Mouse strains developed

2001	C57BL/6-Tg(UBC-GFP)30Scha/J: Ubiquitously expressed EGFP transgene on a pure C57BL/6 background. Repository strain at Jackson Laboratory (stock# 004353). This strain has been used and cited in hundreds of publications.

C. Contributions to Science

1. My Ph.D. thesis studies involved identification of the molecular mechanisms controlling distinct latency-associated Epstein Barr virus (EBV) transcriptional programs. Prior to my dissertation work, it was known that there were three forms of EBV latency which displayed two distinct phenotypes with regard to expression of EBV nuclear antigens (EBNAs): Type III latency is characterized by expression of all six EBV nuclear antigens (EBNA1-EBNA6) whereas Type I and Type II latency is characterized by expression of EBNA1 only. The molecular basis restricted EBNA expression in Types I and II latency was not known. My work demonstrated the existence of a novel EBV promoter "Qp," responsible for driving exclusive expression of EBNA1 in Types I and II latency. My studies also showed that the choice between latency programs was contingent on whether the Type III latency promoter, Cp, is methylated in the host cell. Collectively, this work contributed significantly to the current understanding of regulation of EBV latency, which is relevant to elucidating how EBV persists for life in immunocompetent individuals, how EBV emerges as a life-threatening pathogen in specific immunodeficiencies, and how specific programs of EBV latency may be related to EBV-associated tumors, such as Burkitt lymphoma.

    a. #**Schaefer BC**, Woisetschlaeger M, Strominger JL, and Speck SH. Exclusive expression of Epstein-Barr virus nuclear antigen 1 in Burkitt lymphoma arises from a third promoter, distinct from the promoters used in latently infected lymphocytes. Proc. Natl. Acad. Sci. USA 1991; 88:6550-6554.
    #Cited >130 times.
    b. ***Schaefer BC,** Strominger JL, and Speck SH. Redefining the Epstein-Barr virus-encoded nuclear antigen EBNA1 gene promoter and transcription initiation site in group I Burkitt's lymphoma cell lines. Proc. Natl. Acad. Sci. USA 1995; 92:10560-10564
    ***Cited >190 times.**
    c. †**Schaefer BC**, Strominger JL, and Speck SH. Host-cell-determined methylation of specific Epstein-Barr virus promoters regulates the choice between distinct viral latency programs. Mol. Cell Biol. 1997; 17:364-377.
    †Cited >100 times.
    d. ‡**Schaefer BC**, Paulson E, Strominger JL, and Speck SH. Constitutive activation of Epstein-Barr virus (EBV) nuclear antigen 1 gene transcription by IRF1 and IRF2 during restricted EBV latency. Mol. Cell Biol. 1997; 17:873-886
    ‡Cited >85 times.

2. My early work as an independent investigator involved determining whether NF-κB signaling proteins undergo molecular redistribution in response to TCR signaling. Previous work had shown that several different signaling molecules are recruited to "supramolecular activation clusters (SMACs)," membrane-proximal enrichments of signaling proteins that form on the cytoplasmic face of the antigen-engaged TCR. An assumption in the field, prior to our work, was that SMAC recruitment would be a shared feature of all cytoplasmic proteins important in TCR signaling. Surprisingly, our investigations demonstrated that NF-κB signaling proteins, including Bcl10 and Malt, redistribute to punctate cytoplasmic structures, which we named POLKADOTS. Our ongoing signal transduction work has involved mechanistic studies connecting the POLKADOTS signalosome to TCR activation of NF-κB. Key findings include demonstration that (i) TCR activation of NF-κB is *digital*. In other words, in contrast to pharmacological stimulation of NF-κB (PMA + ionomycin), which results in graded activation of NF-κB as the stimulus is increased, increasing TCR ligand changes only the *number* of T cells that respond, but not the *intensity* of the response; (ii) polyubiquitination of Bcl10 leads to recruitment to pre-existing p62 "speckles," forming the POLKADOTS signalosome; (iii) the POLKADOTS signalosome is required for activation of the IKK complex; (iv) the POLKADOTS signalosome contemporaneously directs a signal dampening activity that involves a highly novel regulatory mechanism: *selective* autophagy of Bcl10, which results in degradation of Bcl10, but not its direct binding parther, Malt1; (v) effector T cells form POLKADOTS signalosomes and use autophagy to degrade Bcl10, whereas naïve T cells use a distinct mechanism of NF-κB activation that appears to be independent of p62, involving recruitment of signaling proteins to SMACs. Collectively, these contributions provide key mechanistic details regarding how signals are transmitted from the TCR to the NF-κB transcription factor. Most recently, we have collaborated with Drs. Wolfgang Losert and Arpita Upadhyaya, biophysicists at the

University of Maryland, to define biophysical features of TCR-mediated activation and degradation of Bcl10. Our study with Dr. Losert in PLOS Computational biology has shown that the regulated polymerization and degradation of Bcl10 has characteristics of an excitable system, providing new insights regarding the digital behavior of this signaling module.

   a. #Kingeter LM, Paul S, Maynard SK, Cartwright NG, and **Schaefer BC**. Cutting Edge: T cell receptor ligation triggers digital activation of NF-κB. Journal of Immunology. 2010; 185:4520-4.
      #Cited >90 times.
   b. *Paul S, Kashyap AK, Jia W, He Y-W, and **Schaefer BC**. Selective autophagy of the adaptor protein Bcl10 modulates T cell receptor activation of NF-κB. Immunity. 2012; 36:947-58.
      ***Cited >200 times; *Faculty of 1000* recommended**
   c. Paul S, Traver MK, Kashyap AK, Washington MA, Latoche JR, and **Schaefer BC**. T cell receptor signals to NF-κB are transmitted by a cytosolic p62-Bcl10-Malt1-IKK signalosome. Science Signaling. 2014; 7:ra45.
   d. Campanello L, Traver MK, Shroff H, †**Schaefer BC**, †Losert W. Signaling through polymerization and degradation: Analysis and simulations of T cell activation mediated by Bcl10. PLOS Computational Biology. 2021 May 20;17(5):e1007986.
      †co-corresponding authors

3. During our studies on TCR signaling to NF-κB, we became interested in the understudied CARD protein, CARD19. Although CARD19 was initially described as a cytoplasmic negative regulator of TCR signaling to NF-κB, we found that this work was based on analysis of a partially spliced cDNA clone, and that the conclusions of this initial study were therefore spurious. Our work with a properly spliced CARD19 cDNA showed that CARD19 is a mitochondrial protein that plays no role in TCR activation of NF-κB. Our latest work has shown that CARD19 interacts with the mitochondrial cristae organizing system (MICOS), and that it plays a role in determining cristae morphology. Notably, 3 of the 7 current publications focused on CARD19 were generated by my laboratory or as a collaboration with my group. We are currently performing further studies to determine whether CARD19 regulates other aspects of mitochondrial function via its interaction with MICOS components.

   a. Rios KE, Kashyap AK, Maynard SK, Washington M, Paul S and **Schaefer BC**. CARD19, the protein formerly known as BinCARD, is a mitochondrial protein that does not regulate Bcl10-dependent NF-κB activation after TCR engagement. Cell. Immunol., 2020; 356:104179.
   b. Bjanes E, Sillas RG, Matsuda R, Demarco B, Fettrelet T, DeLaney AA, Rodriguez Lopez EM, Grubaugh D, Wynosky-Dolfi M, Philip NH, Krespan E, Tovar D, Joannas L, Beiting DP, Henao-Meija J, **Schaefer BC**, Chen KW, Broz P, and Brodsky I. Genetic targeting of Card19 is linked to disrupted NINJ1 expression, impaired cell lysis, and increased susceptibility to Yersinia infection. PLoS Pathog. 2021;17(10):e1009967.
   c. Rios KE, Zhou M, Lott N, Beauregard C, McDaniel DP, Conrads TP, and **Schaefer BC**. CARD19 interacts with mitochondrial contact site and cristae organizing system constituent proteins and regulates cristae morphology. Cells, 2022 11(7):1175.

4. A second major research focus in my lab is the study of immune responses in the central nervous system. Our initial studies focused on inflammatory responses in traumatic brain injury (TBI), using mice as a model system: The first study demonstrated that the gene expression program induced by mild vs. severe TBI is remarkably similar, with the main distinction being that the expression of certain inflammatory genes remains persistently elevated in severe TBI. The second study presented evidence that the anti-inflammatory drug salsalate is a candidate therapy for TBI, reducing inflammation and improving functional recovery, as assessed by behavior assays. Our more recent work has focused on developing novel animal models and immunotherapies for neurotropic viral infections. Our published work on this project includes a description of our methodology for longitudinal tracing of lyssavirus infections and a description of isolation and in vitro characterization of two anti-lyssavirus neutralizing mAbs.

   a. *Lagraoui M, Latoche JR, Cartwright NG, Sukumar G, Dalgard CL, **Schaefer BC**. Controlled cortical impact and craniotomy induce strikingly similar profiles of inflammatory gene expression, but with distinct kinetics. Front Neurol. 2012; 3:155.
      ***Cited >100 times**

   b. Lagraoui M, Sukumar G, Latoche JR, Maynard S, Dalgard CL, **Schaefer BC**. Salsalate treatment following traumatic brain injury reduces inflammation and promotes a neuroprotective and neurogenic transcriptional response with concomitant functional recovery. Brain, Behavior and Immunity. 2017; 61:96-109.
   c. Mastraccio KE, Huaman C, Warrilow D, Smith GA, Craig SB, Weir DL, Laing ED, Smith IL, Broder CC, and **Schaefer BC**. Establishment of a longitudinal pre-clinical model of lyssavirus infection. J. Virol. Methods. 2020; 281:113882.
   d. Weir DL, Coggins SA, Vu B, Coertse J, Yan L, Smith I, Laing ED, Markotter S, Broder CC and **Schaefer BC**. Isolation and Characterization of Cross-Reactive Human Monoclonal Antibodies That Potently Neutralize Australian Bat Lyssavirus Variants and Other Phylogroup 1 Lyssaviruses. Viruses. 2021; 13:391.

5. A distinct area of emphasis in my career has been the development of new tools for research and the optimization of existing tools. Among many publications in this area, I have edited a protocols book and written a review on methodology for identification of cDNA ends by RACE (which included an optimized protocol). I produced a C57BL/6 transgenic mouse strain ubiquitously expressing GFP, which has been a highly utilized tool for cell transfer studies. I also generated a set of retroviral vectors for rapid and reliable creation of stable cell lines. My lab has published optimized protocols for visualizing NF-κB signaling intermediates via confocal microscopy, and for quantifying NF-κB activation using imaging flow cytometry. We have most recently described our methodology for using bioluminescence imaging to semi-quantitatively trace lyssavirus infection in laboratory mice. A central goal of this work is to facilitate progress across the biomedical research community, through the development and distribution of better tools and clear explanation of how to reliably execute challenging techniques.

   a. **†Schaefer BC**. Revolutions in rapid amplification of cDNA ends: new strategies for polymerase chain reaction cloning of full-length cDNA ends. Anal Biochem. 1995 May 20;227(2):255-73.
      †Cited >475 times
   b. **§Schaefer BC**, Schaefer ML, Kappler JW, Marrack P, Kedl RM. Observation of antigen-dependent CD8+ T-cell/ dendritic cell interactions in vivo. Cell Immunol. 2001 Dec 15;214(2):110-22.
      §Describes creation and use of Jackson stock strain 004353 - C57BL/6-Tg(UBC-GFP)30Scha/J. Publication cited >400 times, strain cited many additional times.
   c. Traver MK, Paul S, and **Schaefer BC**. T Cell Receptor Activation of NF-κB in Effector T Cells: Visualizing Signaling Events Within and Beyond the Cytoplasmic Domain of the Immunological Synapse. Methods in Molecular Biology, 2017; 1584:101-27.
   d. Mastraccio KE, Huaman C, Laing ED, Broder CC, and **Schaefer BC**. Longitudinal Tracing of Lyssavirus Infection in Mice via In Vivo Bioluminescence Imaging. In: *Bioluminescence*. Methods in Molecular Biology, Kim, SB. (ed.), 2022; **2524**:369-394.

Complete publications from My Bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/brian.schaefer.1/bibliography/public/

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

NAME: Broder, Christopher C.

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6)

POSITION TITLE: Professor of Microbiology, Immunology and Emerging Infectious Diseases

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Florida Institute of Technology, Melbourne, FL	B.S.	1983	Biological Science
Florida Institute of Technology, Melbourne, FL	M.S.	1985	Molecular Biology
University of Florida, Gainesville, FL	Ph.D.	1989	Immunology and Med-Micro

A. Personal Statement

The main objective of this ReVAMPP proposal is to support Pandemic Preparedness through the study and advancement of broadly effective countermeasures for pathogenic henipaviruses and bunyaviruses, through advanced machine learning-enabled design of prototype pathogen vaccines and antibodies. I will serve as co-investigator, with **Thomas Geisbert** (Project-Lead) of **Research Project 5 (RP5): Animal models and vaccine evaluation.** I have researched enveloped virus-host cell interactions for 34 years. 1999, I established a collaborative international group of experts in Hendra and Nipah virus biology, and over an 11-year period I was the PI of two multi-component, international, NIAID, U01 Cooperative Agreements, and the Co-PI of a third U01 with RP5 Lead (Geisbert); all focused on Nipah virus and Hendra virus research. Collaborative henipavirus work includes structural biochemistry on the viral G and F glycoproteins including most recently with **David Veesler,** Overall Center Co-PI; animal models and *in vivo* pathogenesis, and the development and testing of vaccines and therapeutics; the discovery of the entry receptors for Hendra and Nipah (ephrin-B2/B3) and the development of the feline, ferret and African green monkey models (with **Geisbert**) of Hendra and Nipah pathogenesis; development of antiviral mouse and human monoclonal antibodies (mAb) including mAb m102.4 that successfully completed a Phase 1 trial in Australia (2020) and to date has been used by emergency protocol in 17 people in Australia and 1 person in the US for Hendra/Nipah post-exposure treatment; developed the Hendra/Nipah subunit vaccine based on soluble Hendra G (HeV-sG); one version called Equivac® HeV (Zoetis, Inc.), the first commercialized vaccine to a BSL-4 agent. The HeV-sG vaccine is in Phase I trial in the US (Auro Vaccines/PATH/CEPI). More recently I developed a reverse genetics platform for generating recombinant Cedar henipavirus (rCedV) that can be utilized at BSL-2 for both high-throughput antiviral drug discovery and rCedV chimeras for performing rapid neutralization tests for Nipah and Hendra. I have the expertise in many aspects of the henipavirus-related project goals, and for the past 24 years I have led both basic and translational research projects in a broad area of study on Nipah and Hendra virus. My lab's role will be focused on the development of new reporter viruses with a focus on the recently discovered Langya henipavirus as a new 'prototype henipavirus' distinct from the Nipah/Hendra/Cedar henipaviruses; and my lab will use these new tools, and existing and recently developed platforms to assess new henipavirus vaccines and neutralizing antibodies that will be developed by the Center's Scientific Cores and Research Projects.

Ongoing and recently completed projects that I would like to highlight:

U19 AI142764. NIAID, Center of Excellence for Translational Research (CETR): Role: PI
03/20/19-02/29/24. "Advancement of Vaccines and Therapies for Henipaviruses"

U19 AI171413. NIAID AViDD: "UTMB-Novartis Alliance for Pandemic Preparedness": PIs: Diagana and Shi.
05/01/2022-04/30/2027; Sub-project 3: Henipaviruses. Role: Co-PI with Geisbert

CRADA; Auro Vaccines, LLC. Egan (PI). CEPI (Coalition for Epidemic Preparedness Innovations): Role: Co-PI
07/01/18-06/30/2023; "A Subunit Vaccine (HeV-sG) to Protect against Nipah and Hendra Diseases"

Citations:

1. Amaya M, Cheng H, Borisevich V, Navaratnarajah CK, Cattaneo R, Cooper L, Moore TW, Gaisina IN, Geisbert TW, Rong L, **Broder CC***. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral Res. 2021 May 30:105084. doi: 10.1016/j.antiviral.2021.105084. PMCID: PMC8631057
2. Playford EG, Munro T, Mahler SM, Elliott S, Gerometta M, Hoger KL, Jones ML, Griffin P, Lynch KD, Carroll H, El Saadi D, Gilmour ME, Hughes B, Hughes K, Huang E, de Bakker C, Klein R, Scher MG, Smith IL, Wang LF, Lambert SB, Dimitrov DS, Gray PP, **Broder CC**. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. Lancet Infect Dis. 2020 Apr;20(4):445-454. PMID: 32027842
3. Geisbert TW, Bobb K, Borisevich V, Geisbert JB, Agans KN, Cross RW, Prasad AN, Fenton KA, Yu H, Fouts TR, **Broder CC***, Dimitrov AS*. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. NPJ Vaccines. 2021 Feb 8;6(1):23. doi: 10.1038/s41541-021-00284-w. PMCID: PMC6789926
4. Amaya, M, Yin R, Yan L, Borisevich V, Adhikari BN, Bennett A, Malagon F, Cer RZ, Bishop-Lilly KA, Dimitrov AS, Cross RW, Geisbert TW, **Broder CC***. A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. Viruses, Apr, 2023.

B. Positions, Scientific Appointments

Positions and Employment

2018-pres.	Chair, Department of Microbiology and Immunology, USU, Bethesda, Maryland.
2006-2018	Director, Emerging Infectious Diseases Graduate Program, USUHS, Bethesda, Maryland.
2005-pres.	Professor, Department of Microbiology and Immunology, Joint appointment, Emerging Infectious Diseases Graduate Program, USUHS, Bethesda, Maryland.
2000-2005	Associate Professor, Department of Microbiology and Immunology, Joint appointment, Emerging Infectious Diseases Graduate Program, USUHS, Bethesda, Maryland.
1996-2000	Assistant Professor, Department of Microbiology and Immunology, Joint appointment, Molecular and Cell Biology Graduate Program, Uniformed Services University, Bethesda, Maryland.
1993-1996	IRTA Fellow, LVD, NIAID, NIH, Bethesda, Maryland.
1990-1992	National Research Council, Research Associate, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

Other Experience and Professional Affiliations

2022-	Member, Virus Expert Panel for Disease X Vaccine Libraries (CEPI).
2018-	Nipah Task Force (CEPI).
2018-	Nipah Therapeutics Protocol Team; ICMR, NIAID, WHO.
2011-	Member, Discontools Nipah Virus Infection Panel Expert Group. Gap analysis. International Federation for Animal Health Europe, Brussels, Belgium.
	Editorial boards, dates since joining: *Journal of Virology-07’*, *Virology-10’*, *Viruses-11’*, *Pathogens-11;* V*irologica Sinica-12’*
2009-	Member, National Veterinary Stockpile Nipah virus Countermeasures Workshop; United States Department of Agriculture. Geelong, Australia.

Honors and Awards

2022	The Carol Johns Medal (highest honor USU faculty can bestow on a fellow faculty member).
2021	Military Health System Research Symposium (MHSRS) 2021 Outstanding Research Accomplishment Team Award. (COVID-19 / SARS-CoV-2 serology research).
2020	Military Health System Research Symposium (MHSRS) 2020 Outstanding Individual Research Accomplishment by an Individual Senior Researcher. (Hendra and Nipah virus countermeasures).
2020	Inaugural, 2020 Federal Laboratory Consortium (FLC) Impact Award.

2019	USU Outstanding Biomedical Graduate Educator Award.
2019	The University of Florida, College of Medicine, “Wall of Fame”.
2019	Dean’s Award for Leadership, University of Florida, College of Medicine.
2019	Federal Laboratory Consortium (FLC) Award for Excellence in Technology Transfer.
2016	The James J. Leonard Award for Excellence in Translational/Clinical Research.
2014	The Cinda Helke Award for Excellence in Graduate Student Advocacy.
2013	The 3rd Sidney Pestka Lecture; 22nd Annual Philadelphia Infection & Immunity Forum.
2013	Federal Laboratory Consortium (FLC) Award for Excellence in Technology Transfer.
2013	Second Finalist for the Australian Infectious Diseases Research Centre Eureka Prize.
2013	The CSIRO Chairman’s Medal. The Commonwealth Scientific and Industrial Research Organisation (CSIRO); Australia's national science agency.
2008	The Henry Wu Award for Excellence in Basic Science Research.
1996	The Fellows Award for Research Excellence, Office of Science Education, NIH
1996-1997	AAAS: Breakthrough of the Year, Science Magazine; and the Newcomb Cleveland Prize
1990-1992	National Research Council Research Associateship Award
1989	Medical Guild Graduate Research Award, University of Florida College of Medicine

Patents issued and significant technology licenses

- Bacterial plasmin receptors as fibrinolytic agents: U.S. Pat. No. 5,237,050.
- Oligomeric HIV-1 envelope glycoproteins (gp140): U.S. Pat. Nos. 6,039,957 and 6,171,596.
- HIV-1 Envelope Glycoprotein Oligomer and Methods of Use. U.S. Pat. No. 8,597658.
- CCR5 DNA, new animal models and therapeutic agents for HIV infection: U.S. Pat. No. 7,151,087.
- Cells expressing both human CD4 and CXCR4 associated with HIV infection: U.S. Pat. No. 6,197,578.
- Compositions and methods for the inhibition of membrane fusion by paramyxoviruses: U.S. Pat. Nos. 7,666,431 and 8,114,410.
- Soluble Forms of Hendra and Nipah Virus G Glycoprotein. Australian Pat. No. 2005327194. U.S. Pat. Nos. 8,865,171; 9,045,532; 9,056,902; 9,533,038, 10,053,495.
- Soluble Forms of Hendra and Nipah Virus F Glycoprotein and Uses Thereof: Australian Patent No. 2013276968. U.S. Pat. Nos. 10,040,825; 10,590,172.
- Human monoclonal antibodies against Hendra and Nipah viruses. U.S. Pat. Nos. 7,988,971; 8,313,746, 8,858,938.
- Antibodies against F glycoprotein of Hendra and Nipah viruses. U.S. Pat. Nos. 9,982,038 and 10,738,104
- Cedar virus and Methods of Use. U.S. Patent No., 10,227,664.
- Recombinant Cedar Virus Chimeras. Provisional. Atty. Dkt. No. 103783-0276.
- Hendra sG: Licensed to Zoetis, Inc. (Pfizer Animal Health). Equivac ® HeV; Nov, 2012, Australia.
- Hendra sG: Licensed to AuroVaccines, Aurobindo Pharma USA., Nipah/Hendra human vaccine candidate.
- Monoclonal antibodies to Henipaviruses. Absolute Antibody Ltd, United Kingdom.
- Soluble Nipah and Hendra virus F and G glycoproteins. The Native Antigen Company Ltd, United Kingdom.

C. Contributions to Science

1. My Ph.D. thesis studies centered on the discovery and characterization of a specific receptor for human plasmin on Group A Streptococci during my rotation project as a first year student. My studies revealed that certain group A streptococci elaborated surface receptors that could bind selectively a key fibrinolytic enzyme, plasmin. The bacterium-bound plasmin remained enzymatically active including its ability to hydrolyze a fibrin clot and could not be inhibited by its physiological regulator, alpha 2-plasmin inhibitor. Since these organisms produced streptokinase producing an active enzyme that can convert plasminogen to plasmin, they could accelerate the destruction of the extracellular matrix environment: a model for the "flesh-eating streptococci".

a. Lottenberg, R., **C.C. Broder**, and M.D.P. Boyle. Identification of a Specific Receptor for Plasmin on a Group A Streptococcus. Infection and Immunity. 55(8):1914-1918, 1987.

b. **Broder, C.C.**, R. Lottenberg, and M.D.P. Boyle. Mapping of the Domain of Human Plasmin Recognized by its Unique Group A Streptococcal Receptor. Infection and Immunity. 57(9): 2597-2605, 1989.

c. **Broder, CC**, R Lottenberg, GO vonMering, K. Johnston and MDP Boyle. Isolation of a prokaryotic plasmin receptor: relationship to a plasminogen activator produced by the same microorganism. J. Biol. Chem. 266:4922-28, 1991.
d. Lottenberg, R., **C.C. Broder**, M.D.P. Boyle, S.J. Kain, B.L. Schroeder, and R. Curtiss III. Cloning, Sequence Analysis, and Expression in *Escherichia coli* of a Streptococcal Plasmin Receptor. J. Bacteriology. 174:5204-5210, 1992.

2. My independent work as a postdoctoral fellow focused on the early stages of HIV-1 envelope glycoprotein mediated membrane fusion as a surrogate model of HIV-1 entry. I established a vaccinia virus-based reporter gene assay for measuring viral (HIV-1) glycoprotein-mediated membrane fusion and generated the first panel of T-cell tropic and Macrophage-tropic HIV-1 envelope glycoprotein (Env) encoding recombinant vaccinia virus vectors and I used these tools to be the first to hypothesize that the cellular tropism of HIV-1 could be explained by specific membrane fusion factors required for the different classes of HIV-1 Envs. I also developed the first soluble and secreted full-length oligomeric HIV-1 gp140 glycoprotein and explored the importance of its native oligomeric structure in terms of its presentation of conformational and virus-neutralizing epitopes through the development and characterization of more than 100 murine monoclonal antibodies.

a. **Broder, C.C.,** D.S. Dimitrov, R. Blumenthal, and E.A. Berger. The block to HIV-1 envelope glycoprotein-mediated membrane fusion in animal cells expressing human CD4 can be overcome by a human cell component(s). Virology. 193:483-491, 1993.
b. Nussbaum, O., **C.C. Broder,** and E.A. Berger. HIV-1 Envelope Glycoprotein/CD4 Mediated Cell Fusion: A Novel Recombinant Vaccinia Virus-Based Assay Measuring Activation of a Reporter Gene by Bacterio-phage T7 RNA Polymerase Selectively In Fused Cells. J.Virol. 68:5411-5422, 1994.
c. **Broder, C.C.,** P.L. Earl, D. Long, B. Moss, and R.W. Doms. Antigenic Implications of HIV-1 Envelope Glycoprotein Quaternary Structure: Oligomer-Specific and -Sensitive mAbs. PNAS. 91:11699-11703, 1994.
d. **Broder, C.C.** and E.A. Berger. Fusogenic Selectivity of the Envelope Glycoprotein is a Major Determinant of HIV-1 Tropism for CD4+ T-Cell Lines vs. Macrophages. PNAS. USA. 92:9004-08, 1995.

3. My early studies on the cellular and viral membrane fusion tropism of HIV-1 and the development of a sensitive and specific reporter gene assay of cell-cell membrane fusion facilitated the discovery of the first membrane fusion accessory factor (“fusin”; CXCR4) that I earlier hypothesized existed, and this rapidly led to the discovery by us and others of the second factor for macrophage-tropic Envs (CCR5); the HIV-1 coreceptors. These findings were a significant breakthrough in HIV research leading to numerous new directions in understanding HIV-1 pathogenesis as well as new therapeutic strategies.

a. Feng, Y., **C.C. Broder**, P.E. Kennedy, and E.A. Berger. HIV-1 Entry Cofactor: Functional cDNA Cloning of a Seven-Transmembrane, G Protein-Coupled Receptor. Science. 272:872-877, 1996.
b. Alkhatib*, G., C. Combadiere*, **C.C. Broder***, Y. Feng*, P.E. Kennedy*, P.M. Murphy, and E.A. Berger. CC CKR5: a RANTES, MIP-1α , MIP-1β Receptor as a Fusion Cofactor for Macrophage-Tropic HIV-1. Science. 272:1955-1958, 1996. (*equal contribution).
c. Rucker, J., M. Samson, B. J. Doranz, F. Libert, J. F. Berson, Y. Yi, R. G. Collman, **C. C. Broder**, G. Vassart,, R. W. Doms, and M. Parmentier. Regions in β-chemokine Receptors CCR-5 and CCR-2b that Determine HIV-1 Cofactor Specificity. Cell. 87:1-10, 1996.
d. Edinger, A.L., A. Amedee, K. Miller, B.J. Doranz, M. Endres, M. Sharron, M. Samson, Z-h. Lu, J.E. Clements, M. Murphey-Corb, S.C. Peiper, M. Parmentier, **C.C. Broder**, and R.W. Doms. Differential utilization of CCR5 by macrophage and T cell tropic SIV strains. PNAS. 94:4005-4010, 1997.

4. My initial work on HIV-1 entry led to further independent studies which focused on follow-up investigations characterizing the roles of the HIV-1 coreceptors in the virus entry process. These studies revealed the interplay between the HIV-1 entry receptors, mapped important domains of the coreceptors involved in HIV-1 Env interaction, and also revealed possible avenues of how an HIV-1 Env might engage and differently utilize the CXCR4 and CCR5 coreceptors for infection. In addition, I also engaged in collaborative follow-up studies exploring the utility of soluble oligomeric HIV-1 envelope glycoproteins as subunit vaccine immunogens (gp140) which I initiated at NIH while a postdoctoral fellow.

a. Chabot, D.J., P-F. Zhang, G.V. Quinnan, and **C.C. Broder***. Mutagenesis of CXCR4 Identifies Important Domains for HIV-1 X4 Isolate Envelope-Mediated Membrane Fusion and Virus Entry and Reveals Cryptic Coreceptor Activity for R5 Isolates. J. Virol. 73:6598-6609, 1999.
b. Xiao, X., L. Wu, T.S. Stantchev, Y-R. Feng, S. Ugolini, H. Chen, Z. Shen, **C.C. Broder**, Q.J. Sattentau, and D.S. Dimitrov. Constitutive cell surface association between CD4 and CCR5. PNAS. 96:7496-7501, 1999.

c. Chabot, D.J., H. Chen, D.S. Dimitrov, and **C.C. Broder***. N-linked Glycosylation in CXCR4 Masks Coreceptor Function for CCR5-Dependent HIV-1 Isolates. J. Virol. 74:4404-4413, 2000.
d. Zhang, P.F., Cham, F., Dong, M., Choudhary, A., Bouma, P., Zhang, Z., Shao, Y., Feng, Y.R., Wang, L., Mathy, N., Voss, G., **Broder, C.C**., Quinnan, G.V., Jr. Extensively cross-reactive anti-HIV-1 neutralizing antibodies induced by gp140 immunization. PNAS. USA. 104(24):10193-8. 2007.

5. My recent efforts have focused on emerging viruses that impact human and domestic livestock populations; including lyssaviruses, filoviruses, and henipaviruses for the past 23 years. I obtained the first NIAID funded biodefense project providing fiscal support on select agent research to an overseas laboratory (2003; U01 AI056423). I developed the first henipavirus peptide-based fusion inhibitors, subunit vaccines, and neutralizing human and humanized monoclonal antibodies (mAb), and my NIAID grants supported the development of the feline, ferret and African green monkey models of Hendra and Nipah pathogenesis. Henipavirus countermeasures development is a major a focus of my lab and supported by a NIAID U19 CETR. Here, we are developing next-generation mAb therapies and next-generation vaccines and delivery methods against Nipah and Hendra for both human and livestock use. I am also heavily engaged in collaborative virus surveillance activities focusing on henipaviruses, filoviruses, coronaviruses, and others, in wildlife and human populations in more than 10 countries in South Asia, Southeast Asia, West Africa and Southern Africa; using the Luminex xMAP-based platform which has proven to be more sensitive than ELISAs for the detection of antibodies to viral infections. The key feature of our multiplex microsphere immunoassays (MMIA) are the use of my lab's production of recombinant, soluble, native-like, oligomeric viral glycoproteins, designed similar to our HeV-sG subunit vaccine. Our MMIA array presently contains more than 20 different soluble glycoprotein ectodomains. These programs are funded by the NIAID and the Defense Threat Reduction Agency (DTRA), an agency within the US Department of Defense.

a. Bonaparte, M. I., A. S. Dimitrov, K. N. Bossart, G. Crameri, B. A. Mungall, K. A. Bishop, V. Choudhry, D. S. Dimitrov, L.-F. Wang, B. T. Eaton, and **C. C. Broder*.** Ephrin-B2 Ligand is a Functional Receptor for Hendra Virus and Nipah Virus. Proc Natl Acad Sci U S A. 102(30):10652-7. 2005. (***from the cover***).
b. Bossart, KN, Geisbert, TW, Feldmann, H, Zhu, Z, Feldmann, F, Geisbert, JB, Yan, L, Feng, Y-R, Brining, D, Scott, D, Wang, Y, Dimitrov, AS, Callison, J, Chan, Y-P, Hickey, AC, Dimitrov, DS, **Broder, CC***, and Rockx, B. A neutralizing human monoclonal antibody protects African Green monkeys from Hendra virus challenge. Sci. Transl. Med. 3, 105ra103. 2011. *corresponding author. (***from the cover***). PMID: 22013123
c. Bossart KN, Rockx B, Feldmann F, Brining D, Scott D, Lacasse R, Geisbert JB, Feng YR, Chan YP, Hickey AC, **Broder CC*,** Feldmann H, Geisbert TW. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med. 4(146):146ra107. 2012 (***from the cover***) PMID: 22875827.
d. Geisbert TW, Mire CE, Geisbert JB, Chan YP, Agans KN, Feldmann F, Fenton KA, Zhu Z, Dimitrov DS, Scott DP, Bossart KN, Feldmann H, **Broder CC***. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med. 2014, (***from the cover***) 6(242):242ra82. PMID: 24964990.

Total publications: >200; citations: >25,800; h-index 72 (google scholar).

A more complete list of published work in: MyBibliography:

https://www.ncbi.nlm.nih.gov/myncbi/181k8EOf_FNkT/bibliography/public/

BIOGRAPHICAL SKETCH

NAME: Amaya, Moushimi

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6) [redacted]

POSITION TITLE: Research Assistant Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Kwa-Zulu Natal, Durban, South Africa	B.S.	12/2004	Biological Science
George Mason University, Manassas, VA	M.S.	12/2012	Molecular Biology
George Mason University, Manassas, VA	Ph.D.	12/2015	Cell and Molecular Bio
Uniformed Services University, Bethesda, MD	Postdoctoral Fellow	02/2019	Molecular Virology
Uniformed Services University, Bethesda, MD	Scientist	09/2022	Molecular Virology

A. Personal Statement

As an active researcher in the development of novel therapeutics and vaccine candidates against emerging and re-emerging henipaviruses in relation to pandemic preparedness, I am uniquely qualified to participate as a Co-Investigator in **Research Project 2: Vaccines and antibodies to henipaviruses** of the present U19 application (ReVAMPP). The extensive experience in henipavirus reverse genetics platforms necessary to conduct the proposed studies began in my postdoctoral training, under the guidance and mentorship of **Dr. Christopher Broder**, a leading expert in henipavirus biology. During my fellowship, I rescued and fully characterized a replication competent recombinant Cedar virus (rCedV) as non-reporter and luciferase expressing reporter versions that can safely be utilized at BSL-2. This work resulted in a first co-author publication (see below). In addition, I generated replication competent chimeric viruses as non-reporter and reporter versions that express the envelope glycoproteins of either Nipah virus Bangladesh (NiV-B) (rCedV-NiV-B) or Hendra virus (HeV) (rCedV-HeV) instead of those of CedV. All 6 chimeric viruses were fully characterized in in vitro studies. Together with **Dr. Thomas Geisbert**, at the University of Texas Medical Branch (UTMB), with whom I have a 5-year collaboration, we demonstrated that the chimeras can serve as suitable surrogate viruses for authentic NiV-B or HeV as determined by plaque reduction neutralization tests. Moreover, the fluorescent reporter chimeric viruses were further characterized in a high throughput fluorescence reduction neutralization test (FRNT) to analyze neutralization potencies of sera from henipavirus immunized animals and monoclonal antibodies. This work resulted in a first author manuscript that was very recently published (see below). The FRNT has also been implemented in several neutralization and synergistic neutralization tests that resulted in 2 co-author publications (see below). The highly productive collaborations between Drs. Broder, Geisbert and I underscore the feasibility of the proposed project. The research proposed in this application will not only build on my work developed thus far but also strengthen my scientific portfolio to achieve my long-term goal of vaccine development for priority pathogens.

Citations:

1. **Amaya, M.**, Yin, R., Yan, L., Borisevich, V., Adhikari, B.N., Bennett, A., Malagon, F., Cer, R.Z., Bishop-Lilly, K.A., Dimitrov, A.S., Cross, R.W., Geisbert, T.W., Broder, C.C. (2023). A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses*, *15*(5). https://doi.org/10.3390/v15051077
2. Wang, Z.*, Dang, H. V.*, **Amaya, M.***, Xu, Y., Yin, R., Yan, L., Hickey, A. C., Annand, E. J., Horsburgh, B. A., Reid, P. A., Smith, I., Eden, J. S., Xu, K., Broder, C. C., & Veesler, D. (2022).

Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. *Proc Natl Acad Sci U S A*, *119*(22), e2122769119. https://doi.org/10.1073/pnas.2122769119 (*equal contribution)

3. Wang, Z., **Amaya, M.**, Addetia, A., Dang, H. V., Reggiano, G., Yan, L., Hickey, A. C., DiMaio, F., Broder, C. C., & Veesler, D. (2022). Architecture and antigenicity of the Nipah virus attachment glycoprotein. *Science*, *375*(6587), 1373-1378. https://doi.org/10.1126/science.abm5561
4. **Amaya, M.***, Cheng, H.*, Borisevich, V., Navaratnarajah, C. K., Cattaneo, R., Cooper, L., Moore, T. W., Gaisina, I. N., Geisbert, T. W., Rong, L., & Broder, C. C. (2021). A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res*, *193*, 105084. https://doi.org/10.1016/j.antiviral.2021.105084 (*equal contribution)

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2022-pres	Research Assistant Professor, Department of Microbiology and Immunology, USU, Bethesda, MD.
2019-2022	Scientist, Henry M. Jackson Foundation Inc., Department of Microbiology and Immunology, USU, Bethesda, MD.
2016-2019	Postdoctoral Research Fellow, Department of Microbiology and Immunology, USU, Bethesda, MD.
2016	Adjunct Professor for BIOL 306 Biology of Microorganisms Lab, George Mason University, Fairfax, VA.
2012-2015	Graduate Teaching Assistant for BIOL 306 Biology of Microorganisms Lab, George Mason University, Fairfax, VA.
2011-2012	Graduate Teaching Assistant for BIOL 104 Introduction to Biology I and II Lab, George Mason University, Fairfax, VA.

Other Experience and Professional Memberships

2019-2020	Member, American Society of Cell Biology
2018-2019	Ad hoc reviewer - Emerging Microbes & Infections
2012-2015	Member, American Society of Microbiology

Honors and Awards

2015	College of Science Summer Fellowship Recipient, George Mason University.
2014	College of Science Summer Fellowship Recipient, George Mason University.
2014-2015	Outstanding Doctoral Student Award, George Mason University, School of Systems Biology.
2013-2015	Bioscience Fellowship Recipient, George Mason University, School of Systems Biology.
2013	PhD Impact Award, George Mason University, School of Systems Biology.

Patents issued

- Recombinant Cedar Virus Chimeras. Provisional. Atty. Dkt. No. 103783-0276.

C. Contributions to Science

1. **Graduate career:** My Ph.D. thesis research involved characterizing the host viral interactions in human cells with a focus on the least characterized non-structural protein, nsP3, from VEEV. I initially constructed a tagged version of nsP3 both as an overexpression construct and in the context of the viral genome. Mass spectrometry analyses revealed that VEEV-nsP3 interacted with multiple host factors. Those of particular interest were chosen for additional studies, namely the kinase IKKβ and the RNA helicases DDX1 and DDX3. I validated that VEEV-nsP3 interacted and complexed with IKKβ, DDX1 and DDX3 and furthermore, siRNA knockdown studies indicated a reliance of these host factors for productive and continuous viral replication. These findings formed the basis of the functional model where VEEV-nsP3 interacts with the host translational machinery via DDX3 to potentially aid in translation of viral proteins that could be part of the replication complex, and that this interaction could be essential in the viral life cycle. This contribution will aid in elucidating the functionality of nsP3 in the viral life cycle as well as to promote the development of host based therapeutic options to treat RNA viral infections or disease. Results from my research were highly relevant as they provided new details and insight in understanding the role of the uncharacterized VEEV protein, nsP3, in

the new world alphavirus life cycle. As such, this work resulted in two first author research publications and one review article (see below). My Ph.D. research on host viral interactions was expanded to include host pathways that were altered during viral infection by utilizing small molecule inhibitors. Such pathways included the host phospho-signaling and the ubiquitin proteasome pathways. I validated, as a collaboration, that the MAPK/ERK signaling pathway was activated and necessary for the infectious process in VEEV infected human cells through utilization of the small molecule inhibitor, Ag-126. This joint effort resulted in a second author publication. In a different study, I had revealed with the use of an FDA approved proteasome inhibitor, Bortezomib that VEEV requires the ubiquitin proteasome pathway in the viral life cycle. My results indicated the likely mechanism was that K48 ubiquitination of VEEV capsid protein may be necessary for the early stages of infection. This study was the first to demonstrate that the capsid protein was post translationally modified with ubiquitin and this resulted in a first author publication (see below). In addition, I had also collaborated to investigate the requirement of the ubiquitin proteasome pathway in the negative stranded RNA virus, RVFV infection. We showed that with Bortezomib treatment, the formation of the characteristic nuclear filaments formed by the virulence factor NSs was abolished, thereby preventing NSs interaction with host repressor proteins SAP30 and mSin3A. This study resulted in a second author publication. Cumulatively, these studies would pave the way for developing host-based therapies and/or repurposing FDA approved drugs to treat these re-emerging diseases.

1. **Amaya, M.**, Brooks-Faulconer, T., Lark, T., Keck, F., Bailey, C., Raman, V., & Narayanan, A. (2016). Venezuelan equine encephalitis virus non-structural protein 3 (nsP3) interacts with RNA helicases DDX1 and DDX3 in infected cells. *Antiviral Res*, *131*, 49-60. https://doi.org/10.1016/j.antiviral.2016.04.008
2. **Amaya, M.**, Keck, F., Bailey, C., & Narayanan, A. (2014). The role of the IKK complex in viral infections. *Pathog Dis*, *72*(1), 32-44. https://doi.org/10.1111/2049-632X.12210
3. **Amaya, M.**, Keck, F., Lindquist, M., Voss, K., Scavone, L., Kehn-Hall, K., Roberts, B., Bailey, C., Schmaljohn, C., & Narayanan, A. (2015). The ubiquitin proteasome system plays a role in venezuelan equine encephalitis virus infection. *PLoS One*, *10*(4), e0124792. https://doi.org/10.1371/journal.pone.0124792
4. **Amaya, M.**, Voss, K., Sampey, G., Senina, S., de la Fuente, C., Mueller, C., Calvert, V., Kehn-Hall, K., Carpenter, C., Kashanchi, F., Bailey, C., Mogelsvang, S., Petricoin, E., & Narayanan, A. (2014). The role of IKKbeta in Venezuelan equine encephalitis virus infection. *PLoS One*, *9*(2), e86745. https://doi.org/10.1371/journal.pone.0086745

2. **Postdoctoral career:** My postdoctoral training involved the development and characterization of a recombinant henipavirus platform that can safely be used at BSL-2 to investigate and understand henipavirus infection with respect to pathogenesis, neutralization, drug discovery, therapeutics and vaccine potential. The reverse genetics system for the rCedV platform resulted in a first co-author publication (see below). A collaborative research project entailed the optimization of a replication competent rCedV-Luc for high throughput screening assays to identify small drug like molecules that can target multiple stages in the henipavirus life cycle, which resulted in a first co-author publication (see below). Building on the rCedV platform, I designed and characterized a robust recombinant chimeric henipavirus reverse genetics system to be used as a surrogate system for pathogenic henipaviruses. The envelope glycoproteins of rCedV were replaced with those of either NiV-B (rCedV-NiV-B) or HeV (rCedV-HeV) and non-reporter and reporter versions were generated. I optimized and implemented a rapid high throughput cell-based fluorescence reduction microneutralization assay using rCedV chimeras to determine monoclonal antibody neutralization potencies and detect the presence of neutralizing antibodies and measure neutralizing titers in henipavirus immunized animal sera. This work resulted in a first author manuscript (see below). In a collaborative effort, I generated recombinant henipavirus chimeric virus escape mutants to understand the structure of henipavirus envelope glycoproteins. This collaboration resulted in a co-author manuscript (see below). In addition, my work has been recently presented at multiple meetings such as the Keystone Symposia, Bat Infectious Diseases Symposium, and the World One Health Congress.

1. **Amaya, M.**, Yin, R., Yan, L., Borisevich, V., Adhikari, B.N., Bennett, A., Malagon, F., Cer, R.Z., Bishop-Lilly, K.A., Dimitrov, A.S., Cross, R.W., Geisbert, T.W., Broder, C.C. (2023). A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses*, *15*(5). https://doi.org/10.3390/v15051077
2. Wang, Z., **Amaya, M.**, Addetia, A., Dang, H. V., Reggiano, G., Yan, L., Hickey, A. C., DiMaio, F., Broder, C. C., & Veesler, D. (2022). Architecture and antigenicity of the Nipah virus attachment glycoprotein. Science, 375(6587), 1373-1378. https://doi.org/10.1126/science.abm5561
3. **Amaya, M.**, Cheng, H., Borisevich, V., Navaratnarajah, C. K., Cattaneo, R., Cooper, L., Moore, T. W., Gaisina, I. N., Geisbert, T. W., Rong, L., & Broder, C. C. (2021). A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. Antiviral Res, 193, 105084. https://doi.org/10.1016/j.antiviral.2021.105084
4. Laing, E. D., **Amaya, M.**, Navaratnarajah, C. K., Feng, Y. R., Cattaneo, R., Wang, L. F., & Broder, C. C. (2018). Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. Virol J, 15(1), 56. https://doi.org/10.1186/s12985-018-0964-0

Complete list of published work in MyBibliography: MAmaya_Bibliography.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Antony S. Dimitrov

eRA COMMONS USER NAME (credential, e.g., agency login): (b) (6) [redacted]

POSITION TITLE: Senior Staff Scientist

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Sofia State University, Bulgaria	M.Sci.	06/1987	Chemistry
The University of Tokyo, Japan	Ph.D.	04/1995	Physical Chemistry
NCI-Frederick, NIH, Frederick, MD	Postdoctoral Training	04/2004	Biochemistry, Viral entry

A. Personal Statement

I have been familiar with HeV-sG since its discovery. I am a co-author of the HeV-sG characterization article in JVI 2005. It shows that HeV and NiV as well G-glycoproteins, membrane attached and soluble as well, exist as dimers and dimers of dimers. Under the supervision of Dr. Broder I played a major role in the discovery of ephrin B2 as a functional receptor for Nipah and Hendra viruses, sharing an equal contribution with Dr. Bonaparte in the related PNAS article (2005). I had been also characterizing the binding site of ephrin B2 to HeV and NiV G glycoprotein and found aminoacid residues on ephrin B2 that are critical for the virus receptor binding.

I was a PI (MPI, with Dr. Fouts) of two pre-clinical development programs – one for the HeV-sG as subunit vaccine against henipavirus infections and the other for the human antibody, m102.4, as postexposure treatment against such infections. I am currently involved in the clinical investigation of the HeV-sG subunit vaccine led by Auro Vaccines LLC. I am also actively involved in coordination the work of the research projects and cores within the CETR U19- AI142764 “Advancement of Vaccines and Therapies for Henipaviruses” (Christopher Broder, PI), where I wrote the proposal for RP1 related to the next generation HeV-sG human vaccine. Although the subunit vaccine in its current formulation has an excellent efficacy, its stability at ambient condition and efficacy are expected to be significantly enhanced within RP1 of the above CETR U19, there is still room to explore the immunization efficiency by decreasing the cost of storage and application procedure. Within one of the latest awards we are developing patches of soluble microneedle arrays for intradermal delivery of HeV-sG. The vaccine is expected to be stable at ambient conditions and ready for easy self-administration by pressing the patch to a bare skin place, like on the shoulder. During the past 2 decades I accumulated relevant experience related to GLP, GMP, and partly GCP and believe that I can significantly contribute to the proposed project.

B. Positions, Scientific Appointments, and Honors

2019 –	Senior Scientist, USUHS, Bethesda, MD 20814 / HJF, Bethesda, MD 20817

2006 – 2019 Senior Staff Scientist, Profectus BioSciences, Inc., 6411 Beckley Str., Baltimore, MD 21224
2004 – 2006 Research Associate, Dept. of Microbiology at USUHS, Bethesda, MD 20814
1999 – 2004 Post-Doctoral Fellow, Laboratory of Experimental and Computational Biology, CCR, NCI, NIH, Bldg. 469, Rm. 211, P.O. Box B, Frederick, MD 21702-1201
1995 – 1999 Scientist, L'ORÉAL Tsukuba Center, Tokodai 5-5, Tsukuba, Japan
1992 – 1995 Senior Research Associate, Nagayama Protein Array Project, ERATO, JRDC, Tokodai 5-5, Tsukuba, Japan
1987 – 1993 Research Specialist (Chemist), Faculty of Chemistry, Sofia State University, Sofia, Bulgaria

C. Contributions to Science

1. *Development of HeV-sG as a human subunit vaccine against henipavirus infection.*
Relates to my current activities described above in the Personal Statement.

A. Geisbert TW, Bobb K, Borisevich V, Geisbert JB, Agans KN, Cross RW, Prasad AN, Fenton KA, Yu H, Fouts TR, Broder CC, **Dimitrov AS**. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. **NPJ Vaccines**, 2021 Feb 8;6(1):23. doi: 10.1038/s41541-021-00284-w

2. *Discovery that ephrin B2 is a functional receptor for both HeV and NiV. Characterization of HeV-sG.*
Dr. Broder (USUHS, Bethesda) pioneered the research on HeV and NiV starting the beginning of the century. I joint his group in 2004. Dr. Bossart had already created soluble versions of HeV and NiV G glycoproteins and was spending time on a search for the viral receptors. It was anticipated that both viruses share common receptor indicated by immune response crossreactivity and anti-G antibody crossreactivity, including m102. Dr. Broder suggested me to join in the efforts of receptor discovery. We mainly attempted to co-immunoprecipitate the receptor with soluble G from lysates of permissive and non-permissive cells using coomassie stain, radiolabeling, and different modification. It later became clear that the S-agarose we used for co-IP with the S-tagged soluble G produces a non-specific band overlapping ephrin B2 and, thus, the band was considered false positive. When Matthew Bonaparte joint the team proposed to use gene chip technology developed at his university, SUNY. He sent lysates from permissive and non-permissive cells for analyses. Using the gene chip data he successfully narrowed the possibilities for the receptor down to low 20s. Farther on, narrowing couldn't be justified by the available data. Then, I proposed to purchase plasmids with the identified genes from Origene, transfect non-permissive cells, and find out which gene will render them permissive to HeV or NiV. Thus, ephrin B2 was discovered as receptor for HeV and NiV. Looking back into those data ephrin B3 shows a signal higher than background, but we have ignored it at that time, being excited of ephrin B2 identification.

A. Bonaparte MI, **Dimitrov AS**, Bossart KN, Crameri G, Mungall BA, Bishop KA, Choudhry V, Dimitrov DS, Wang LF, Eaton BT, Broder CC. From the Cover: Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. **PNAS** 2005; 102: 10652-10657.
B. Bossart KN, Crameri G, **Dimitrov AS**, Mungall BA, Feng YR, Patch JR, Choundhary A, Wang LF, Eaton BT, Broder CC. Receptor Binding, Fusion Inhibition, and Induction of Cross-Reactive Neutralizing Antibodies by a Soluble G Glycoprotein of Hendra Virus. **J. Virol.** 2005; 79: 6690-6702.

3. *Having a contribution in the discovery of m102.4 by identifying its parent m102 as a crossreactive neutralizer.*
Being in the laboratory of Dr. Broder I was using HeV and NiV mediated cell-cell fusion assays to study mutant G glycoproteins created by inactivating glycosylation sites. At that time, Dr. Zhu had found Fab binders to HeV-sG and asked me to test those for inhibitory activities in the cell-cell fusion assay. His Fabs showed different inhibitory potency to HeV and NiV with Fab m102, although not the best against either virus, showing a balanced inhibition to both viruses. Then, Dr. Zhu optimized m102 by in vitro maturation to m102.4.

A. Zhu Z, **Dimitrov AS**, Bossart KN, Crameri G, Bishop KA, Choudhry V, Mungall BA, Feng YR, Choudhary A, Zhang MY, Feng Y, Wang LF, Xiao X, Eaton BT, Broder CC, Dimitrov DS. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. **J. Virol.** 2006; 80: 891-9.

B. Zhu Z, **Dimitrov AS**, Chakraborti S, Dimitrova D, Xiao X, Broder CC, Dimitrov DS. Development of human monoclonal antibodies against diseases caused by emerging and biodefense-related viruses. **Expert Rev Anti Infect Ther**. 2006; 4: 57-66.
C. Zhu Z, Bossart KN, Bishop KA, Crameri G, **Dimitrov AS**, McEachern JA, Feng Y, Middleton D, Wang LF, Broder CC, and Dimitrov DS. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. **J Infect Dis** 2008; 197:846-853.
D. Prabakaran P, Zhu Z, Xiao X, Biragyn A, **Dimitrov AS**, Broder CC, and Dimitrov DS. Potent human monoclonal antibodies against SARS CoV, Nipah and Hendra viruses. **Expert Opin Biol Ther** 2009; 9:355-368.
E. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng YR, Brining D, Scott D, Wang Y, **Dimitrov AS**, Callison J, Chan YP, Hickey AC, Dimitrov DS, Broder CC, Rockx B. “A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge” Sci. Transl. Med. 2011; v. 3 (105), p. 105ra103.

4. *Editing a volume from the Methods of Molecular Biology series*
It was an exciting time finding appropriate chapter topics covering the discovery and preclinical development of therapeutic antibodies, then finding authors and reviewing and editing the chapters.

A. **Dimitrov AS**, Editor; Therapeutic antibodies. Methods and protocols, *Methods Mol. Biol.* v. 525; March 2009, Humana Press / Springer, New York, NY 10013. ISBN: 978-1-934115-92-3
B. **Dimitrov AS**. Methods in molecular biology. Therapeutic antibodies. Methods and protocols. Preface. Methods Mol Biol 2009; 525: vii-viii, xiii.
C. Prado I, Fouts TR, **Dimitrov AS**. (2009) Methods Mol Biol. v. 525 pp. 517-31. “*Neutralization of HIV by antibodies.*”

5. *Studying the mechanism of HIV entry. Applying mAbs to inhibit HIV entry*
While postdoc under the supervision of Dr. Blumenthal, we proposed that HIV-1 Env gp41 destabilizes target cell or its own membrane upon activation of gp120 by CD4 and respective coreceptor, CCR5 or CXCR4, complexes, resulting in fusion or viral inactivation, respectively. In relation to HIV-1 entry to cells we detected intermediates of the fusion process by using conformation specific mAb. Later, at Profectus I worked with anti-HIV antibodies as entry inhibitors. Results from the completed grant awards identify sweet epitopes that are simultaneously targeted by inhibitory antibodies show synergistic effect and an exceptional efficacy in vitro.

A. **Dimitrov AS**, Xiao X, Dimitrov DS, Blumenthal R. Early Intermediates in HIV-1 Envelope Glycoprotein-mediated Fusion Triggered by CD4 and Co-receptor Complexes. J. Biol. Chem. 2001; 276: 30335-30341.
B. **Dimitrov AS**, Rawat SS, Jiang S, Blumenthal R. The Role of the Fusion Peptide and Membrane-proximal Domain in HIV-1 Envelope Glycoprotein-mediated Membrane Fusion. Biochemistry 2003;42:14150-14158.
C. **Dimitrov AS**, Jacobs A, Finnegan CM, Stiegler G, Katinger H, Blumenthal R. Exposure of the membrane-proximal external region of HIV-1 gp41 in the course of HIV-1 envelope glycoprotein-mediated fusion. Biochemistry. 2007 Feb 6;46(5):1398-401.
D. **Dimitrov A**, Drug evaluation: Ibalizumab, a CD4-specific mAb to inhibit HIV-1 infection. Current Opinion in Investigational Drugs 2007 Aug; 8(8):653-61.

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Christopher	C.	Broder	Ph.D.

Position/Title: Professor and Chair

Department: Microbiology & Immunology

Organization Name: Uniformed Services University of the Health Sciences

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Credential, e.g., agency login: (b) (6) [redacted]

*** Project Role:** Other (Specify)

Other Project Role Category: Site Principal Investigator

Degree Type: PhD

Degree Year: 1989

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Broder_TO_BE_EDITED1032988677.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
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Position/Title: Professor

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*** Project Role:** Other (Specify)

Other Project Role Category: Site Principal Investigator

Degree Type: Ph.D.

Degree Year: 1995

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_RP3_Schaefer_v21032988678.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Moushimi		Amaya	Ph.D.

Position/Title: Research Assistant Professor
Department: Microbiology & Immunology

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Division: School of Medicine

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Credential, e.g., agency login: (b) (6) [redacted]

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type:
Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Amaya_v21032988679.pdf	application/pdf
Attach Current & Pending Support		

PROFILE - Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix
	Antony		Dimitrov	Ph.D.

Position/Title: Senior Staff Scientist
Department: Microbiology & Immunology

Organization Name: The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.
Division: School of Medicine

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Credential, e.g., agency login: (b) (6) [redacted]

*** Project Role:** Co-Investigator
Other Project Role Category:

Degree Type:
Degree Year:

	File Name	Mime Type
***Attach Biographical Sketch**	Biosketch_Dimitrov_Broder_RP2_v11032988680.pdf	application/pdf
Attach Current & Pending Support		

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

Additional Senior/Key Person Form Attachments

When submitting senior/key persons in excess of 8 individuals, please attach additional senior/key person forms here. Each additional form attached here, will provide you with the ability to identify another 8 individuals, up to a maximum of 4 attachments (32 people).

The means to obtain a supplementary form is provided here on this form, by the button below. In order to extract, fill, and attach each additional form, simply follow these steps:

- Select the "Select to Extract the R&R Additional Senior/Key Person Form" button, which appears below.
- Save the file using a descriptive name, that will help you remember the content of the supplemental form that you are creating. When assigning a name to the file, please remember to give it the extension ".xfd" (for example, "My_Senior_Key.xfd"). If you do not name your file with the ".xfd" extension you will be unable to open it later, using your PureEdge viewer software.
- Using the "Open Form" tool on your PureEdge viewer, open the new form that you have just saved.
- Enter your additional Senior/Key Person information in this supplemental form. It is essentially the same as the Senior/Key person form that you see in the main body of your application.
- When you have completed entering information in the supplemental form, save it and close it.
- Return to this "Additional Senior/Key Person Form Attachments" page.
- Attach the saved supplemental form, that you just filled in, to one of the blocks provided on this "attachments" form.

Important: Please attach additional Senior/Key Person forms, using the blocks below. Please remember that the files you attach must be Senior/Key Person Pure Edge forms, which were previously extracted using the process outlined above. Attaching any other type of file may result in the inability to submit your application to Grants.gov.

1) Please attach Attachment 1 ☐
2) Please attach Attachment 2 ☐
3) Please attach Attachment 3 ☐
4) Please attach Attachment 4 ☐

ADDITIONAL SENIOR/KEY PERSON PROFILE(S)	**Filename** **MimeType**
Additional Biographical Sketch(es) (Senior/Key Person)	**Filename** **MimeType**
Additional Current and Pending Support(s)	**Filename** **MimeType**

Tracking Number:

Project/Performance Site Location(s)

Project/Performance Site Primary Location

UEI: Z83LJK5NWBC1

Organization Name: Uniformed Services University of the Health Sciences

* Street1: 4301 Jones Bridge Road		Street2:
* City: Bethesda	County: Montgomery	* State: MD: Maryland
Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 20814-4799
DUNS Number: 931258755	* Project/Performance Site Congressional District: MD-008	

Project/Performance Site Location 1

Organization Name: The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

UEI: UYLKBRENAPG5

* Street1: 6720-A Rockledge Drive		Street2: Suite 100
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Province:	* Country: USA: UNITED STATES	* Zip / Postal Code: 20817-0000
DUNS Number: 144676566	* Project/Performance Site Congressional District: MD-008	

	File Name	Mime Type
Additional Location(s)		

TITLE: Cedar henipavirus animal model

Funding announcement: RFA-AI-23-020

Funding Title: Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picronaviridae

PRINCIPAL INVESTIGATOR: Brian Schaefer, Ph.D., Site Principal Investigator

Prime PI: Thomas Geisbert, Ph.D., UTMB

Period of Performance: March 1, 2024 - February 28, 2029

Abstract

Henipaviruses are single-stranded, negative-sense enveloped RNA viruses of the paramyxovirus family. Two henipaviruses, Nipah virus (NiV) and Hendra virus (HeV), cause a systemic and often fatal respiratory and/or encephalitic disease in humans and ten other mammalian species. Importantly, NiV and HeV are significant biothreats to humans and economically important livestock in Australia and Southeast Asia. There are currently no vaccines or therapeutics approved for human use. Notably, development of countermeasures for NiV and HeV is hampered by the fact that these viruses require BSL-4 containment, meaning that very few research groups worldwide have access to the required biocontainment facilities to perform preclinical studies with these important human viral pathogens. To address this problem, we are developing a BSL-2 animal model that is based on Cedar virus (CedV), which is a non-pathogenic henipavirus. Specifically, we are employing recombinant Cedar viruses (rCedVs) in which the NiV and HeV fusion (F) and receptor-binding glycoprotein (G) are expressed in the rCedV genome, replacing CedV F and G. Additionally, we have also incorporated our recently developed *in vivo* bioluminescence methodology to longitudinally trace the dynamics and anatomical progression of rCedV-luciferase (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host innate immune response in mice, we have demonstrated sustained replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Importantly, whereas rCedV-luc does not establish stable expression in the brain, both of the chimeric viruses do. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in specific strains of mice. The rCedV-luc platform is thus an authentic henipavirus system that can be used to study henipavirus *in vivo* biology safely and expediently under BSL-2 containment. Our overall hypothesis is that rCedV-luc infection of mice lacking specific innate immune responses represents a BSL-2 platform for the study of henipavirus biology and pathogenesis, as well as for development and testing of anti-viral countermeasures. We will address this hypothesis through three Specific Aims: Aim 1: To optimize the use of immunodeficient mice and rCedV-NiV-luc/rCedV-HeV-luc chimeras as a robust BSL2 model of pathogenic henipavirus disease; Aim 2: To determine the mechanism of rCedV-NiV neurovirulence; Aim 3: To define the efficacy and mechanisms of mAb-based therapeutics for CNS-resident henipavirus infections. Aims involve synergistic collaborations with several other research projects and cores in this U19 program. Successful completion of these Aims will establish the rCedV-luc mouse model as a robust BSL-2 platform for the exploration of henipavirus pathogenesis and countermeasures.

TITLE: Vaccines and antibodies to henipavirus
Funding announcement: RFA-AI-23-020
Funding Title: Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picronaviridae
PRINCIPAL INVESTIGATOR: Christopher Broder, Ph.D., Site Principal Investigator
Moushimi Amaya, Ph.D and Antony Dimitrov, Ph.D., Co-investigators
Prime PI: Thomas Geisbert, Ph.D., UTMB
Period of Performance: March 1, 2024 - February 28, 2029

Project Summary/Abstract

Pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. Among these are the two emerged zoonotic paramyxoviruses: the ‘prototype’ henipaviruses (HNVs); Nipah virus (NiV) and Hendra virus (HeV), discovered in the mid to late 1990s. The prototype HNVs have a uniquely broad tropism capable of infecting 18 species across 6 orders of mammals using the conserved ephrin-B2 and -B3 ligands as entry receptors. NiV and HeV infection often results in a systemic and fatal respiratory and/or neurological disease in multiple mammalian species including humans. NiV and henipaviral disease are also included in the WHO List of Priority Pathogens, and they are significant biothreats to humans and livestock in South and South East Asia and Australia. HNVs have two membrane glycoproteins: the attachment glycoprotein (G) and the fusion glycoprotein (F) that are the major targets of neutralizing antibodies and the focus of all vaccine and antiviral monoclonal antibody (mAb) strategies. The most extensively studied HNV vaccine is a soluble recombinant form of HeV G (HeV-sG), shown to provide complete protection against lethal challenge by either NiV (both Malaysia (NiV-M) and Bangladesh (NiV-B) or HeV in 4 species including nonhuman primates (NHPs). With no licensed vaccines or treatments available for human use, this project will use the prototype HNVs as a model to develop, test, and translate a novel vaccination platform (a thermostable, needle-free, adaptable, and widely deployable polyphosphazene (PPZ) adjuvanted dissolvable microneedle patch (MNP)). Preliminary data demonstrates potent immunogenicity of a PPZ-MNP-HeV-sG vaccine in mice, inducing robust antigen-specific HNV cross-neutralizing antibody and cellular immune responses. The MNP vaccines will be used to evaluate HeV-sG and compare stabilized soluble forms of HNV G and F (sG_{tet} and sF_{stb}) and combinations in challenge studies. A reverse genetics system has been developed to generate recombinant, nonpathogenic Cedar virus (rCedV) and rCedV chimeric virus tools by swapping the G and F glycoproteins with those of NiV/HeV as a rapid, surrogate, BSL-2 high-throughput neutralization platform to be used to rapidly evaluate HNV vaccine responses. Also, divergent and pathogenic zoonotic HNVs have been discovered including the related rodent borne Mòjiāng virus and Langya virus (LayV)), and the divergent bat-borne Angavokely virus (AngV). This project will develop and test the rapid, adaptable and readily deployable MNP vaccine platform together with new virological tools to facilitate the translational nature of this pandemic preparedness approach. Specifically, we will: 1) Develop PPZ-MNP-based sG_{tet} and sF_{stb} prototype HNV vaccines; 2) Construct new reporter virus tools; 3) Evaluate vaccine-induced immune responses and HNV-specific mAbs and nbs; and 4) Validate the PPZ-MNP platform with another paramyxovirus antigen.

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Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Equipment

The laboratories are located in the Department of Microbiology and Immunology at USU.

The following equipment is supplied in the laboratory:

- Epifluorescence microscopy system (Fully-automated Zeiss AxioObserver epifluorescence microscope)
- BioRad BioPlex 200 HTF multiplexing system
- Luminex MagPIX multiplexing system
- BioRad iQ5 real-time PCR system
- Lonza Nucleofector 4D Shuttle – core unit and X-unit
- Coulter Z1-Dual cell counter
- centrifuges: Eppendorf 5425 microfuges (4); Beckman Allegra 25R; Thermo-Fisher Jouan GR4i; various rotors
- inverted microscopes for tissue culture (3)
- gel electrophoresis equipment for protein (7) and DNA (12) gels
- semi-dry protein transfer apparatus (4)
- Perkin-Elmer and Eppendorf thermal cyclers (5)
- ELISA plate readers (2)
- balances (6)
- pH meter (2)
- refrigerators
- freezers (-20 C, -80 C)
- platform orbital shakers (5)
- sonicator with cup horn and microtip
- bacterial shakers (6)
- heating blocks (16)
- waterbaths (14)
- biosafety cabinets (6)
- CO_2-tissue culture incubators (11)
- Thermo-Fisher Locator 4 liquid nitrogen storage tank with electronic monitoring (6)
- GE-ATKA low pressure chromatography systems, including UV detectors and fraction collectors (2)
- CTL S6 Universal Analyzer

The following equipment is supplied as common support equipment within the department:

- Fuji LAS-4010 chemiluminescence/fluorescence CCD camera imaging system
- IVIS in vivo imaging system (equipped to detect luminescence and fluorescence)
- 2D gel electrophoresis system (protein)
- Alpha-Innotech gel documentation system (EtBr-stained gels)
- MilliQ ultra-pure water system
- Nanodrop spectrophotometer
- Luminometer
- Fluorescence plate readers (2)

The University Biomedical Instrumentation Center, USUHS provides other resources and is also located on- site and provides a wide variety of services, including:

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Translational Imaging Facility

- Siemens Inveon SPECT/PET/CT Scanner
- Bruker In-Vivo Xtreme II: supports bioluminescence, X-ray, and direct radioisotopic imaging
- Bruker Biospec 70/20 USR MRI
- Image Guided Therapy 7T MR guided Focused Ultrasound System for neurological applications

Flow Cytometry

- Becton-Dickinson (10- and 13-parameter) LSRII FACS analyzers (2)
- 15-parameter FACSAria II FACS sorter, including a 541nm laser upgrade for sorting of RFP-expressing cells
- Amnis Image Stream X Mark II imaging flow cytometer with IDEAS statistical image analysis software
- Bio-Rad Bio-Plex 200 multi-analyte bioassay detection system
- Offline data analysis stations (3) with software packages including: FlowJo, WinList, and ModFit

Microscopy

- Zeiss 710NLO confocal system with 405/458/488/514/561/633 conventional lasers and a Coherent Ultra2 Ti-Sapphire laser for multiphoton excitation, continuously tunable over the range of 690 to 1080 nm
- Zeiss 700 confocal system with 405/458/488/514/561/633 laser lines
- Zeiss Elyra PS.1 Superresolution microscope for superresolution structured illumination microscopy (SR-SIM), photoactivated localization microscopy (PALM), and direct stochastic optical reconstruction microscopy (dSTORM). System is fully equipped, including the 3D-PALM/dSTORM option.
- Becker-Hickl 2-detector FLIM system (for FRET – attached to Zeiss 710NLO)
- Zeiss AxioExaminer.Z1 upright confocal system with Coherent Chameleon multiphoton laser for in vivo imaging and electrophysiology
- Zeiss AxioImager.M2 upright microscope and MicroBrightField Stereo Investigator software (stereology analysis).
- Leica AF6000 system, including an inverted microscope equipped with a fully motorized 3-axis stage plus atmosphere and temperature control
- Wide-field fluorescence microscopes (3)
- Transmission electron microscope (Philips CM100 transmission EM)
- Ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment)
- Offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize; Metamorph.

Genomics

- Fuji FLA-5000/LAS-3000 chemiluminescence/fluorescence CCD/laser scanning imaging systems
- ABI 9800 Fast Thermal Cycler
- ABI 7500 Real Time PCR System
- ABI 3500xl Genetic Analyzer
- ABI 394 DNA/RNA Synthesizer (2)
- ABI 3900 DNA Synthesizer
- Eppendorf epMotion 5075 VAC Liquid handling robot
- Thermo Scientific Evolution 300 UV/Vis Spectrophotometer
- Systec MediaPrep & Plate Pourer

Proteomics

- Agilent 1100 HPLC (2)
- AB SCIEX Voyager DSTR MALDI-TOF mass spectrometer

- AB SCIEX Q-TOF tandem mass spectrometer
- Genetix QArray2 Micro array printer

Histopathology
- Fee-for-service histology and immunohistochemistry
- Nanozoomer digital slide scanner

Structural Biology
- X-ray crystallography facility with a Rigaku HighFlux HomeLab X-ray diffraction system, including:
    - MicroMax-007 HF, a microfocus rotating anode generator
    - R-AXIS Imaging Plate detector
    - X-stream 2000 cryogenic system
    - AB Sciex 4000 QTRAP LC/MS/MS System

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Facilities and Other Resources

Overall Scientific Environment: Uniformed Services University (USU) is the medical school at which approximately half of the physicians in the Armed Services receive their graduate training. Research at USU is supported primarily by extramural grants, as in other medical schools. Dr. Broder (PI) is a tenured Professor and Chair of the Department of Microbiology and Immunology, which includes 12 full-time Faculty members. The overall focus of the Department is mechanisms of infectious diseases and the host response/immunology. Faculty interests and active research programs at USU are diverse, with many nationally- and internationally-known investigators. Dr. Broder, the PI, has had and is currently involved in active collaborations within the University, in areas of viral immunology and vaccine and antiviral therapeutics and animal model development. USU is also physically located directly across from the main NIH campus in Bethesda. The overall broad scientific environment at both USU and the NIH is highly conducive to productive collaborations. The PI often uses these resources to his advantage, both for his research objectives and interests and in assisting in adjunct faculty appointments within the Emerging Infectious Diseases graduate program for both on and off-campus scientists interested in participating in graduate education and graduate student training. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional. In addition, multi-lab Immunology journal club, Immunology data club, Virology journal club, and virology data club exist. Approximately 10 groups regularly participate in the immunology journal/data club and 5 groups regularly participate in the virology journal/data club.

Dr. Broder has been well-supported by the Department of Microbiology and Immunology and the USU Office of Research. Although USU is a modest-sized medical school, the technical resources are outstanding, rivaling many very large institutions (see below). The modest size of the school is an advantage, as key pieces of equipment (e.g., Bruker In Vivo Xtreme II; Siemens Inveon SPECT/PET/CT Scanner) are not overbooked and therefore readily available to the laboratory personnel. In addition to University funds, we have successfully used the NIH S10 mechanism to purchase advanced and very expensive equipment, including the Zeiss 710NLO system and the FACSAria 15-parameter cell sorter. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional, as is illustrated by the most recent USU purchase of cutting-edge equipment: the Zeiss Elyra PS.1 super-resolution microscope.

Laboratory: Dr. Broder's laboratories are divided into 3 rooms totaling 1,440 sq. ft. Both investigators possess routine equipment for microbiology, molecular biology, and biochemistry including incubators, centrifuges, inverted microscopes, gel electrophoresis equipment for protein and DNA gels, thermal cyclers, balances, pH meters, refrigerators, freezers, platform orbital shakers, sonicator, UV transilluminator, shakers, heating blocks, water baths, free-standing biosafety cabinets, and CO_2-tissue culture incubators. Additionally, Dr. Broder's lab is equipped with an epifluorescence microscopy system (a Zeiss AxioObserver inverted fluorescence microscope, upgraded in 2015), a BioRad iQ5 real-time PCR system, and a Lonza Nucleofector 4D Shuttle. Dr. Broder's lab also includes two complete GE-ATKA low pressure chromatography systems, with integrated UV detectors, fraction collectors, and pump systems, and gradient fractionator apparatus. Dr.
Broder's lab performs virus based assays that incorporate primarily 2 techniques: plaque assays and neutralization assays. Both of these techniques determine viral concentration and/or viral quantity and require manual counting of plaques/clear areas (plaque assay) or manually counting GFP foci using a fluorescent microscope (neutralization assays). Since a large number of iterations with multiple viruses are performed routinely, Dr. Broder acquired a CTL S6 analyzer, which is an automated plate reader that can read up to 4 fluorescent colors, captures 25 megapixel images and is able to recognize over 11,000 spots per well in a 96-well plate format. The analyzer can efficiently handle a large number of 96-well plates required for assay characterization and development thus allowing for a high-throughput workflow with the use of minimal reagents. Operator bias is also diminished when counting plaques/GFP foci from processed images allowing for consistent, objective and statistically reliable results. Washed formalin fixed 96-well plates are first loaded into the analyzer and the software takes a high quality image of each well. Using the Basic count feature in the software package the user establishes parameters, which is used to enumerate GFP foci in each well of a single 96-well plate or from batch processing multiple plates. The counts can then be exported as an Excel file, which can be used to generate neutralization profiles for example. Conventional plaque assays and virus neutralization assays can take between 5- 7 days to complete, whereas the protocol established with the CTL

S6 analyzer has reduced the time to 3 days. Postdocs, students, and technicians have desks and computers in the laboratory.

Animal: Animals are maintained in University facilities under the supervision of a full-time veterinarian. USUHS maintains a modern AAALC Accredited, Central Animal Facility of about 50,000 square feet. It, and the University's animal care and use program, is managed by the Center for Laboratory Animal Medicine, which is directed by a veterinarian who is an ACLAM Diplomat and staffed with three other veterinarians, a graduate animal husbandry man, and about 30 technicians. The University is able to provide appropriate care for a wide variety of laboratory animal species, from invertebrates to lower vertebrates, to higher vertebrates including non-human primates and domestic livestock, as well as the more commonly used species such as rodents and rabbits. The facility also includes a number of properly equipped ABSL-2 rooms.

Computer: Windows-based computers as well as Apple Mac all within 4 years of life cycle. They are available for routine use by postdoctoral fellows, graduate students, and technicians located in the laboratories. The University maintains site licenses for other image and data analysis software, including Zeiss Axiovision, VisioPharm, and OriginPro.

Office: Dr. Broder has an office separate from but proximal to the laboratories. Trainees and technicians have desks in the laboratory. The department employs one senior program manager, administrative officer, administrative specialist and a microbiologist. Other personnel can be provided as necessary to support the program.

Clinical: N/A

Other: The Biostatistics Consulting Center (BCC), a service of the Department of Preventive Medicine and Biometrics, provides statistical consulting to USUHS scientific investigators. We routinely consult with Cara Olsen, Research Assistant Professor (the full-time Biostatistics Consultant of the BCC), regarding proper design of experiments for statistical testing and for statistical analysis of the resulting data.

The USU Translational Imaging Facility (TIF) houses state-of-the art equipment for live animal imaging, including a Siemens Inveon SPECT/PET/CT Scanner, a Bruker Biospec 70/20 USR Magnetic Resonance Imaging system, and a Bruker In-Vivo Xtreme II bioluminescence and X-ray imaging sytem.

The USU Biomedical Instrumentation Center (BIC) houses core equipment for use by investigators throughout the University. Instrumentation is available either free or on a fee-for-service basis, depending on which instruments have annual service contracts (which are paid largely through per-hour use fees). The BIC Flow Cytometry Core includes two Becton-Dickinson (10- and 13-parameter) LSRII FACS analyzers, one 15-parameter FACSAria FACS sorter, and one Amnis Image Stream X Mark II imaging flow cytometer, as well as off-line analysis workstations.

The BIC Imaging Core houses three confocal microscopes, including a Zeiss 700 inverted system with 405/458/488/514/561/633 laser excitation; a Zess 710NLO inverted system with 405/458/488/514/561/633 conventional lasers and a Coherent Ultra2 Ti-Sapphire laser for multiphoton excitation, continuously tunable over the range of 690 to 1080 nm; and a Zeiss AxioExaminer-Z1 upright microscope equipped with a direct-coupled Coherent Chameleon tunable infrared laser for ex vivo and in vivo mutliphoton imaging projects. A Becker-Hickl two-detector FLIM system (for FRET analyses) is connected to the inverted Zeiss 710NLO system. Recently, the BIC has also acquired a Zeiss Elyra PS.1 super-resolution microscope, which is capable of 4-parameter SR-SIM (super-resolution structured illumination) imaging, 3-parameter PALM (Photoactivation localization microscopy) and dSTORM (direct stochastic optical reconstruction microscopy), as well as 3D-PALM/dSTORM. The BIC also houses a Leica AF6000 system, consisting of an inverted microscope equipped with a fully motorized 3-axis stage plus atmosphere and temperature control, allowing extended term (days) live cell analyses. Additionally, there is a stereology system consisting of a Zeiss AxioImager.M2 upright microscope connected to MicroBrightField's Stereo Investigator software package. The facility also includes several additional wide-field fluorescence microscopes, and three offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize; Metamorph Basic. The Imaging Core also includes a transmission electron microscope (Philips CM100 transmission EM) and an ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment).

The BIC Genomics core includes an ABI 3900 DNA synthesizer, an ABI3500xl Genetic Analyzer (for sequencing), a RochLightCycler 480 for real-time PCR, and Systec Mediaprep and Plate Pourer instrument. There is also an integrated Fuji FLA-5000/LAS-3000 imaging system for many applications that involve fluorescence and chemiluminescence imaging of gels and blots. The BIC Proteomics Core includes two Agilent 1100 HPLCs, an AB SCIEX Voyager DSTR MALDI-TOF mass spectrometer, and an AB SCIEX Q-TOF tandem mass spectrometer.

The BIC Structural Biology Core includes a Rigaku HighFlux HomeLab X-ray diffraction system, with a MicroMax-007 HF microfocus rotating anode generator, an R-AXIS Imaging Plate detector, and an X-stream 2000 cryogenic system. Other available BIC instruments and services include histopathology and PET/CT instrumentation for small animal research.

A full-time statistician is available at the University for consultation on data analysis and to help with statistical analysis.

The University also has an equipment repair service, central duplicating service, audiovisual service, and microcomputer support service. The University Learning Resource Center is a high quality medical and scientific library with additional microcomputers and support. A wide variety of scientific journals are available in print and via remote computer access. A central autoclave/glassware washroom serves the Department of Microbiology and Immunology and is maintained through extramural grant support.

USUHS

Site Principal Investigator: Brian Schaefer, Ph.D.

EQUIPMENT

Schaefer Laboratory
Epifluorescence microscopy system (Fully-automated Zeiss AxioObserver epifluorescence microscope)
BioRad CFX Connect real-time PCR system
Lonza Nucleofector 4D Shuttle – core unit and X-unit
Coulter Z1-Dual cell counter
DeNovix CellDrop-FL cell counter with fluorescence
centrifuges: Eppendorf 5425 microfuges (3); Beckman Allegra 25R; Thermo-Fisher Jouan GR4i; various rotors NestAdvance Bullet Blender bead mills (2)
Miltenyi QuadroMACS separator (2)
inverted microscopes for tissue culture (2)
gel electrophoresis equipment for protein (3) and DNA (5) gels
semi-dry protein transfer apparatus (2)
Bio-Rad thermal cyclers (2)
balances (4)
pH meter
refrigerators
freezers (-20 C, -80 C)
platform orbital shakers (5)
sonicator with cup horn and microtip
bacterial shaker
heating blocks (6)
waterbaths (8)
biosafety cabinets (2)
CO_2-tissue culture incubators (3)
Thermo-Fisher Locator 4 liquid nitrogen storage tank with electronic monitoring

Departmental
GE Healthcare Life Sciences Amersham Imager 680 (for imaging of protein and DNA gels and blots)
Alpha-Innotech gel documentation system (EtBr-stained gels)
2D gel electrophoresis system (protein)
MilliQ ultra-pure water system
Nanodrop spectrophotometer
Luminometer
Fluorescence plate readers (2)

USU CORE FACILITIES (fee for service or free)

Biomedical Research Imaging Core (BRIC)

IVIS® Spectrum CT preclinical *in vivo* imaging system (2D and 3D bioluminescence and CT imaging)
Siemens Inveon SPECT/PET/CT Scanner
Bruker Biospec 70/20 USR MRI
Image Guided Therapy 7T MR guided Focused Ultrasound System for neurological applications

The American Genome Center (TAGC)

Illumina NovaSeq 6000 systems (7)
Illumina HiSeq X systems (6)
Illumina NextSeq 500 system
10x Genomics Visium CyAssist transcriptomic probe transfer system
10x Genomics Chromium Connect automated single-cell NGS library workstation
10x Genomics Chromium Controller massively parallel sample partitioning platform
Agilent Fragment Analyzer automated parallel capillary electrophoresis systems (2)
Bio-Rad Experion LabChip Platform automated electrophoresis system
PRIMER Data Science Core - direct connection to a high-speed network switch for data storage on a petabyte-scale Isilon SAN

Biomedical Instrumentation Center (BIC)

Flow Cytometry
Cytek Aurora 5-laser, 64-channel Spectral Analyzer
Becton-Dickinson 5-laser, 30-parameter FACSymphony A5
Becton-Dickinson 4-laser, 15-parameter LSRII FACS analyzer
BD FACSAria Fusion 4-laser, 18-parameter flow sorter
BD FACSAria IIIu 4-laser, 17-parameter flow sorter
Amnis Image Stream X Mark II imaging flow cytometer with IDEAS statistical image analysis software
Bio-Rad Bio-Plex 200 multi-analyte bioassay detection system
Agilant Seahorse XPe96 Analyzer
Offline data analysis stations (3) with software packages including: FlowJo, WinList, and ModFit

Microscopy
Zeiss 980 Confocal with Airyscan2
Zeiss 700 confocal system with 405/458/488/514/561/633 laser lines
Zeiss 7 MP Multi-photon Confocal Electrophysiology System
Zeiss Elyra PS.1 Superresolution microscope for superresolution structured illumination microscopy (SR-SIM), photoactivated localization microscopy (PALM), and direct stochastic optical reconstruction microscopy (dSTORM). System is fully equipped, including the 3D-PALM/dSTORM option.
Zeiss Lightsheet Z.1
Zeiss Axioscan Z1 Slide Scanner
Zeiss AxioImager M2 upright microscope and MicroBrightField Stereo Investigator software (stereology analysis).
Leica AF6000 system, including an inverted microscope equipped with a fully motorized 3-axis stage plus atmosphere and temperature control
Transmission electron microscope (JEOL JEM-1011)
Ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment)
Grid stainer (Leica EM AC20)
Wide-field fluorescence microscopes (3)
Offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize; Metamorph.

Genomics
3500 XL Applied Biosystems Genetic Analyzer
Gene Forge 3900 and ABI 3900 and DNA Synthesizer
U-3000 Thermo-Scientific HPLC system
Agilent 1200 HPLC
QX200 Droplet Digital PCR System (ddPCR)
Roche LightCycler 480 Real-Time PCR System
Agilent Capillary Electrophoresis Fragment Analyzer

Structural Biology/Proteomics
AB Sciex 4000 QTRAP LC/MS/MS System
Agilent 1200 HPLC
Wyatt DynaPro NanoStar Dynamic Light Scattering
GE Healthcare Biacore 3000 Surface Plasmon Resonance
Art Robbins Instruments Phoenix Crystallization Robot
JANSi UVEX Fluorescence Microscope

Histopathology
Leica Autostainer XL
Leica CV 5030 Robotic Coverslipper
Sakura Tissue-Tek VIP tissue Processor
Leica CM 3050 S Cryostat
Microm HM 325 Microtome

Facilities and Other Resources

Overall Scientific Environment: Uniformed Services University (USU) is the medical school at which approximately half of the physicians in the Armed Services receive their graduate training. Research at USU is supported primarily by extramural grants, as in other medical schools. Dr. Schaefer is a tenured Professor in the Department of Microbiology and Immunology, which includes 12 full-time Faculty members. The overall focus of the Department is mechanisms of infectious diseases and the host response/immunology. Faculty interests and active research programs at USU are diverse, with many nationally- and internationally-known investigators, including the Department of Microbiology and Immunology chair, Dr. Christopher Broder, who is an internationally-known virologist (and centrally involved in this proposal).

USU is directly across the street from the main NIH campus and close to Johns Hopkins, the University of Maryland and several other outstanding institutions, facilitating opportunities to establish productive collaborations with world-class investigators. For example, Dr. Schaefer has had a multi-PI U01 grant with Dr. Wolfgang Losert (Dept of Physics, University of Maryland) as the co-PI and Dr. Hari Shroff (NIH/NIBIB) as a collaborator. Dr. Schaefer is also primary PI on a multi-PI R01 from NIAID with Dr. YouWen He at Duke University Medical Center, and Dr. Thomas Conrads (Women's Health Integrated Research Center). Dr. Schaefer and his staff have also collaborated with the Advanced Imaging Center (AIC) at the Howard Hughes Medical Institute (HHMI) for some of their imaging-based research. Overall, the broader scientific environment at USU and the greater Bethesda/Washington D.C. metropolitan region is highly conducive to productive collaborations. Dr. Schaefer has been using these resources to his advantage, and has been very active in establishing collaborative relationships to facilitate his research objectives. Notably, Dr. Schaefer and Dr. Broder have been collaborating for several years on lyssavirus- and henipavirus-related project, and they have a very strong working relationship.

Members of the Schaefer lab participate in a weekly multi-lab Immunology journal club, Immunology data club, virology journal club, and virology data club. Approximately 10 groups regularly participate in the immunology journal/data club and 5 groups regularly participate in the virology journal/data club. Also, members of the Schaefer lab participate in a bi-weekly data presentation/discussion group which meets monthly.

Dr. Schaefer has been well-supported by the Department of Microbiology and Immunology and the USU Office of Research. Although USU is a modest-sized medical school, the technical resources are outstanding, rivaling many very large institutions (see below). The modest size of the school is an advantage, as key pieces of equipment (e.g., The IVIS® Spectrum CT preclinical in vivo imaging system; Cytek Aurora spectral flow cytometer), and 10x Genomics Visium Spatial Transcriptomics instruments are not overbooked and therefore readily available to laboratory personnel. Dr. Schaefer is also Chair of the USU Biomedical Instrumentation Center (BIC), and thus plays a central role in directing equipment purchases, ensuring that needed technologies are available for the research of the PI and his USU colleagues. In addition to University funds, we have successfully used the NIH S10 mechanism to purchase core facility equipment. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional, as is illustrated by recent USU purchases of cutting-edge equipment including a Perkin-Elmer IVIS® Spectrum CT preclinical in vivo imaging system, a Zeiss Elyra PS.1 super-resolution microscope, an Agilant Seahorse XPe96 Analyzer, a BD FACSAria Fusion flow sorter, and many other pieces of advanced research equipment. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional.

USU has several Ph.D. programs, including an Emerging Infectious Diseases (EID) program, a Molecular and Cellular Biology (MCB) program and a Neuroscience (NES) program. Dr. Schaefer holds secondary appointments in all three programs and is currently Interim Director of the Emerging Infectious Diseases Graduate Program. Ds. Schaefer has been very active in training graduate students, having trained >10 Ph.D. students. USU recruits strong graduate students, and Dr. Schaefer's students have been very productive, during and beyond their dissertation studies. The availability of talented graduate students is a further resource, promoting productivity and enhancing the overall vibrancy of the scientific environment at USU.

Laboratory: Dr. Schaefer's laboratory area consists of three rooms of 450 sq. ft, 300 sq. ft and 225 sq.ft., and a dedicated microscopy suite of 110 sq. ft. The Schaefer lab contains routine equipment for microbiology, molecular biology, and biochemistry including incubators, centrifuges, inverted microscopes, gel electrophoresis equipment for protein and DNA gels, thermal cyclers, balances, pH meters, refrigerators, freezers, platform orbital shakers, sonicator, UV transilluminator, shakers, heating blocks, waterbaths, free-standing biosafety cabinets, and CO_2-tissue culture incubators. Additionally, Dr. Schaefer's lab is equipped

with an epifluorescence microscopy system (a Zeiss AxioObserver inverted fluorescence microscope), a BioRad CFX Connect real-time PCR system, a Coulter Z1-Dual cell counter, a DeNovix CellDrop-FL cell counter and a Lonza Nucleofector 4D Shuttle. Postdocs, students, and technicians have desks and computers in the laboratory.

Animal: Animals are maintained in University facilities under the supervision of a full-time veterinarian. The USUHS maintains a modern AAALC Accredited, Central Animal Facility of about 50,000 square feet. The facility also includes two preclinical behavior cores (one for mice, one for rats). The facility and the University's animal care and use program is managed by the Department of Laboratory Animal Medicine (DLAR), which is directed by a veterinarian who is an ACLAM Diplomate and staffed with three other veterinarians, a graduate animal husbandryman, and about 30 technicians. The University is able to provide appropriate care for a wide variety of laboratory animal species, from invertebrates and lower vertebrates to higher vertebrates including non-human primates and domestic livestock, as well as more commonly used species such as rodents and rabbits. The facility also includes a number of properly equipped ABSL-2 rooms.

Computer: Windows-based computers (Intel i5/i7 or similar processors and ≥8GB of RAM) are available for routine use by postdoctoral fellows, graduate students, and technicians located in the laboratories of both investigators. Dr. Schaefer's laboratory also has a dedicated workstation for use for analysis of microscopy and in vivo imaging data. Image analysis software in the Dr. Schaefer's lab includes Zeiss Zen, with a digital deconvolution module and numerous tools for quantitative image analysis. The University maintains site licenses for other image and data analysis software, including Zeiss Axiovision, VisioPharm, and OriginPro. Dr. Schaefer also maintains a license for FlowJo software, which can be used on any networked computer in the laboratory. The computers are connected by a central server to each other and to the Internet. A variety of USU-supported software programs are available, including EndNote, Microsoft Office, Adobe Creative Cloud, Geneious Prime and Graph Pad Prism 9.

Office: Drs. Schaefer's office is separate from but proximal to each of his laboratories. Trainees and technicians have desks in the laboratory. Four full-time administrative officers and one part-time administrator are available to provide support within the department.

Clinical: N/A

Other: The Biostatistics Consulting Center (BCC), a service of the Department of Preventive Medicine and Biometrics, provides statistical consulting to USUHS scientific investigators. We routinely meet with BCC consultants regarding proper design of experiments for statistical testing and for statistical analysis of the resulting data.

The USU Biomedical Research Imaging Core (BRIC) houses state-of-the art equipment for pre-clinical studies, including a IVIS® Spectrum CT, a Siemens Inveon SPECT/PET/CT Scanner and a Bruker Biospec 70/20 USR Magnetic Resonance Imaging system. A Scientist and several staff provide technical support and manage the facility.

The USU American Genome Center (TAGC) is one of only four academic genome centers in the United States. It is the Department of Defense operations subsidiary of the Collaborative Health Initiative Research Program (CHIRP) established between the National Heart, Lung, and Blood Institute (NHLBI) and USU. Since inception, TAGC has generated more than 40,000 whole Genome Sequences across 107 research studies sourced from NHLBI, other NIH Institutes, DoD and USU investigators. TAGC is also the sequencing center and genome data analysis center for the APOLLO Cancer Moonshot project, and collaborates for this purpose with the Murtha Cancer Center (MCC) at Walter Reed, the National Cancer Institute, and the Veterans Administration. Through a collaborative relationship with 10x Genomics, USU has early access to newly released platforms such as Visium Spatial Transcriptomics (including improvements to that platform).

The USU TAGC includes a large array of next-generation sequencing instruments including Illumina NovaSeq 6000 systems, Illumina HiSeq X systems, and an Illumina NextSeq 500 system. A 10x Genomics Chromium Connect automated single-cell NGS library workstation is available for automated single cell partitioning, barcoding and NGS library preparation. A 10x Genomics Chromium Controller massively parallel sample partitioning platform is available for barcoding single cell RNA for library preparation and for single cell transcriptome profiling, including CITE-seq workflows. 10x Genomics Visium CyAssist transcriptomic probe transfer system is available to facilitate spatial transcriptomics workflows. Electrophoresis systems include Agilent Fragment Analyzers and a Bio-Rad Experion LabChip Platform. Sequencing platforms are connected to a high-speed network switch for data storage on a petabyte-scale Isilon SAN.

The USU Biomedical Instrumentation Center (BIC) consists of five core equipment facilities for use by investigators throughout the University. Instrumentation is available either free or on a fee-for-service basis, depending on which instruments have annual service contracts (which are paid largely through per-hour use fees). Each core is run by a full-time Director with an advanced science background (most hold a PhD) and a high degree of technical expertise. The majority of cores have at least one additional employee to help manage the core and provide guidance and assistance to users.

The BIC Flow Cytometry Core includes three analyzers (Cytek Aurora 5-laser, 64-channel Spectral Analyzer; Becton-Dickinson 5-laser, 30-parameter FACSymphony A5; Becton-Dickinson 4-laser, 15-parameter LSRII) and two sorters (BD FACSAria Fusion 4-laser, 18-parameter flow sorter; BD FACSAria IIIu 4-laser, 17-parameter flow sorter) and an Amnis Image Stream X Mark II imaging flow cytometer. Other equipment includes a Bio-Rad Bio-Plex 200 multi-analyte detection system and an Agilant Seahorse XPe96 Analyzer. The core has several off-line analysis workstations with FlowJo and other data analysis platforms.

The BIC Imaging Core houses a wide array of microscopes, including a Zeiss Elyra PS.1 super-resolution microscope, which is capable of 4-parameter SR-SIM (super-resolution structured illumination) imaging, 3-parameter PALM (Photoactivation localization microscopy) and dSTORM (direct stochastic optical reconstruction microscopy), as well as 3D-PALM/dSTORM. Other microscopes include aZeiss 980 Confocal with Airyscan2, a Zeiss 700 confocal system, a Zeiss 7 MP Multi-photon Confocal Electrophysiology System, a Zeiss Lightsheet Z.1 and a Zeiss Axioscan Z1 Slide Scanner. The facility also includes several additional wide-field fluorescence microscopes, and three offline data analysis stations with software packages including: Zeiss Zen software and full Physiology package; Media Cybernetics' 3D Constructor, Image Pro Analyzer, Autodeblur, and Autovisualize. For electron microscopy needs, the core is equipped with a JEOL JEM-1011 transmission electron microscope, an ultramicrotome (Leica EM UC6 with EM FC6 cryo attachment), and a grid stainer (Leica EM AC20).

The BIC Genomics core includes a 3500 XL Applied Biosystems Genetic Analyzer, a Gene Forge 3900 and an ABI 3900 and DNA Synthesizer, a U-3000 Thermo-Scientific HPLC system, an Agilent 1200 HPLC, a QX200 Droplet Digital PCR System, a Roche LightCycler 480 Real-Time PCR System and an Agilent Capillary Electrophoresis Fragment Analyzer.

The BIC Structural Biology and Proteomics Core includes a GE Healthcare Biacore 3000 Surface Plasmon Resonance instrument, an AB Sciex 4000 QTRAP LC/MS/MS System, an Agilent 1200 HPLC, a Wyatt DynaPro NanoStar Dynamic Light Scattering analyzer, an Art Robbins Instruments Phoenix Crystallization Robot, and a JANSi UVEX Fluorescence Microscope (for crystal inspection).

The BIC Histopathology Core includes a Leica Autostainer XL, a Leica CV 5030 Robotic Coverslipper, a Sakura Tissue-Tek VIP tissue Processor, a Leica CM 3050 S Cryostat, and a Microm HM 325 Microtome.

The University also has an equipment repair service, central duplicating service, audiovisual service, and microcomputer support service. The University Learning Resource Center is a high quality medical and scientific library with additional microcomputers and support. A wide variety of scientific journals are available via remote computer access. A central autoclave/glassware washroom serves the Department of Microbiology and Immunology.

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Resource Sharing Plan (to include sharing research data, model organisms and data)

We routinely present data derived from our studies at scientific meetings such as the American Society for Virology (ASV), American Society for Microbiology, Keystone Symposia among others, and when components are completed, invention disclosures filed, and following consultation with our appropriate technology transfer offices, we publish and disclose our findings. We have always made our reagents and data available to other investigators, and when appropriate, complete MTAs in a timely fashion. If research findings we deem sensitive are requested by outside parties, prior to publication mutual non-disclosure agreements will be put in place. In addition, we will have a free exchange of findings and data with all our consultants and collaborators. We will adhere to all institutional, state and federal resource sharing guidelines and agree to share knowledge, research materials, and any other resources necessary and relevant to the particular focus of the grant, recognizing that final authorization will be cleared with all appropriate organizational parties. We will make all data gathered available quickly and in an easy-to-interpret format, adhering to confidentiality regulations, provided that this does not conflict with our local requirements.

Research resources, research tools, research procedures and protocols, and novel materials such as recombinant viruses like VSV and rCedV based reporters for LayV and other emerging viruses that may arise out of the funded studies will be made available to others in the private and public sector as soon as appropriate agreements covering such transfer can be executed, or in the case of project data, shall be published as soon as possible or otherwise shared with other researchers pursuant to NIH policies regarding data sharing.

The USU and HJF Joint Office of Technology Transfer (JOTT) will coordinate all technology transfer activities for data and resources generated by USU and UTMB and Novartis. The JOTT will adhere to all applicable policies, principles, guidelines and procurement rules in making unique research resources readily available for research purposes to qualified individuals and entities, and in ensuring that there is no more than a short–term restriction on publication or public dissemination of data in order for the JOTT to evaluate and file for patent protection on any subject invention that may arise from the funded study. Dissemination of unpublished data that is considered to be proprietary or confidential shall occur pursuant to a suitable confidential disclosure agreement prepared by the JOTT as required. Material transfer agreements, license agreements, and Cooperative Research and Development Agreements (CRADAs), as appropriate, will be used to transfer material property. Material transfer agreements will be established to share resources among the research community for noncommercial research use.

Vertebrate Animals

1. The following mouse strains will be used in this proposal:
   - **i)** **B6-albino-Ifnar1-/- mice**. We maintain a breeding colony of B6-albino Ifnar1-/- mice at Charles River Labs (CRL). We generated these mice by crossing the B6-albino strain (Jackson Laboratory B6(Cg)-Tyrc-2J/J, Strain #:000058) with the Ifnar1-/- strain (Jackson Laboratory B6(Cg)-Ifnar1<tm1.2Ees>/J, Strain # 028288), followed by crossing F1 mice back to B6-albino and then intercrossing white progeny that carried the Infar1- allele. This colony is now producing only albino mice that are homozygous Ifnar1-/-.
   - **ii)** **B6-albino-STAT1-/- mice.** We are currently establishing a breeding colony of B6-albino STAT1-/- mice at CRL. We are currently crossing the B6-albino strain (Jackson Laboratory B6(Cg)-Tyrc-2J/J, Strain #:000058) with the STAT1-/- strain (Jackson Laboratory B6.129S(Cg)-Stat1tm1Dlv/J, Strain # 012606), followed by crossing F1 mice back to B6-albino and then intercrossing white progeny that carry the STAT1- allele. We anticipate establishing a colony of the desired genotype by early 2024..

   We will use mice for in vivo infection with rCedVs, with many experiments including bioluminescent imaging. Animals are housed in an approved ABSL-2 room and all laboratory and animal care staff follow BSL-2 safety precautions. The total number of required mice (including all mice produced during breeding) is 5440. Experiments will be performed with male and female mice. The PI has an IACUC-approved experimental protocol and an IBC-approved biosafety protocol that details the use of the above strains of mice for the described experiments.

2. The goal of this project is to validate the mouse as a BSL-2 model for henipavirus pathogenesis, and as a platform for development and testing of countermeasures. For this work, we must use an experimental animal model to allow the course of in vivo infection and the host immune response to be studied. There is no in vitro model which can model the complex in vivo dynamics of viral infection, moving between distinct cell types and anatomical locations, or the host immune response to infection. We have chosen to utilize mice due to their ease of care, the existence of well-characterized inbred strains and knockout models, and our preliminary data with rCedVs showing robust and reproducible infection of mice which is highly amenable to statistical analysis. The number of animals to be used is based on power analyses, as detailed in our approved IACUC protocols.
3. Experiments will involve the euthanasia of infected animals experiencing pain or distress. Our IACUC protocols also define a weight loss threshold or a combined disease score criterion as end points for virus-infected animals requiring euthanasia. Animals not euthanized for the above reasons will be euthanized for tissue collection, at time points described in our IACUC protocol. Euthanasia will be performed as described in part 4, below.
4. Mice reaching criteria/endpoints described in 3, above, will be euthanized by ketamine or ketamine-xylazine overdose. This method is consistent with AMVA guidelines.

Site Principal Investigator: Brian Schaefer, Ph.D.

Select Agents

No select agents are used in this research.

We will perform animal studies with recombinant forms of Cedar virus. These viruses are categorized as BSL-2. Dr. Schaefer has an approved BSL-2 biosafety protocol for research with Cedar virus, including in vivo ABSL-2 studies. All members of the Schaefer research group have received specific training in laboratory safety and the proper use and disposal of BSL-2 organisms (and two current laboratory members have certifications for BSL-3). Dr. Schaefer has BSL-2 certified laboratories where work with these agents will be conducted. Animal experiments are conducted in approved ABSL-2 rooms within the USU animal facility, conducted under USU IACUC-approved protocols.

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

Select Agents

No select agents are used in this research.

We will generate and characterize recombinant forms of Cedar virus and Vesicular Stomatitis Virus. These viruses are categorized as BSL-2. Drs. Broder and Amaya have approved biosafety protocols for in vitro research with Cedar virus. All members of the Broder lab have received specific training in laboratory safety and the proper use and disposal of BSL-2 organisms. Dr. Broder has BSL-2 certified laboratories where work with these agents will be conducted.

USUHS
Site Principal Investigator: Christopher Broder, Ph.D.
Co-investigators: Moushimi Amaya, Ph.D.
Antony Dimitrov, Ph.D.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Molecular biology tools. Oligonucleotides and gene fragments for PCR and cloning will be purchased and verified by internal quality control at Twist Biosciences, Integrated DNA Technologies or Genscript. All clonal products will be validated by whole plasmid sequencing. All new expression constructs are verified by (1) restriction digest and electrophoretic separation of fragments to ensure fragments of the predicted size are generated and (2) sequencing to ensure the correct sequence is present with no spurious mutations.

Cells. All cells were originally obtained from ATCC or collaborators and maintained by the PI. They are tested monthly for mycoplasma contamination by two complementary methods (qPCR analysis and Lonza MycoAlert biochemical luminescence-based assay). Cells used will be used from the listed initial passage number for a maximum of 50 passages, and then discarded for a fresh vial from the working freezer stock to prevent any long-term selection of altered cell populations. To avoid cross-contamination between different cell lines, only one cell line is used at a time in a biosafety cabinet. Cell lines are routinely validated using either flow cytometry and/or functional tests.

Recombinant Cedar viruses and recombinant vesicular stomatitis viruses (VSV). Viruses are validated through genome sequences or infectivity/functional tests. SOPs, as detailed documents, for work with either recombinant Cedar virus, recombinant Cedar virus chimeras (encoding either Hendra virus or Nipah virus F and G glycoproteins), and recombinant VSVs have been established in the Broder/Amaya laboratory and all laboratory staff are required to be familiar with this documentation. Rescued recombinant viruses are validated through genome sequencing and compared to the original cloned full length viral genomes. Use of recombinant Cedar virus recombinant Cedar virus chimeras has been approved at USU.

Antibodies. Antibodies are tested for specificity as follows: (1) For antibodies used repeatedly in the PI’s lab, the staining/blotting performance of new antibody lots are compared against previous lots; (2) For new antibodies, banding/staining patterns are compared against published data; (3) Whenever possible, new antibodies are also validated using objective controls, such as cells known to lack the protein of interest (e.g., knockout cells) or cells engineered to over-express an epitope-tagged form of the molecule (i.e., staining by the new antibody is compared side-by-side with staining by the epitope-tag antibody).

Commercial chemical reagents. Such reagents are validated by one or both of the following: (1) performance comparison to previous lots of the same reagent; (2) use in an established assay that incorporates one or more validated performance controls.

Rigor and Reproducibility

The Broder laboratory employs quality systems to ensure rigor, reproducibility, and robust and unbiased results in all experiments. All findings in our work have been and will be validated by multiple independent lines of evidence to ensure scientific rigor. All experiments are carried out with several internal controls with multiple replications to guarantee reproducibility.

USUHS

Site Principal Investigator: Brian Schaefer, Ph.D.

Authentication of Key Biological and Chemical Resources

The reagents that are used in the Schaefer lab are routinely authenticated as follows:

Expression constructs: All new expression constructs are verified by (1) restriction digest and electrophoretic separation of fragments to ensure fragments of the predicted size are generated and (2) sequencing to ensure the correct sequence is present with no spurious mutations.

Antibodies: Antibodies are tested for specificity as follows: (1) For antibodies used repeatedly, the staining/blotting performance of new antibody lots are compared against previous lots; (2) For new antibodies, banding/staining patterns are compared against published data; (3) Whenever possible, new antibodies are also validated using objective controls, such as cells known to lack the protein of interest (e.g., knockout cells) or cells engineered to over-express an epitope-tagged form of the molecule (i.e., staining by the new antibody is compared side-by-side with staining by the epitope-tag antibody).

Cell lines: Cell lines are routinely validated using flow cytometry for key cell surface markers and/or functional tests. Cell lines transfected for virus production are validated via measuring titers of virus in the resultant supernatants.

Viruses: Viruses are validated through a combination of genome sequencing, infectivity and functional testing. Rescued recombinant viruses are validated through genome sequencing, with comparison to the original cloned full length viral genomes.

Mice: Every new litter of knockout animals is PCR screened for both the wild-type and inactivated allele, with validated wild-type and knockout control DNAs included in every screen. For transgenic animals, presence of the transgene is PCR verified in every litter, with validated controls included in the screen. Additional screens include periodic western blotting and/or FACS analysis of tissues/cells from knockout animals, to validate that PCR-identified knockouts indeed fail to express the protein product of the inactivated gene.

Commercial chemical reagents: Such reagents are validated by one or both of the following: (1) performance comparison to previous lots of the same reagent; (2) use in an established assay that incorporates one or more validated performance controls.

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	55,000.00	16,181.00	71,181.00
1	Graduate Students	12.00	0.00	0.00	40,514.00	11,919.00	52,433.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	71,250.00	20,962.00	92,212.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	40,521.00	11,921.00	52,442.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	12,056.00	3,547.00	15,603.00

5 **Total Number Other Personnel**

Total Other Personnel 283,871.00

Total Salary, Wages and Fringe Benefits (A+B) 283,871.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**
Ultralow temp (-80C) Freezer	12,123.00

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	12,123.00

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	3,000.00
2.	Foreign Travel Costs	3,000.00
	Total Travel Cost	6,000.00

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	48,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	34,728.00
9.	Translational Imaging Core (BRIC)	23,400.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	169,416.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	471,410.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	471,410.00	173,196.00
FY23 HJF Companywide G&A	16.70	644,606.00	107,649.00
		Total Indirect Costs	280,845.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number): USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	752,255.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	752,255.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	57,145.00	16,812.00	73,957.00
1	Graduate Students	12.00	0.00	0.00	42,094.00	12,384.00	54,478.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	74,029.00	21,779.00	95,808.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	42,101.00	12,386.00	54,487.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	12,526.00	3,685.00	16,211.00

5 **Total Number Other Personnel**

Total Other Personnel 294,941.00

Total Salary, Wages and Fringe Benefits (A+B) 294,941.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: ☐ Add Attachment ☐ Delete Attachment ☐ View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	48,000.00
2.	Publication Costs	5,000.00
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal Costs (procurement and husbandry)	48,046.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	203,334.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	498,275.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	498,275.00	183,067.00
FY23 HJF Companywide G&A	16.70	681,342.00	113,784.00
		Total Indirect Costs	296,851.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	795,126.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	795,126.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5

Enter name of Organization: The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium

Budget Period: 3 **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file:

Total Senior/Key Person: 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	59,374.00	17,468.00	76,842.00
1	Graduate Students	12.00	0.00	0.00	43,736.00	12,867.00	56,603.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	76,916.00	22,629.00	99,545.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	43,743.00	12,869.00	56,612.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	13,015.00	3,829.00	16,844.00

5 **Total Number Other Personnel**

Total Other Personnel: 306,446.00

Total Salary, Wages and Fringe Benefits (A+B): 306,446.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file

Total Equipment

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	43,200.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	48,048.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	193,536.00

G. Direct Costs

Funds Requested ($)

Total Direct Costs (A thru F) 499,982.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	499,982.00	183,693.00
FY23 HJF Companywide G&A	16.70	683,673.00	114,173.00

Total Indirect Costs 297,866.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

Funds Requested ($)

Total Direct and Indirect Institutional Costs (G + H) 797,848.00

J. Fee

Funds Requested ($)

K. Total Costs and Fee

Funds Requested ($)

Total Costs and Fee (I + J) 797,848.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	61,689.00	18,149.00	79,838.00
1	Graduate Students	12.00	0.00	0.00	45,441.00	13,369.00	58,810.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	79,916.00	23,511.00	103,427.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	45,449.00	13,371.00	58,820.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	13,522.00	3,978.00	17,500.00

5 **Total Number Other Personnel**

Total Other Personnel 318,395.00

Total Salary, Wages and Fringe Benefits (A+B) 318,395.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	33,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	48,048.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	183,336.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	501,731.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	501,732.00	184,336.00
FY23 HJF Companywide G&A	16.70	686,068.00	114,573.00

Total Indirect Costs 298,909.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	800,640.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	800,640.00

L. Budget Justification

(Only attach one file.) Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 5

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 5** **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Brian		Schaefer	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00

Project Role: Site Principal Investigator

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 0.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
1	Post Doctoral Associates	12.00	0.00	0.00	64,095.00	18,857.00	82,952.00
1	Graduate Students	12.00	0.00	0.00	47,214.00	13,890.00	61,104.00
	Undergraduate Students						
	Secretarial/Clerical						
1	Scientist II	9.00	0.00	0.00	83,032.00	24,428.00	107,460.00
1	Research Assistant II (Clouse)	9.00	0.00	0.00	47,222.00	13,893.00	61,115.00
1	TAGC Research Associate (Alba)	1.85	0.00	0.00	14,049.00	4,133.00	18,182.00

5 **Total Number Other Personnel**

Total Other Personnel 330,813.00

Total Salary, Wages and Fringe Benefits (A+B) 330,813.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	20,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Animal costs (procurement and husbandry)	48,048.00
9.	Translational Imaging Core (BRIC)	39,000.00
10.	Biomedical Instrumentation Core (BIC)	10,608.00
11.	The American Genome Center (TAGC)	52,680.00
12.	Data Management and sharing costs	0.00
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	170,336.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	501,149.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	501,149.00	184,122.00
FY23 HJF Companywide G&A	16.70	685,272.00	114,440.00

Total Indirect Costs 298,562.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number) USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	799,711.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	799,711.00

L. Budget Justification

(Only attach one file.) [Add Attachment] [Delete Attachment] [View Attachment]

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		0.00
Section B, Other Personnel		1,534,466.00
Total Number Other Personnel	25	
Total Salary, Wages and Fringe Benefits (A+B)		1,534,466.00
Section C, Equipment		12,123.00
Section D, Travel		6,000.00
1. Domestic	3,000.00	
2. Foreign	3,000.00	
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		919,958.00
1. Materials and Supplies	192,200.00	
2. Publication Costs	5,000.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	226,918.00	
9. Other 2	179,400.00	
10. Other 3	53,040.00	
11. Other 4	263,400.00	
12. Other 5	0.00	
13. Other 6		
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		

Section G, Direct Costs (A thru F)	2,472,547.00
Section H, Indirect Costs	1,473,033.00
Section I, Total Direct and Indirect Costs (G + H)	3,945,580.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	3,945,580.00

Budget Justification – RP3 – Schaefer

The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. (HJF) in partnership with the Uniformed Services University of the Health Sciences (USUHS) will manage this proposal, if awarded.

Key Personnel:

Brian. C. Schaefer, Ph.D. (Site Principal Investigator, 1.2 calendar months, 10% effort) is a Professor of Microbiology and Immunology at USUHS. His expertise is in cellular immunology, animal models of immune responses in the central nervous system and applying advanced imaging methods to biological questions. He and Dr. Broder have been collaborating for nearly a decade on creating animal models and investigating therapeutic interventions for neurotropic viral infections. The preliminary data in this application resulted from this collaboration, through an NIH-funded U19 grant (with aims that do not overlap the aims of this proposal). Dr. Schaefer will direct all animal studies in this project, managing and advising staff and overseeing data collection and data analysis. Dr. Schaefer will manage the overall experimental direction, the writing and editing of manuscripts for publication and the preparation of progress reports and other administrative functions related to this project, with assistance from the Senior Staff Scientist. (Dr. Schaefer is a full-time Federal employee. Hence, no salary is requested for this project).

Other Personnel

†**To be named** (Scientist II, 9 calendar months, 75% effort). A Scientist with experience in animal models and transcriptomics analysis will be recruited for this project. This Scientist will supervise animal research studies, coordinate, conduct and supervise data analyses for transcriptomics studies. The scientist will also coordinate overall data analysis and will participate with Dr. Schaefer in preparation of manuscripts for funding and in preparing progress reports and performing other administrative tasks related to the conduct of this project. The Scientist II will be employed by the Henry M. Jackson Foundation, and salary support is requested on this project.

†**To be named** (Postdoctoral fellow, 12 calendar months, 100% effort). A Postdoctoral fellow with experience in animal research and virology will be recruited for this project. The Postdoctoral fellow will perform approximately half of the animal experiments proposed for this study. The Postdoctoral fellow will be employed by the Henry M. Jackson Foundation, and salary support is requested on this project.

†**Celeste Huaman** (Graduate Student, 12 calendar months,100% effort) will perform approximately half of the proposed in vivo experiments described in this proposal. Ms. Huaman is a senior graduate student in Dr. Schaefer's lab, who has been working collaboratively with members of the Broder lab on this project. Notably, Ms. Huaman generated the majority of preliminary data in this proposal, and has extensive experience in all procedures and analysis techniques required for the execution of the proposed experiments. Ms. Huaman worked in Dr. Schaefer's lab as a Research Assitant II for 5 years before joining the USU Emerging Infectious Diseases graduate program, and she acquired a very deep technical skill set during that time. Ms. Huaman completed the CEEZAD BSL-3 training course at the Biosecurity Research Institute of Kansas State University in 2021, and she has trained most current laboratory staff in proper BSL-2 technique. Ms. Huaman is currently employed by the Henry M. Jackson Foundation, and salary support is requested for her on this project.

†**Caitlyn Clouse** (Research Assistant II, 9 calendar months, 75% effort) will provide general technical support, performing routine tissue culture, ordering reagents, and maintaining databases, as required for the project. Ms. Clouse will also perform genotyping of animals breeding at Charles River Lab and she will maintain detailed records of animal genotypes. She will also assist the postdoctoral fellow and Ms. Huaman with all animal experiments. Ms. Clouse completed the CEEZAD BSL-3 training course at the Biosecurity Research Institute of Kansas llent technical support, including assistance to Ms. Huaman in gathering the preliminary data for this project. Ms. Clouse is an employee of the Henry M. Jackson Foundation and we are requesting salary support for her on this project.

[†]**Camille Alba** (Research Associate, The American Genome Center (TAGC), 1.85 calendar months, 15.4% effort) will be responsible for leading the collaborative performance task for this study. She will oversee performance of library preparation, library quality assessment, library clustering and whole genome sequencing of the samples across the entire project. She will report Illumina NovaSeq platform performance metrics to the collaborating staff scientist and oversee data transfer batches. Ms. Alba is employed as a Henry Jackson Foundation employee therefore salary support is requested.

[†]A merit increase has been added to years 2-5 of the personnel budget to account for salary increases for Henry M. Jackson Foundation personnel.

Equipment:

An ultralow (-80 C) freezer, ThermoFisher Scientific Model TSX60086A ($12,123), is needed for storage of the many RNA samples, viral stocks and frozen tissues (for sectioning) and slides with frozen sections. Dr. Schaefer's current -80 freezers are filled to capacity.

Travel: (Year 1)

Funds are requested to attend two domestic scientific conferences or one international conference. This travel is for data presentation, exchange of ideas with colleagues, and for keeping up-to-date regarding the latest research findings relevant to the proposed project. Meeting attendance and data presentation is a key component of postdoctoral and graduate student training.

Materials and Supplies:

For the laboratory to successfully complete the experiments, the project will need to procure the following reagents and supplies:

General Laboratory Supplies

Serological pipettes, microscope slides and coverslips, pipet tips, microcentrifuge tubes, sterile conical tubes, ultracentrifuge tubes, syringes, needles, gloves, respirators, gowns, buffers, flasks, plates, etc.

Materials

Trizol reagents, RNAse-free DNAse, RNAse-free water and buffers, Reverse Transcriptase, Taq Polymerase, qPCR master mix, RNAse-free consumables for tissue homogenization, magnetic beads for cell sorting, tissue culture media, fetal bovine serum, antibiotics, etc

Real-time PCR probes

DNA oligonucleotide

Labeled antibodies for histology and flow cytometry

Animal costs (procurement and husbandry):

Animal Breeding at Charles River Labs

The breeding of experimental mice at USU is contracted to Charles River Labs (CRL). All aspects of animal care and breeding are managed by CRL, while genotyping of progeny is performed by Dr. Schaefer's group, using biosamples collected and shipped by CRL. We maintain a colony of B6-albino Ifnar1-/- mice and B6-albion STAT1-/- mice in our breeding colony at CRL. For this project, we expect to maintain 12 breeding cages and 12 progeny cages to supply the animals needed for the proposed experiments. ($1.80/cage/day x 24 cages x 365 days = $15,768 + Transportation charges $1,500 = $20,768).

Animal Husbandry at USU

Animal facility (DLAR) mouse per diems:
USU DLAR FY24 per diem for biohazardous mice is $4.78/cage/day. We expect to have an average of 8 experimental cages housed in the facility (8 cages x 365 days x $4.78/cage/day = $13,957.60/yr)

Translational Imaging Core (BRIC):
BRIC – IVIS Spectrum CT bioluminescence imaging (9 hr/week x 52 weeks/yr x $50/hr = $23,400/yr)

Biomedical Instrumentation Core (BIC):
BIC – flow cytometry (4 hr/week x 52 weeks/yr x $36/hr = $7,488/year).
BIC – confocal microscopy (2 hr/week x 52 weeks/yr x $30/hr =$3,120/year)

The American Genome Center (TAGC):

Transcriptomics studies performed at the USU American Genome Center

Total RNAseq on brain slices from 32 animals/year x $300 per animal = $9,600/year

Supplies for Visium Spatial Gene Expression:

Visium CytAssist Spatial Gene Expression for FFPE, Mouse Transcriptome 6.5mm slides (quantity of 16 per year) $24,500/year

Visium CytAssist Tissue Slide Cassettes = $1,800/year

Dual INdex Kit TS set A = $620/year

S1 Novaseq Flow cells (for spatial transcriptomics sequencing), 2 flow cells/year = $8,600/year

Supplies for Single-cell RNAseq:

Chromium Next GEM Automated Single Cell 3' Library and Gel Bead Kit v3.1 = $27,130/year

Chromium Next GEM Automated Chip G Single Cell Kit = $630/year

S1 Novaseq Flow cells (for single-cell RNAseq), 1 flow cell/year = $4,300/year

Publication costs: (Year 2)
We estimate 2 manuscripts per year for an average cost of $2,500 per publication. This cost estimate is based on our average fee paid per open access journal publication for the last 3 years.

Indirect Costs:

HJF indirect cost (IDC) is calculated based on the value-added cost base overhead rates. The fringe rate used is 29.42 % for Tier 1 employees and 7.29% for Tier 2 employees (all employees in this proposal are Tier 1).

The HJF indirect cost is calculated based on the value-added cost base overhead rates. The IDC rate is 36.74% USU Onsite Overhead. Additionally, 16.70% Companywide G&A and applied on the total direct cost less subaward plus the USU Onsite Overhead. For proposals including subawards, an additional 1.93% is applied on total external subaward costs.

The above fringe benefits and indirect cost rates for FY2023 were approved by the U.S. Army Medical Research Acquisition Activity on September 1, 2022.

RESEARCH & RELATED BUDGET - Budget Period 1

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 1** **Start Date:** 03/01/2024 **End Date:** 02/28/2025

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role:	Site Principal Investigator										
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	86,601.00	25,478.00	112,079.00
Project Role:	Co-investigator										
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role:	Co-investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 112,079.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant (Thammavong)	4.00	0.00	0.00	15,585.00	4,585.00	20,170.00
1	Research Associate III (Yan)	6.00	0.00	0.00	41,560.00	12,227.00	53,787.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	31,669.00	9,317.00	40,986.00
1	Research Assistant (TBD)	12.00	0.00	0.00	42,000.00	12,356.00	54,356.00

4 **Total Number Other Personnel**

Total Other Personnel 169,299.00

Total Salary, Wages and Fringe Benefits (A+B) 281,378.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**
Steriguard Biosafety cabinet thermo scientific: 48in, 1300Series	6,450.00
CTL Analyzer	79,000.00

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	85,450.00

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	55,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
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16.		
17.		
	Total Other Direct Costs	55,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	421,828.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	421,828.00	154,980.00
FY23 HJF Companywide G&A	16.70	576,808.00	96,327.00
		Total Indirect Costs	251,307.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	673,135.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	673,135.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 2

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 2** **Start Date:** 03/01/2025 **End Date:** 02/28/2026

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role:	Site Principal Investigator										
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	89,978.00	26,472.00	116,450.00
Project Role:	Co-investigator										
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role:	Co-investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 116,450.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant(Thammavong)	4.00	0.00	0.00	16,193.00	4,764.00	20,957.00
1	Research Associate III (Yan)	6.00	0.00	0.00	43,181.00	12,704.00	55,885.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	32,904.00	9,680.00	42,584.00
1	Research Assistant (TBD)	12.00	0.00	0.00	43,638.00	12,838.00	56,476.00

4 **Total Number Other Personnel**

Total Other Personnel 175,902.00

Total Salary, Wages and Fringe Benefits (A+B) 292,352.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs

	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
Total Participant/Trainee Support Costs	

Number of Participants/Trainees

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	55,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
10.		
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17.		
	Total Other Direct Costs	55,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	347,352.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	347,352.00	127,617.00
FY23 HJF Companywide G&A	16.70	474,969.00	79,320.00
		Total Indirect Costs	206,937.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	554,289.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	554,289.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 3

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 3** **Start Date:** 03/01/2026 **End Date:** 02/28/2027

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role: Site Principal Investigator											
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	93,488.00	27,504.00	120,992.00
Project Role: Co-investigator											
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role: Co-investigator											

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 120,992.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant(Thammavong)	4.00	0.00	0.00	16,824.00	4,950.00	21,774.00
1	Research Associate III (Yan)	6.00	0.00	0.00	44,865.00	13,199.00	58,064.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	34,187.00	10,058.00	44,245.00
1	Research Assistant (TBD)	12.00	0.00	0.00	45,340.00	13,339.00	58,679.00

4 **Total Number Other Personnel**

Total Other Personnel 182,762.00

Total Salary, Wages and Fringe Benefits (A+B) 303,754.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: ☐ Add Attachment Delete Attachment View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees	
	Total Participant/Trainee Support Costs	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	55,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
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16.		
17.		
	Total Other Direct Costs	55,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	358,754.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	358,754.00	131,806.00
FY23 HJF Companywide G&A	16.70	490,560.00	81,924.00
		Total Indirect Costs	213,730.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number): USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	572,484.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	572,484.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Budget Period 4

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 4** **Start Date:** 03/01/2027 **End Date:** 02/29/2028

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role:	Site Principal Investigator										
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	97,134.00	28,577.00	125,711.00
Project Role:	Co-investigator										
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role:	Co-investigator										

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 125,711.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Assistant (Thammavong)	4.00	0.00	0.00	17,480.00	5,143.00	22,623.00
1	Research Associate III (Yan)	6.00	0.00	0.00	46,615.00	13,714.00	60,329.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	35,521.00	10,450.00	45,971.00
1	Research Assistant (TBD)	12.00	0.00	0.00	47,108.00	13,859.00	60,967.00

4 **Total Number Other Personnel**

Total Other Personnel 189,890.00

Total Salary, Wages and Fringe Benefits (A+B) 315,601.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	Funds Requested ($)

Additional Equipment: ☐ Add Attachment ☐ Delete Attachment ☐ View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	35,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
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16.		
17.		
	Total Other Direct Costs	35,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	350,601.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	350,601.00	128,811.00
FY23 HJF Companywide G&A	16.70	350,601.00	80,062.00

Total Indirect Costs 208,873.00

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	559,474.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	559,474.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED BUDGET - Budget Period 5

OMB Number: 4040-0001
Expiration Date: 11/30/2025

UEI: UYLKBRENAPG5 **Enter name of Organization:** The Henry M. Jackson Fdn for the Adv'mt of Mil Med, Inc.

Budget Type: ☐ Project ☒ Subaward/Consortium **Budget Period: 5** **Start Date:** 03/01/2028 **End Date:** 02/28/2029

A. Senior/Key Person

Prefix	First	Middle	Last	Suffix	Base Salary ($)	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Christopher		Broder	Ph.D.		1.20	0.00	0.00	0.00	0.00	0.00
Project Role: Site Principal Investigator											
	Antony		Dimitrov	Ph.D.		6.00	0.00	0.00	100,922.00	29,691.00	130,613.00
Project Role: Co-investigator											
	Moushimi		Amaya	Ph.D.		2.00	0.00	0.00	0.00	0.00	0.00
Project Role: Co-investigator											

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

Total Funds requested for all Senior Key Persons in the attached file

Total Senior/Key Person 130,613.00

B. Other Personnel

Number of Personnel	Project Role	Months Cal.	Months Acad.	Months Sum.	Requested Salary ($)	Fringe Benefits ($)	Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Research Assistant(Thammavong)	4.00	0.00	0.00	18,162.00	5,343.00	23,505.00
1	Research Associate III (Yan)	6.00	0.00	0.00	48,433.00	14,248.00	62,681.00
1	Program Manager III (Boxley)	3.00	0.00	0.00	36,906.00	10,858.00	47,764.00
1	Research Assistant (TBD)	12.00	0.00	0.00	48,945.00	14,399.00	63,344.00

5 **Total Number Other Personnel** **Total Other Personnel** 197,294.00

Total Salary, Wages and Fringe Benefits (A+B) 327,907.00

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment item	**Funds Requested ($)**

Additional Equipment: ☐ Add Attachment ☐ Delete Attachment ☐ View Attachment

Total funds requested for all equipment listed in the attached file	
Total Equipment	

D. Travel

		Funds Requested ($)
1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	
2.	Foreign Travel Costs	
	Total Travel Cost	

E. Participant/Trainee Support Costs

		Funds Requested ($)
1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	

Number of Participants/Trainees

Total Participant/Trainee Support Costs

F. Other Direct Costs

		Funds Requested ($)
1.	Materials and Supplies	35,000.00
2.	Publication Costs	
3.	Consultant Services	
4.	ADP/Computer Services	
5.	Subawards/Consortium/Contractual Costs	
6.	Equipment or Facility Rental/User Fees	
7.	Alterations and Renovations	
8.	Data Management and sharing costs	0.00
9.		
10.		
11.		
12.		
13.		
14.		
15.		
16.		
17.		
	Total Other Direct Costs	35,000.00

G. Direct Costs

	Funds Requested ($)
Total Direct Costs (A thru F)	362,907.00

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)
FY23 USU Onsite Overhead	36.74	362,907.00	133,333.00
FY23 HJF Companywide G&A	16.70	496,242.00	82,872.00

Total Indirect Costs 216,205.00

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)
USAMRAA, Jennifer C. Jackson, +1.301.619.2054

I. Total Direct and Indirect Costs

	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	579,112.00

J. Fee

	Funds Requested ($)

K. Total Costs and Fee

	Funds Requested ($)
Total Costs and Fee (I + J)	579,112.00

L. Budget Justification

(Only attach one file.) Budget Justification v4.pdf

Add Attachment Delete Attachment View Attachment

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		605,845.00
Section B, Other Personnel		915,147.00
Total Number Other Personnel	21	
Total Salary, Wages and Fringe Benefits (A+B)		1,520,992.00
Section C, Equipment		85,450.00
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		235,000.00
1. Materials and Supplies	235,000.00	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	0.00	
9. Other 2		
10. Other 3		
11. Other 4		
12. Other 5		
13. Other 6		
14. Other 7		
15. Other 8		
16. Other 9		
17. Other 10		

Section G, Direct Costs (A thru F)	1,841,442.00
Section H, Indirect Costs	1,097,052.00
Section I, Total Direct and Indirect Costs (G + H)	2,938,494.00
Section J, Fee	
Section K, Total Costs and Fee (I + J)	2,938,494.00

Budget Justification - RP 2 - Broder

The Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. (HJF) in partnership with the Uniformed Services University of the Health Sciences (USUHS) will manage this proposal, if awarded.

Key Personnel:

Christopher Broder, Ph.D., (Principal Investigator, 1.2 calendar months, 10% effort), is a Professor of Microbiology and Immunology at USUHS and will serve as PI on the proposed project. He is a leading expert in henipavirus biology and genetics, as well as development of glycoprotein-based vaccines and monoclonal antibody-based therapeutics against these viruses; many tested in collaboration with Dr. Thomas Geisbert (Project Lead on this application). Dr. Broder has more than 15 years of collaborative research projects with Dr. Geisbert focused on henipaviruses and together have published on a variety of henipavirus countermeasures including the use of recombinant CedVs. Dr. Broder's group recently developed the reverse genetics platform for designing and rescue of recombinant Cedar henipaviruses (rCedV) that can be utilized at BSL-2, some of which have been successfully used in drug library HTS assays and one report on the successful use of Cedar virus in antiviral drug discovery for henipaviruses including Nipah (NiV), Hendra (HeV) and CedV has been published in collaboration with Dr. Geisbert. Dr. Broder's group generated and characterized the recombinant Cedar chimeric viruses that were used to generate the preliminary data for this application. He will oversee all aspects of the recombinant Cedar chimeric virus construction and characterization. In addition, he and Dr. Amaya will collaborate on the design of all experiments and the preparation of progress reports and manuscripts. Dr. Broder is a federal employee and no salary support is requested.

Moushimi Amaya, Ph.D., (Co-investigator, 2.0 calendar months, 16.67% effort). Dr. Amaya is an experienced biochemist and molecular virologist with expertise in a variety of areas including gene cloning and expression and mutagenesis specifically with viruses and viral culture and propagation, and viral protein purification and analysis. She has considerable experience in henipavirus reverse genetics systems. Using the rCedV platform, Dr. Amaya designed, generated and characterized replication competent chimeric viruses as non-reporter and reporter versions that express the envelope glycoproteins of either NiV-Bangladesh (NiV-B) (rCedV-NiV-B) or HeV (rCedV-HeV) instead of those of CedV. In a collaborative effort, Drs. Amaya, Broder and Geisbert demonstrated that the chimeras can serve as suitable surrogate viruses for authentic NiV and HeV viruses. Dr. Amaya developed and characterized the fluorescent reporter chimeric viruses in a high throughput fluorescence reduction neutralization test (FRNT) to analyze neutralization potencies of sera from henipavirus immunized animals and monoclonal antibodies. She will design, oversee and conduct the research plans related to the generation and characterization of the rCedV chimeric viruses and VSV reporter viruses outlined in this proposal. She will develop, perform quality assurance and test the new chimeric viruses for use in henipavirus neutralization assays. Dr. Amaya is a federal employee and no salary support is requested.

Antony S. Dimitrov, Ph.D., (Co-investigator, 6.0 calendar months, 50% effort). In addition to Dr. Dimitrov's duty to the day-to-day operations of this ReVAMPP and the Admin Core, he will oversee and conduct the research plans related to the production and characterization of all rCedV chimeric viruses and VSV reporter viruses; including generation, quality control, and in vitro cell-based studies. Dr. Dimitrov is highly familiar with all scientific and translational development activities of the proposed ReVAMPP. He was a former member of Broder laboratory and played major roles in the development and biochemical characterization of the HeV-sG subunit glycoprotein (JVI 2005), the discovery of the NiV and HeV entry receptor ephrin B2 (PNAS 2005) and in the development, characterization and efficacy testing of the first neutralizing human monoclonal antibody to NiV and HeV, the latter studies conducted with Profectus Biosciences in collaboration with Dr. Geisbert. When at Profectus Bio, Dr. Dimitrov led their program in the pre-clinical development of HeV-sG as a subunit vaccine and the human antibody, m102.4, for treatment of henipavirus infections. Dr. Dimitrov is an employee of the Henry M. Jackson Foundation and salary support is requested.

Other Personnel

TBD (Research Assistant, 12.0 calendar months, 100% effort). This individual will provide technical laboratory support for this project. In addition, this individual will also be responsible for ordering supplies, organizing shipments, and other laboratory support as instructed by the senior staff. As an employee of the Henry M. Jackson Foundation salary support is requested for this individual.

Lianying Yan (Linda) (Manager/Lead Research Associate 3, 6 calendar months, 50% effort) has been in Dr. Broder's laboratory since 2003 and is well-versed in all aspects of the research in his laboratory. Ms. Yan is a highly trained molecular biologist skilled in expression construct design, screening techniques, and stable cell line development for protein expression using immunoprecipitation and Western blot techniques. Ms. Yan is highly experienced in designing soluble and secreted viral glycoprotein constructs and establishing stable cell-lines to express and produce, purify and characterize the proteins using protocols that include Bio-Plex bead coupling and assay development. She is also expert in confocal and fluorescent microscopy techniques and has been co-author of several manuscripts. Ms. Yan is an employee of the Henry M. Jackson Foundation and salary support is requested.

Madison Thammavong (Research Assistant I, 4 calendar months, 33% effort). Mr. Thammavong will assist Ms. Yan in the production of proteins. He will be trained as a back-up support with various laboratory responsibilities. Mr. Thammavong is an employee of the Henry M. Jackson Foundation and salary support is requested.

Kimberly Boxley, (4.0 calendar months, 33% effort), serves as the Center Financial and Post-Award Administrative Manager. Ms. Boxley, is currently a Program Manager III with the Henry M. Jackson Foundation and her office is located adjacent to Dr. Broder within the Department of Microbiology, USU. Ms. Boxley has been working in grants management for over 15 years and successfully completed the Grants Management Certificate Program (GMCP) from Management Concepts in both the federal and pass-thru tracks. She is enrolled in the Program Management Program (PMP) at the Program Management Institute. She currently manages a department portfolio of approximately $32M in direct funded research awarded during the last 3 years. Her day to day grants management includes budget analysis, employee supervision, training of project managers, forecasting burn rates and compliance issues. Ms. Boxley is an employee of the Henry M. Jackson Foundation and salary support is requested.

Supplies

The costs for materials and supplies are based on historical and current supply needs for these types of studies and on current costs for these supplies. The research teams will be engaged with numerous recombinant gene cloning strategies to produce new expression plasmids and recombinant viral stocks. Several large genomic segments will need to be synthesized and the final clones of all these viral genomes assembled and cloned into expression plasmids.

There will be five different chimeric virus stocks that will need to be prepared, qualified, stored and characterized: three rCedV chimeric viruses and two VSV reporter virus stocks. Piloting and rescuing these new viruses will require significant amounts of materials for all the plasmid preparations and transfection reagents and the multiple anticipated variations in plasmid ratios to achieve virus rescues. We estimate $2000 per construct for gene synthesis and sequencing and $4000 per construct for reagents. Once rescued and cloned each virus genome (the viral stock) will have to be deep sequenced. In addition, the proposed studies will require a significant amount of both cell line and virus culture as well consumables, disposables and reagents (enzyme substrate for the luciferase reporter assays) for the serum neutralization tests with rCedV chimeric reporter viruses. The reporter viruses will be designed and rescued within the first 2 years. The budget for supplies includes molecular biology reagents as well as bacterial culture media and supplements, although reagents and chemicals will be needed to run the assays with the newly created reporter chimeric viruses.

Categories of supplies:

Cell and virus culture media, sera and reagents
Molecular biology reagents; enzymes, primers, plasmid purification kits, ligation kits, gene synthesis, whole plasmid sequencing, virus genome sequencing
Chemicals,
Consumables; flasks, plates, pipettes, gloves, tips

Steady-Glo® Luciferase Assay System for in vitro luciferase assays
CTL S6 analyzer (Fluorescent neutralization tests) and Promega GloMax (luciferase based assays)
Other miscellaneous expenses

Indirect Costs:

HJF indirect cost (IDC) is calculated based on the value-added cost base overhead rates. The fringe rate used is 29.42 % for Tier 1 employees and 7.29% for Tier 2 employees (all employees in this proposal are Tier 1).

The HJF indirect cost is calculated based on the value-added cost base overhead rates. The IDC rate is 36.74% USU Onsite Overhead. Additionally, 16.70% Companywide G&A and applied on the total direct cost less subaward plus the USU Onsite Overhead. For proposals including subawards, an additional 1.93% is applied on total external subaward costs.

The above fringe benefits and indirect cost rates for FY2023 were approved by the U.S. Army Medical Research Acquisition Activity on September 1, 2022.

From: Boxley, Kimberly on behalf of Boxley, Kimberly <kimberly.boxley.ctr@usuhs.edu>
To: Haller, Sherry
Cc: Stutz, Sonja J.; Geisbert, Thomas W.; CHRISTOPHER BRODER; Brian Schaefer
Subject: Re: ReVAMPP RP2/RP3 Document Reminder
Date: Friday, May 26, 2023 2:38:55 PM
Attachments: Biosketch-RP3-Schaefer-v2.pdf
Biosketch_Broder-TO BE EDITED.pdf
Biosketch_Amaya_UTMB-USU-ReVAMPP_RP2-v2.pdf
Biosketch_Dimitrov_Broder_RP2_v1.pdf
Key Persons_RP2 and RP3.pdf
Project-Performance Site_UTMB.pdf
Abstract-RP3-Schaefer-v2.pdf
Abstract-RP2-Broder-v2pdf.pdf
Equipment_Broder_RP2-v2.pdf
Facilities_ Broder_RP2-v2.pdf
Equipment-RP3-Schaefer-v2.pdf
Facilities-RP3-Schaefer-v2.pdf
Resource Sharing plan_Broder_RP2-v2.pdf
Vertebrate Animals-RP3-Schaefer-v3.pdf
Select Agents-RP3-Schaefer-v2.pdf
Select Agents_Broder_RP2-v2.pdf
Authentication_Broder_RP2-v2.pdf
Authentication of Key Resources Plan-RP3-Schaefer-v2.pdf
RR Budget_UTMB_Schaefer-v2.pdf
Budget justification-Schaefer-RP3 5-24-2023 v4.pdf
RR Budget_UTMB_Broder-v2.pdf
Budget Justification_Broder_RP2-v3.pdf
image001.png

Hello Sherry,

Most of our documents are pretty good draft's. Maybe minor editorial changes from our grants office.

1. Key Person Biosketches – 5 pages, NIH format– please address your role and expertise for this U19 in personal statement **(Attached) Dr. Broder would like to make some revisions to his.**
2. Key Person Information (including person to be contacted on matters for this application for your institution) **Attached.**
3. Site Information **Attached**
4. Abstract/Project Summary (30 lines) **Attached**
5. Facilities & Resources (Word doc) **pdf documents attached**
6. Equipment (Word doc) **pdf documents attached**
7. Resource Sharing Plan (Word doc). **pdf document attached for Broder, will send document for Schaefer**
8. Vertebrate Animals (Word doc *as applicable*) **pdf attached**
9. Select Agent Research Plan (*as applicable*) **pdf attached**
10. Authentication of Key Biological/Chemical Resources **pdf attached**
11. Budget (SF424) & Justification (Word doc) – *please include justification for Core A effort as well*
    - Project Leads at least 1.2 person months effort **attached, 1.2 cal months verified, no Core A effort; pdf attached of justification**
12. Signed Letter of Intent/Consortium Agreement- for projects that include only effort but no salary or other costs, we will provide a collaborative agreement for signature instead. **this will be provided by grants office no later than June 2, 2023**
13. Specific Aims- 1 page *(draft received 5/18/23 for RP2; draft received 5/12/23 for RP3)*
14. Research Strategy: 12 pages for Research Projects **pdf for Broder attached (draft); will send**

document for Schaefer

Thanks much.....Kim

On Fri, May 26, 2023 at 11:51 AM Haller, Sherry <shhaller@utmb.edu> wrote:

> Hello Kim,
>
> Could you please send us your most current/near final versions of the documents below for USUHS for RP2 and RP3 as soon as possible? Apologies if you have sent any of these items already, but I only have the draft aims for these components so far in my folders.
>
> 1. Key Person Biosketches – 5 pages, NIH format– please address your role and expertise for this U19 in personal statement
> 2. Key Person Information (including person to be contacted on matters for this application for your institution)
> 3. Site Information
> 4. Abstract/Project Summary (30 lines)
> 5. Facilities & Resources (Word doc)
> 6. Equipment (Word doc)
> 7. Resource Sharing Plan (Word doc)
> 8. Vertebrate Animals (Word doc *as applicable*)
> 9. Select Agent Research Plan (*as applicable*)
> 10. Authentication of Key Biological/Chemical Resources
> 11. Budget (SF424) & Justification (Word doc) – *please include justification for Core A effort as well*
>   - Project Leads at least 1.2 person months effort
> 12. Signed Letter of Intent/Consortium Agreement- for projects that include only effort but no salary or other costs, we will provide a collaborative agreement for signature instead.
> 13. Specific Aims- 1 page *(draft received 5/18/23 for RP2; draft received 5/12/23 for RP3)*
> 14. Research Strategy: 12 pages for Research Projects
>
> Thank you!
>
> Sherry
>
> **Sherry L. Haller, PhD**
>
> Associate Director
>
> Center for Biodefense and Emerging Infectious Diseases
>
> The University of Texas Medical Branch
>
> Keiller Bldg., Rm. 1.104D
>
> 301 University Blvd., Galveston, TX 77555-0609

C: (785) 554-1713 O: (409) 747-0766

Teams: Call Chat

E: shhaller@utmb.edu

utmb Health
Center for Biodefense &
Emerging Infectious Diseases

From: Haller, Sherry
Sent: Friday, May 19, 2023 2:53 PM
To: Boxley, Kimberly <kimberly.boxley.ctr@usuhs.edu>
Cc: Stutz, Sonja J. <sjstutz@utmb.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Brian Schaefer <brian.schaefer@usuhs.edu>
Subject: ReVAMPP RP2/RP3 Document Reminder

Hello Kim,

This is a reminder as you are working on collecting the required documents for RP2 and RP3 on Dr. Geisbert and Dr. Broder's ReVAMPP U19 application. If you need us to resend any templates for the documents below, please let us know as soon as possible so we can get that over to you. Otherwise, we hope to receive final or near final versions of everything listed below (expect strategy and aims, final due May 26) by end of day **Monday, May 22**.

1. Key Person Biosketches – 5 pages, NIH format– please address your role and expertise for this U19 in personal statement
2. Key Person Information (including person to be contacted on matters for this application for your institution)
3. Site Information
4. Abstract/Project Summary (30 lines)
5. Facilities & Resources (Word doc)
6. Equipment (Word doc)
7. Resource Sharing Plan (Word doc)
8. Vertebrate Animals (Word doc *as applicable*)
9. Human Subjects Study Record (*as applicable*)
10. Select Agent Research Plan (*as applicable*)

11. Authentication of Key Biological/Chemical Resources
12. Budget (SF424) & Justification (Word doc) – *please include justification for Core A effort as well*
    1. 5 years
    2. Project Leads at least 1.2 person months effort, Scientific Core Leads at least 0.6 person months effort, Data Mgmt. Core Lead at least 1.2 person months effort
    3. NIH salary cap, M&O, travel, publications, service fees, indirects, etc.
13. Signed Letter of Intent/Consortium Agreement- for projects that include only effort but no salary or other costs, we will provide a collaborative agreement for signature instead.
14. Specific Aims- 1 page *(draft received 5/18/23 for RP2; draft received 5/12/23 for RP3)*
15. Research Strategy: 12 pages for Research Projects; 6 pages for Scientific and Data Mgmt. Cores
    1. Milestone Plan: Annual Go/No Go's to be met by end of Y3 (Phase I); Gantt chart/Timeline, PhI/PhII
    2. Tie in Industry Expertise
    3. Address Regulatory Barriers
    4. Bibliography (no page limit)

Please let us know if you have any questions or concerns about meeting this deadline for any of these documents. If you have anything completed, feel free to send us what you have ready, so we may begin our review process and start uploading documents to the application.

Thank you!

Sherry L. Haller, PhD

Associate Director

Center for Biodefense and Emerging Infectious Diseases

The University of Texas Medical Branch

Keiller Bldg., Rm. 1.104D

301 University Blvd., Galveston, TX 77555-0609

C: (785) 554-1713 O: (409) 747-0766

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utmb Health
Center for Biodefense &
Emerging Infectious Diseases

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Kim N. Boxley l Program Manager 3 l Henry M Jackson Foundation
Uniformed Services University of the Health Sciences l Microbiology and Immunology
4301 Jones Bridge Road, Room B4122 l Bethesda, Maryland 20814
Phone: **301-295-1941** l Fax: **301-295-1545** l Cell: **301-793-1883**
Email: KIMBERLY.BOXLEY.CTR@USUHS.EDU

SPECIFIC AIMS

The viral order *Bunyavirales* is composed of several viral families known to contain several high priority human pathogens. Notably, **Arenaviridae** and **Nairoviridae** families contain viruses which cause severe hemorrhagic diseases in humans across the world with mortality rates up to XX and significant, long-term sequelae in survivors. These viruses are on the World Health Organization's (WHO) List of Priority Pathogens where six arenaviruses – Lassa, Lujo, Chapare, Guanarito, Junin and Machupo (LASV, LUJV, CHPV, GTOV, JUNV, MACV) and one Nairovirus-Crimean-Congo Hemorrhagic Fever Virus (CCHFV)– are identified as NIAID Category A pathogens due to ease of dissemination or transmission person-to-person, produce significant morbidity and mortality, have potential for major public health impact, and require special action for public health preparedness. The threat is further heightened for these agents due to lack of internationally approved vaccines to address threats of natural epidemics as well as the potential bio-weaponization of these agents. Threats to public health are further heightened due to **lack of internationally approved vaccines** to address threats of natural epidemics as well as the potential bio-weaponization of these viruses. To address this unmet need, **Research Project 1 (RP1)** will leverage combined expertise in high-containment virology, immunology, and biological product development, to develop arenavirus and nairovirus research tools and vaccine approaches **which can be adapted across members of each viral family in a "plug-and-play" manner**. Much of the work developing vaccines against high containment viruses has relied heavily on viral isolates derived over 40 years ago or longer, which creates a concerns tied to efficacy as newer related viruses continue to emerge and cause disease in humans. Recent advances in reverse genetics of viruses have allowed the generation of infectious genetic clones of several arenaviruses (LASV, MACV, LUJV, JUNV) and nairoviruses (CCHFV) which may make vaccine testing of genetically divergent isolates more feasible. Vaccine development for most arenaviruses and nairoviruses has largely centered on the understanding of the critical role for viral glycoproteins (GP) and nucleoproteins (NP) to drive natural immunity. We have recently successfully engineered a recombinant, stabilized-trimeric antigen for the LASV GP to act as an antigenic mimic of viral surface displayed GP. We have found that this trimeric GP co-delivered with LASV NP subunits or NP subunits alone, **can fully protect guinea pigs against lethal challenge by LASV** underscoring the value of these antigens as vaccine components. Subunit vaccines are prime candidates for alternative vaccination approaches such as microneedle patches (MNP) which harness the potently immunoresponsive cutaneous microenvironments using dissolvable MNPs coupled to recombinant viral glycoprotein antigens and adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The importance of humoral immunity for affording potent protection or treatment against viral infections cannot be understated as evidenced by the recent success using monoclonal antibody therapies to treat Ebola virus disease or COVID-19, yet little is known for the potential for pre-exposure prophylactic (PREP) administration of antibody therapies and what kind of prophylactic windows are possible. The **goals** of this proposal are two-fold: (1) Develop reverse genetics tools to evaluate emerging arenaviruses and nairoviruses for which no isolates are available. (2) Develop novel vaccination approaches utilizing subunit-MNP vaccines developed in or monoclonal antibody PREP strategies which can provide protective immunity against Old and New World arenaviruses or emerging nairoviruses Specifically, we will:

AIM 1: Rescue recombinant arenaviruses and nairoviruses circulating in nature with no available isolates:

We will generate recombinant infectious clones (and reporter-gene versions) of LASV, MACV, and CCHFV isolates for which isolates are not available using reverse genetics. Rescued viruses will be characterized in cell-based assays. Recombinant viruses will be examined *in vivo* (animal model development) within **Core E**.

AIM 2: Develop novel arenavirus and nairovirus vaccination approaches:

2.1 Subunit immunogens and MNP vaccination: Working with **Core C, D**, and **E**, we will determine optimal immunogen/MNP combination that affords pan-lineage protection against LASV, MACV, and CCHFV disease in animal models. We will define detailed multi-parameter cellular and systems level humoral correlates of protection of candidate vaccination approaches. We will then utilize lessons learned to address related "test case" arenaviruses and nairoviruses to develop a subunit/MNP approach for an old world arenavirus (LUJV) a new world arenavirus (CHPV), and an emerging bat borne nairovirus, Kasokero virus.

2.2 Pre-exposure Prophylaxis (PREP) using half-life extension antibody treatments: Working with **Core D**, we will develop vaccination approaches employing a potent therapeutic anti-JUNV monoclonal antibody re-engineered with YTE and LA Fc point mutation predicted to extend pharmacokinetics and determine protective efficacy and estimate conferred immunity correlates in animal studies performed in **Core E**.

SPECIFIC AIMS

The emergence and reemergence of pathogenic RNA viruses represent continuous infectious disease and pandemic threats to public health. Notable examples are the two emerged zoonotic paramyxoviruses, the ‘prototype’ henipaviruses; Nipah virus (NiV) and Hendra virus (HeV), discovered in the mid to late 1990s. NiV and HeV have a broad host tropism capable of infecting 18 animal species across 6 orders of mammals using the highly conserved ephrin-B2 and -B3 ligands as entry receptors. NiV and HeV infections result in a systemic and often fatal respiratory and/or neurological disease in 11 mammalian species including humans. They are significant biothreats to humans and economically important livestock in Australia and South East Asia. Henipaviruses have two membrane glycoproteins: the attachment glycoprotein (G) and the fusion glycoprotein (F) that are the major targets of neutralizing antibodies and the focus of all vaccine strategies. The most extensively studied NiV/HeV vaccine has been the soluble recombinant G glycoprotein of HeV (HeV-sG), which can provide complete protection against lethal challenge by either NiV or HeV in four animal species including nonhuman primates (NHPs). We have recently tested the immunogenicity of HeV-sG delivered using an advanced dissolvable microneedle patch (MNP) technology. Preliminary data reveal potent NiV/HeV neutralizing antibody responses and this new approach may offer a novel, highly stable and rapid vaccination modality. In parallel, we have extensively characterized the nonpathogenic henipavirus, Cedar virus (CedV); develop a reverse genetics platform, generated recombinant CedV (rCedV) and rCedV chimeras by swapping G and F glycoproteins for the respective NiV or HeV G and F, and developed a surrogate BSL-2 virus neutralization platform. More recently, novel henipaviruses, including genetically related Mòjiāng virus and Langya virus (LayV) have been discovered, and in contrast to HeV/NiV, both appear to be rodent borne and do not use ephrin ligands as entry receptors, and each has infected humans causing disease. Further, a most recently discovered bat-borne and divergent henipavirus, Angavokely virus, from wild Madagascar fruit bats, has also been described. Here, we propose to develop the MNA patch delivery platform, create LayV and other reporter virus tools to complement the HeV/NiV chimera tool, and use these approaches to developed high throughput BSL-2 based virus neutralization platforms to evaluate the MNP subunit vaccine approach; and apply the MNP vaccine patch technology to other emerging and/or potential zoonotic paramyxovirus pathogens. Specifically, we will:

Aim 1: Develop and evaluate sG_{tet} and sF_{stb} prototype henipavirus subunit vaccine candidates by dissolvable MNP vaccination.

We will compare NiV vaccine immunogens (sGtet and sFstb) and HeV (HeV-sFstb) to HeV-sG in heterologous challenge studies using Nipah-Bangladesh and Hendra-Redlands viruses when delivered by microneedle vaccine patch. Immunogenicity will be tested in mice and NiV/HeV neutralizing potencies evaluated. Challenge studies will be conducted in ferrets. Antivirus humoral responses will be evaluated by,,,,, (Gunn). Selected best-in-class vaccine candidate(s) will be evaluated in the NHP NiV/HeV model.

Aim 2: Rescue recombinant Langya virus (rLayV) and LayV glycoprotein reporter virus platforms.

We will generate the new henipavirus LayV as an additional ‘prototype’ henipavirus using reverse genetics. Recombinant LayV (and reporter-gene versions) will be constructed and virus rescue procedures will be conducted by the Scientific Core at UTMB to rLayV. Alternate, virus tools will also be generated using reporter-gene versions and rCedV chimeras encoding the LayV F and G. Rescued viruses will be characterized in cell-based assays. The rLayV virus will be examined in vivo (animal model development) within the Center Core.

Aim 3: Evaluate antibodies and nanobodies to henipaviruses developed by the Center’s RPs.

Henipavirus tools and high-throughput virus neutralization platforms will be utilized to test and evaluate MNP vaccine induced immune sera and individual virus specific monoclonal antibodies against henipaviruses discovered and/or developed by the research projects in the Center. (Needs defining here, RP4 and 5, and also AngV F/G mab discovery – Genovac)

Aim 4: Adapt the MNP vaccination approach in Center Phase 2 to other paramyxoviruses.

Here, we will develop, characterize, and validate this *plug-and-play* polyphosphazene (PPZ) adjuvanted dissolvable MNP platform with different recombinant paramyxovirus glycoprotein antigens.

Specific Aims

Henipaviruses are single-stranded, negative-sense enveloped RNA viruses of the paramyxovirus family[1]. Two henipaviruses, Nipah virus (NiV) and Hendra virus (HeV), cause a systemic and often fatal respiratory and/or encephalitic disease in humans and ten other mammalian species[2-4]. Importantly, NiV and HeV are significant biothreats to humans and economically important livestock in Australia and Southeast Asia[5]. There are currently no vaccines or therapeutics approved for human use. Notably, development of countermeasures for NiV and HeV is hampered by the fact that these viruses require BSL-4 containment, meaning that very few research groups worldwide have access to the required biocontainment facilities to perform preclinical studies with these important human viral pathogens[6].

To address this problem, we are developing a BSL-2 animal model that is based on Cedar virus (CedV), which is a non-pathogenic henipavirus[7]. Specifically, we have engineered recombinant Cedar viruses (rCedVs) in which the NiV and HeV fusion (F) and receptor-binding glycoprotein (G) are expressed in the rCedV genome, replacing CedV F and G. Additionally, we have also incorporated our recently developed *in vivo* bioluminescence methodology[8, 9] to longitudinally trace the dynamics and anatomical progression of rCedV-luciferase (rCedV-luc) infections in individual animals. Using various approaches to inhibit the host interferon response in mice, we have demonstrated sustained replication of rCedV-luc and the rCedV-NiV-luc and rCedV-HeV-luc chimeras. Importantly, whereas rCedV-luc does not establish stable expression in the brain, both of the chimeric viruses do. Moreover, preliminary findings show that rCedV-NiV-luc causes neurological dysfunction and death in STAT1-/- mice. The rCedV-luc platform is thus an authentic henipavirus system that can be used to study henipavirus *in vivo* biology safely and expediently under BSL-2 containment. Our **overall hypothesis** is that rCedV-luc infection of mice lacking interferon responses represents a BSL-2 platform for the study of henipavirus biology and pathogenesis, as well as for development and testing of anti-viral countermeasures. We will address this hypothesis through three Specific Aims:

Aim 1: To optimize the use of Stat1-/- mice and rCedV-NiV-luc/rCedV-HeV-luc chimeras as a robust BSL2 model of pathogenic henipavirus disease. Hypothesis: *Infection of Stat1-/- mice with recombinant rCedVs will recapitulate major hallmarks of pathogenic NiV and HeV infections in humans*. We will establish a breeding colony of Stat1-/- mice on a B6-albino background. We will infect Stat1-/- mice with rCedV-luc chimeras. We will use flow cytometry, spatial transcriptomics approaches and histological methods to define cell types infected, gene expression changes and immune phenotypes that characterize pathological (Stat1-/- model; rCedV-luc chimeras) vs. non-pathological (Ifnar1-/- model; rCedV-luc) infections.

Commented [BS1]: -Collaboration with Core D (systems serology - Gunn)
-Collab with RP2 (other rCedV chimeras)
-Possible exploration of hamster infection (rCedV-NiV-luc), using much larger dose than what was used for UTMB studies to determine if (1) efficient CNS infection can be achieved and (2) if CNS infection is highly correlated with lethality. Hamsters may represent a rCedV lethal model employing a host that is not immunocompromised. Limitations: few specific analysis tools (mAbs in particular) for the hamster model.

Aim 2: To determine the mechanism of rCedV-NiV neurovirulence in the Stat1-/- mouse model. Hypothesis: *CNS infection by rCedV chimeras requires functional interaction between G and host ephrin B3; neurovirulence is a property shared by henipaviruses in the absence of host IFN-dependent defenses*. We will evaluate whether interaction between NiV/HeV G and host ephrin B3 is required for neurotropism. We will employ direct injection of rCedV into the brains of Stat1-/- mice to determine whether neurological disease is a property shared by brain-resident henipaviruses in the absence of a host interferon response.

Commented [BS2]: -Collab with RP2 (rCedV-NiV ephrin B3 binding mutant)

Aim 3: To define the efficacy and mechanisms of mAb-based therapeutics for CNS-resident henipavirus infections. Hypothesis: *G- and F-directed mAb-based therapeutics will demonstrate Fc-dependent efficacy against CNS-resident henipavirus*. Stat1-/- mice infected with rCedV chimeras will be treated with anti-G and anti-F mAbs and nanobodies. We will employ the approaches of Aim 1 to determine the immunological basis of any observed mAb therapeutic effects. We will also specifically investigate the role of mAb-FcR interactions for therapeutic efficacy.

Commented [BS3]: -Collaboration with RP5, nanobodies
- Collaboration with Core D (systems serology - Gunn)

HeV and NiV are emerging human and livestock pathogens with a high fatality rate, demonstrated human-to-human transmission, and a worrisome recent history of outbreaks in proximity to densely populated areas, including Kerala, India[10-12]. There is thus an urgent need to develop novel therapeutics directed against these viruses. However, work with HeV and NiV requires BSL-4 containment, severely limiting translational research efforts. Through the development and investigation of this longitudinal BSL-2 animal model of authentic and pathological henipavirus infection, we will greatly facilitate future development and preclinical validation of therapeutics directed against these deadly human pathogens.

SPECIFIC AIMS

The COVID-19 pandemic has highlighted the need for fast drug development technologies and new antiviral drugs to address emerging pathogens. One promising approach is the use of nanobodies, which are antigen-binding entities derived from camelid animals' heavy chain-only antibodies. Nanobodies are small (15 kDa), retain full antigen specificity, and can recognize inaccessible epitopes while crossing tissues and blood-brain barriers, making them potential antiviral drugs with high cross-reactivity against multiple viral pathogens.

Henipaviruses and arenaviruses are two viral families that pose severe public health risks worldwide, causing encephalitis and hemorrhagic fever. Currently, there are no licensed treatments that specifically target these viruses, so effective treatments are urgently needed. To address this issue, four prototype viruses were selected from each of the Henipavirus and Arenavirus genera based on their significant impact on public health and the lack of effective treatments against them. This research strategy can be generalized and applied to more members of these two viral groups.

The study focuses on two Henipavirus prototypes: Nipah virus (NiV) and Hendra virus (HeV), which have caused outbreaks in Asia and Australia, respectively, and are responsible for severe respiratory and central nervous system (CNS) illnesses in humans. These viruses have high mortality rates ranging from 40% to 90%. Similarly, two prototypic viruses of the Arenavirus genus that can cause severe illness and death in humans are focused in this study, Lassa virus (LASV) and Machupo virus (MACV). LASV is responsible for causing Lassa fever, which is endemic in West Africa and can lead to hemorrhagic fever, while MACV is responsible for causing Bolivian hemorrhagic fever. These viruses are a global public health threat due to their geographical distribution and diversity.

To develop nanobody-based therapies against Henipaviruses and Arenaviruses, the study proposes to target the viruses' key entry machinery, including the G and F glycoproteins of HNV and the GPC of arenavirus. The study will use a structure-based strategy to understand viral neutralization mechanisms and facilitate nanobody enhancement engineering and design blood-brain barrier-penetrating nanobodies. The hypothesis is that nanobodies will serve as a useful countermeasure against CNS diseases caused by these viruses. The study will leverage three nanobody development platforms: camelid animals, nanomouse, and low-immunogenic human nanobody libraries, which closely resemble the natural human antibody heavy chain variable domain. These libraries will enable the quick identification of nanobody leads that require minimal optimization for clinical use. The study will carry out this project with three specific aims.

Aim 1: To identify cross-reactive nanobodies to Henipaviruses. Given their high degree of glycoprotein (F and G) similarity, it feasible to identify cross-reactive neutralizing nanobodies against both HeV and NiV for broad protection. We will use both immunization in camelids and nanomice and a recently generated naive human nanobody library for nanobody production. We will employ sequential immunization and antigen panning strategies to identify nanobodies that cross-recognize HeV and NiV. Enriched libraries will be deep-sequenced to ensure comprehensive coverage. Finally, candidate nanobodies will be evaluated for specificity by ELISA and neutralizing activity by RP2.

Aim 2: Identification of nanobodies to Arenaviruses. Arenaviruses, such as Old World LASV and New World MACV, contain a heavily glycosylated and conformationally labile surface glycoprotein (GPC), making it challenging to generate monoclonal antibody therapies using conventional antibodies. To overcome this, we plan to develop nanobodies as a therapy option. Nanobodies, being smaller in size, could potentially penetrate the glycan surface of GPC and neutralize the virus. We will generate nanobodies by immunizing camelids and nanomice with a stabilized trimeric GPC immunogen, and also use a naive human nanobody library for screening neutralizing nanobodies. Individual nanobodies will be assessed for target specificity by binding and neutralization activity in RP1.

Aim 3: Nanobody characterization and structure-based engineering. We will first conduct an affinity test for all identified nanobody neutralizers and correlate their affinity and neutralization potency. Using the structure-based analysis, using X-ray crystallography and Electron Microscopy Single Particle Analysis, of nanobody epitopes and neutralization mechanisms, we will rationally design to improve the therapeutic efficacy and engineer the combination of nanobodies that target multiple epitopes. We will also design a nanobody therapy that targets the central nervous system (CNS) by coupling with transferrin receptor 1 (TfR1) for efficient blood-brain barrier (BBB) penetration. Finally, we will evaluate the efficacy of the engineered nanobodies in an in vivo rCedV-NiV-B chimera mouse model in RP3.

SPECIFIC AIMS - Core A (Administrative Core), University of Texas Medical Branch at Galveston

The Administrative Core will function as a critical component of the Research and Development of Vaccines and Monocolonal Antibodies for Pandemic Preparedness (ReVAMPP) “Paramyxoviridae and Bunyavirales Vaccines and Antibodies Center” (PABVAX) by providing support for the implementation of an integrated, product-oriented research program composed of major research projects and core facilities. The Core will be responsible for the overall management and coordination of PABVAX activities, including governance and financial and operational management to facilitate the execution of the ReVAMPP network-wide strategic research goals and Center plans to develop countermeasures for Paramyxoviridae and Bunyavirales infections.

The Specific Aims of the Administrative Core are to:

Aim 1: Manage, coordinate, and supervise all Center activities.

Under the direction of the PD/PIs (Drs. Geisbert and Broder), the Administrative Core will provide organizational and programmatic structure to promote research productivity and scientific interactions in support of the development of countermeasures for Parmyxoviridae and Buynavirales. The Administrative Core will guide the refinement of policies governing PABVAX operations and will ensure compliance with requirements for the responsible conduct of research activities, particularly those involving select agents, vertebrate animals, and human subjects. The Core will coordinate all activities of the Scientific Advisory Board and will oversee the management of Center financial resources.

Aim 2. Provide regulatory expertise as needed for implementing translational research activities.

Regulatory guidance will be provided by the Administrative Core from institutional experts who will assist PABVAX researchers in overcoming nonclinical regulatory barriers.

Aim 3: Coordinate seamless communication within the Center, with NIAID program staff, and throughout the ReVAMPP Network.

The Administrative Core will assure continuous communication within the Center by organizing regular meetings with all Core members and Research Project personnel and will facilitate the dissemination of information among Center scientists, institutional officials, and external stakeholders. The Administrative Core will be in direct contact with each component of the Center to continually review research progress and to maintain detailed, up-to-date records; the core will exploit this relationship to generate all written reports to assure timely delivery of information to NIAID program staff and to complete all annual continuation applications. In conjunction with the ReVAMPP Coordinating and Data Sharing Center (CDSC), the Administrative Core will also coordinate interactions with ReVAMPP network participants to facilitate network-wide communication of Center activities.

Aim 4: Lead collaborative efforts with the CDSC and the ReVAMPP Network to ensure program compliance.

The Administrative Core will be responsible for maintaining communication with the ReVAMPP CDSC to lead and coordinate the Center’s collaborative obligations to the ReVAMPP Network. Further program compliance will be assured through the organization of annual ReVAMPP network-wide meetings for Center personnel, as well as the annual ReVAMPP Center progress meetings for PABVAX personnel and Scientific Advisory Board Members.

Data Management Core: Specific Aims

The Data Management Core (DMC) aims to design a secure, scalable, and redundant data storage and transfer infrastructure that will serve as the central knowledge vault for all research projects and cores. DMC will establish a robust mechanism for "raw" HIPAA-compliant data collection generated by all groups and performs routine quality control activities focusing on 6 dimensions: completeness, accuracy, consistency, validity, uniqueness, and integrity. After a thorough cleaning and error resolution, the data will populate the local RAID6 redundant instance of the SQL server, completely mimicking the Immport system, which will be used for data sharing with the coordinating center and worldwide research communities. Pure and organized data will conform to the FAIR data principles to follow the Findability, Accessibility, Interoperability, and Reuse of digital assets. DMC will utilize the HIPAA-compliant Microsoft SharePoint cloud located behind the secure UTMB firewall to organize a data lake with a dedicated vault for each research project and core. Individual vaults will undergo automatic data assessment routines to identify outliers, gaps in data, and errors and report to individual vault stakeholders. All inconsistencies will be resolved during the organized monthly meetings between stakeholders and the data management group. The data will be prepared to be further deposited to the MariaDB instance following Import schema and table structures. DMC team has already established a similar infrastructure in the West African Center for Emergent Infectious Disease and has experience and contacts developed with the Immport admin team to achieve this goal. DMC core will also assist in the bioinformatics, data science, and statistical analysis required at any project stage. With years of experience in clinical biomedical informatics working with Big Data EMR, our team will help with in-silico validation and external data integration to supplement the research activities of all groups. To implement the proposed infrastructure, we propose the following aims:

Aim 1: **Establish a Robust Data Management System**
1.1 Develop a secure, user-friendly data management system using HIPAA-compliant tools like Microsoft OneDrive and SharePoint Server to store, organize, manage, and share research data.
1.2 Standardize data formats, metadata, and documentation to ensure consistency, interoperability, and reusability. This will involve creating data dictionaries, templates, and guidelines for data entry and quality control.
1.3 Design vault specific PowerBI dashboard that allows continuous monitoring of the state of the data for each of the project and core
Aim 2: **Facilitate Data Sharing and Training**
2.1 Develop tools and platforms for data sharing, collaboration, and communication among project research teams, including data sharing agreements, data access portals, and workshops.
2.2 Provide training and technical assistance to research teams on data management best practices
2.3 Maximize the dissemination and reuse of research data in line with the NIH FAIR mission through strategies such as publishing datasets in public repositories and creating data citation policies.
Aim 3: **Ensure Data Quality Control and Security**
Implement rigorous data quality assurance and control measures to maintain research data's accuracy, completeness, and validity. Ensure data security and compliance with relevant regulations to protect sensitive data from unauthorized access, disclosure, or loss. This will involve regular risk assessments, data backup and encryption, and access controls. Data quality will be assessed across several dimensions: accuracy, completeness, consistency, timeliness, validity, and uniqueness. Accuracy and validity ensure the data correctly represents real-world entities and conforms to HIPAA and FAIR rules, while completeness and uniqueness check for comprehensive data without repetitions. Consistency and timeliness checks will ensure uniform representation across different sources and the data's relevance to the required time point.

Core C - Microneedle Patch Core (Falo, Andrianov, Korkmaz): SPECIFIC AIMS

The ***goal*** of the *Microneedle Patch (MNP) Core* is to develop, characterize, and validate our *plug-and-play* polyphosphazene (PPZ) adjuvanted dissolvable MNP platforms with different recombinant viral glycoprotein antigens that will be developed in the Research Projects (RPs: RP1 and RP2). The MNP Core will develop and batch release PPZ MNP vaccine platforms with RP1- and RP2-specific viral antigens based on the well-defined quantitative release criteria of their validated physicochemical and immunological properties. Ultimately, these PPZ MNP vaccine platforms will enable the development of clinically translatable, highly protective vaccines against several prototype viral pathogens from multiple viral families, including *Arenaviruses (Lassa, Machupo, Lujo, and Chapare viruses)*, *Nairoviruses (Crimean-Congo hemorrhagic fever-CCHF and Kasokero viruses)*, and *Paramyxoviruses (Nipah, Hendra, and Langya viruses)*, through studies outlined in RP1 and RP2.

The MNP Core leverages our unique and broadly deployable vaccination approach enabled by a synergistic combination of plug-and-play, clinically feasible *immunoadjuvant* and *skin-targeted vaccine delivery* platforms, for which we have well-established formulation/production and quality control protocols. Our unique vaccination strategy will effectively harness the highly immunoresponsive cutaneous microenvironments using dissolvable MNPs incorporating recombinant viral glycoprotein antigens and PPZ adjuvants to elicit robust, strong, and long-lasting protective immunity against the target pathogen. The RP-specific *recombinant viral glycoprotein antigens* will drive the generation of virus-specific protective immune responses. *The PPZ macromolecules* will serve as an immunoadjuvant nanoassembly that will provide many immune-potentiating features to complement subunit antigens, such as improved antigen uptake, multivalent antigen presentation, and innate immune stimulation, to rationally augment vaccine-induced immunity. *Dissolvable MNPs* will enable targeting vaccine components to the highly immunoresponsive cutaneous layers in a safe, reproducible, and self-applied manner for efficacious immunization, as well as facilitate the improved vaccine storage and distribution due to their thermostability. ***Our preliminary data strongly support these innovative features of our skin-targeted vaccination strategy.*** Collectively, our PPZ MNP-based skin immunization technology *1)* brings key immunogenicity advantages over conventional immunization routes (intramuscular and subcutaneous) by targeting the highly efficient endogenous immune circuitry of the skin to significantly improve vaccine-specific immune responses, and *2)* shifts vaccination from traditional needle-and-syringe administration of heat-sensitive vaccines by trained healthcare workers to highly effective, widely deployable, needle-free, thermostable, and self-administered vaccines for more effective, sustainable, and equitable mass vaccination campaigns. Ultimately, our clinically translatable PPZ MNP vaccine platforms are readily adaptable to develop efficacious and broadly deployable vaccines against emerging and re-emerging viruses, including those with RP1- and RP2-specific recombinant viral glycoprotein antigens.

Building on our strong preliminary data with PPZ MNP platforms with different antigens, our expertise with MNPs, PPZ adjuvants, skin-targeted vaccines, and murine immunogenicity studies, as well as complementary expertise provided by Core D, we will develop, extensively characterize, immunologically evaluate, and batch release PPZ MNP platforms with RP-specific antigens to enable the development of effective, safe, broadly deployable, and clinically translatable vaccines against the prototype viruses outlined in RP1 and RP2. Our cohesive research support plan involves two Specific Aims in the MNP Core as IND-enabling studies with RP-specific viral antigens:

Specific Aim 1: To Develop And Characterize PPZ MNP Vaccines. We have comprehensive experience with the translational development of PPZ adjuvants and dissolvable MNPs that we have advanced to human clinical trials. *AIM 1* is geared toward the development and physicochemical characterization of PPZ MNP platforms with RP1- and RP2-specific antigens (drug substances or antigens will be extensively characterized in Core D) based on standard operating procedures (SOPs) established for formulation and quality control (QC) of PPZ-complexed antigens and vaccine-loaded MNPs. Ultimately, PPZ MNP platforms with project-specific antigens (drug product) will be batch released based on the quantitative release/pass criteria of their physicochemical features to enable reproducible targeting of the vaccine components to the skin for efficacious immunization.

Specific Aim 2: To Validate The Correlates Of Protection Of PPZ MNP Vaccines In Mice. We have expertise with the evaluation of the correlates of PPZ MNP vaccine-induced protection through murine studies. *AIM 2* is planned toward the evaluation of vaccine-induced humoral (antigen-specific serum binding antibodies [IgM, total IgG, IgG1, IgG2, and IgA] and virus-specific neutralization activity of vaccine-induced serum antibodies via an *in vitro* neutralization assay) and cellular (polyfunctional antigen-specific T-cell responses in vaccine-draining lymph nodes and spleens) immune responses in mice immunized with PPZ MNP vaccine platforms with project-specific viral antigens. Ultimately, PPZ MNP vaccine platforms with RP1- and RP2-specific viral antigens will be advanced to RPs based on the quantitative immunological release/pass criteria to enable the optimal induction of effective, robust, and long-lived virus-specific protective immune responses in studies outlined in RP1 and RP2.

Core C - Microneedle Patch Core (Falo, Andrianov, Korkmaz): RESEARCH SUPPORT PLAN

1. Significance

The threat of emerging and re-emerging infectious diseases highlights the need for continued improvement of vaccination strategies to enable more effective, sustainable, and equitable global immunization campaigns(1-3). Development of efficacious, safe, low-cost, user-friendly, broadly deployable, and rapidly adaptable vaccination platforms, with emerging vaccine adjuvants and vaccine delivery systems, as well as their proactive validation with vaccines against prototype pathogens are of great significance to maximize pandemic preparedness(4-6). The Microneedle Patch (MNP) Core brings together the transformative project components, ***1)*** *skin immunization*, ***2)*** Polyphosphazene (*PPZ) adjuvant*, and ***3)*** *dissolvable MNPs*, with the rationally designed antigens that will be developed in research projects (RPs) against high-priority pathogens (**Fig.1**) to enable the achievement of the goals of the overall project, as well as to support the translational development of a next-generation immunization strategy. Our cohesive project plan designed around these key project components will yield IND-enabling results for PPZ MNP platforms, with RP-specific viral antigens, which will facilitate their rational clinical translation. Ultimately, PPZ MNP platforms will help obviate the existing bottlenecks in the vaccine efficacy, compliance, storage, acceptance, and distribution for more widespread global immunization campaigns.

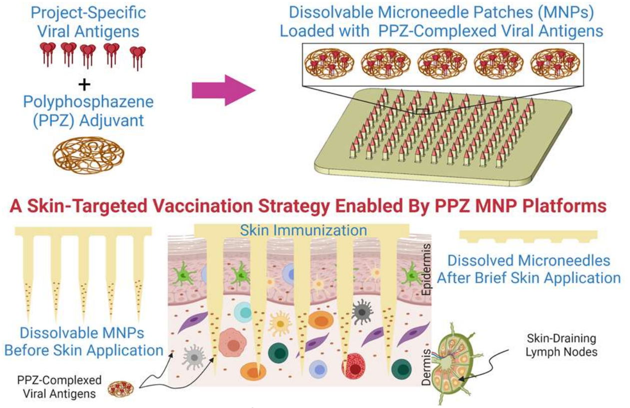

Fig. 1. A paradigm-shifting immunization strategy using heat-stable, self-administered, and widely deployable dissolvable MNPs integrating PPZ-complexed project-specific antigens.

Skin Immunization. Rapidly increasing experimental and clinical evidence strongly supports the notion that skin vaccination offers key biological benefits over conventional vaccination routes (intramuscular and subcutaneous) to induce significantly improved vaccine-specific protective immunity(7-11). The skin offers an immunocompetent tissue microenvironment that hosts a high density of professional antigen-presenting cells (APCs) and immune-accessory cells, with broad immune functions, as well as a rich network of lymphatic vessels that facilitate the continuous immune communications with the skin-draining lymph node(12-15). Unlike the immunologically inert features of muscle and subcutaneous tissues that consist of a substantially lower density of APCs than the skin tissue, the highly efficient immune networks of the skin drive the generation of strong and long-lasting antigen-specific immune responses, enabling more effective immunization compared with conventional intramuscular and subcutaneous vaccination strategies(7-10, 12-14). We and others have demonstrated that skin immunization is capable of inducing strong and long-lasting immune responses in both systemic and mucosal compartments, which are superior to those induced by conventional needle immunization via parenteral routes(9, 10, 16-18). *We have unique expertise with skin immunization and propose to exploit the established advantages of the skin immune system in the MNP Core to enable the development of effective, safe, and broadly deployable skin-targeted vaccines, with the project-specific viral antigens that will be extensively characterized in Core D.*

PPZ Immunoadjuvants. Recombinant subunit protein antigens are often complemented with vaccine adjuvants to mount robust, strong, and durable protective immune responses(19-21). PPZ macromolecules are emerging synthetic water-soluble biodegradable immunoadjuvants that have well-defined molecular properties and are fully compatible with dissolvable MNPs(22-24). PPZ macromolecules have excellent antigen-binding features, spontaneously self-assemble with antigens in aqueous solutions, without the need for sophisticated formulation processes, and form nanoassemblies with virus-mimicking dimensions (60-100 nm)(25, 26). Mounting clinical and experimental evidence supports that PPZ macromolecules serve as a potent and safe adjuvant that provides several immune-potentiating features, including increased antigen uptake, multivalent antigen presentation, and multifaceted innate immunity stimulation to enhance vaccine-induced protective immune responses (22-28). We and others have demonstrated through animal studies that PPZ adjuvants, especially the most commonly used PPZ adjuvants*, clinical-stage poly[di(carboxylato-phenoxy)phosphazene] (PCPP)* and its structural homologue *poly[di(carboxylato-ethylphenoxy) phosphazene] (PCEP)*, significantly augment vaccine immunogenicity, enable antigen dose-sparing, and achieve long-lasting protection in live viral challenge studies(28-30). *We will leverage our comprehensive experience with the translational development of PPZ adjuvants in the MNP Core to enable the development of effective and rapidly deployable PPZ MNP vaccine platforms, with the RP-specific antigens.*

Dissolvable MNPs. Effective and reproducible delivery of vaccine components to the highly immunoresponsive cutaneous microenvironments is pivotal for efficacious skin immunization strategies(12, 13, 31-33). Dissolvable

MNPs have emerged as a clinically feasible delivery platform that can administer vaccines to the immunologically rich milieu of the skin in a safe, patient-friendly, and consistent manner(12, 13, 32). We have previously shown that microneedles of our dissolvable MNPs physically penetrate the outermost skin layers and actively deposit vaccine components in a defined three-dimensional (3D) space within the skin, eliminating the delivery efficiency, formulation, and precision complexities associated with other skin-targeted vaccine delivery approaches(12, 16, 33-35). Importantly, we and others have introduced dissolvable MNPs in human clinical trials in studies that are establishing the feasibility of MNP-based biomolecule delivery to human skin (*NCT02192021, NCT05377905*). Further, the immunogenicity and logistic benefits of self-administered, temperature-stable MNP vaccines over needle-and-syringe delivery of heat-sensitive vaccines by trained healthcare workers have been demonstrated by several laboratories, including our own laboratory(12, 13, 32). Collectively, our dissolving MNPs offer a widely applicable vaccine delivery platform to enable more effective, sustainable, and equitable global immunization campaigns against infectious pathogens. *We will exploit our unique expertise with the development of clinically feasible dissolvable MNPs in the MNP Core to enable the development of effective, safe, and widely deployable skin-targeted vaccines (drug products), with the project-specific viral antigens (drug substances from Core D).*

Together, the MNP Core will deliver PPZ MNP vaccine platforms with project-specific viral antigens, with the goal of unlocking the full potential of the cutaneous immune system to capacitate the development clinically viable vaccines for effective, safe, and widespread immunization against high-priority viruses.

2. Innovation

The MNP Core will exploit our transformative, rapidly translatable, self-administered, needle-free, thermostable, and widely applicable vaccination strategy consisting of next-generation vaccination platforms, such as emerging PPZ adjuvants and dissolvable MNP-based skin-targeted vaccine delivery systems, to enable the translational development of novel, highly protective vaccines against several prototype viral pathogens outlined in RPs. Key factors contributing to the innovation of the MNP Core include:

- *The skin tissue* is a more potent vaccination site than conventional vaccination routes, including muscle and subcutaneous tissue, since it contains a more immunoresponsive environment than the latter tissues.
- *PPZ macromolecules* are next-generation immunoadjuvants that are compatible with dissolvable MNPs. Importantly, PPZ macromolecules offer remarkable immune-potentiating features, including increased antigen uptake and stability, multivalent antigen presentation, and efficient skin innate immune stimulation to enhance both humoral and cellular protective immune responses induced by vaccine antigens.
- *Dissolvable MNPs* are a next-generation vaccine delivery system that offers a needle-free, thermostable, self-applied, and broadly deployable platform that can reproducibly target vaccine components (antigen and adjuvant) to the immunologically rich milieu of the skin. Importantly, we will leverage our unique and clinically tested dissolvable MNPs to enable precise and reproducible delivery of PPZ-complexed project-specific antigens to the highly immunocompetent cutaneous layers for the optimal induction of protective humoral and cellular immunity. Dissolvable MNPs will not only improve the vaccine immunogenicity and efficacy but also eliminate the need for needles, syringes, medical expertise, and cold-chain dependence to reduce cost, and to improve vaccine storage and distribution for increased vaccine coverage.
- Translational feasibility is enabled by our next-generation vaccination strategy synergistically combining the clinically tested PPZ adjuvants and dissolvable MNPs with project-specific viral antigens. The success of our translational efforts will be ensured by the clinical product development (GMP Manufacturing, GLP Testing, Target Product Profiles) support that will be provided by highly experienced investigators of Mapp Biopharmaceutical (Core D), Emergent Biosolutions and Moderna (see the attached letters of support).

The outcome of this project will be IND-enabling results for PPZ MNP platforms, with RP-specific antigens, which will facilitate the clinical translation of a paradigm-shifting vaccination strategy against high-priority pathogens.

3. Experimental Plan

3.1. Studies Supporting The Feasibility Of The MNP Core

Our published (12, 13, 16, 33-35) and preliminary data demonstrate our expertise with the proposed activities in the MNP Core and herein we show representative data supporting that we have the expertise to enable the development of PPZ MNP vaccine platforms with various antigens.

Over the past decade, the research teams of Dr. Falo and Dr. Korkmaz at UPITT have pioneered the translational development, analysis, and human application of dissolvable MNPs for effective skin-targeted delivery of diverse biocargos, including vaccine components (antigen + adjuvant). **Fig.2** shows representative MNPs designed and developed at the UPITT, and their skin-targeted vaccine delivery performance. These user-friendly MNPs consist of micron-scale, obelisk-shaped, ultra-sharp (<1 μm in tip radii) protrusions with filleted bases, which maximize biocargo volume and microneedle strength for consistent delivery of vaccine components to the defined skin layers. Our MNPs are manufactured from water-soluble materials that are designated as safe for human use (categorized as GRAS) by the FDA. Upon a brief (~5 min) cutaneous application, these high-quality MNPs **(Fig.2B)** penetrate the highly host-protective cutaneous layers, quickly dissolve **(Fig.2C)** in the aqueous skin environments upon penetration, and effectively deliver the integrated vaccine components to the immune-responsive layers in murine **(Fig.2D-F)** and human **(Fig.2G-I)** skin. *<u>We will exploit this expertise to develop PPZ MNP platforms with RP-specific viral antigens based on established guidelines and inspection checklists, which we are now using for creation and quality control of MNPs that are being tested in our own investigator-initiated clinical trials.</u>*

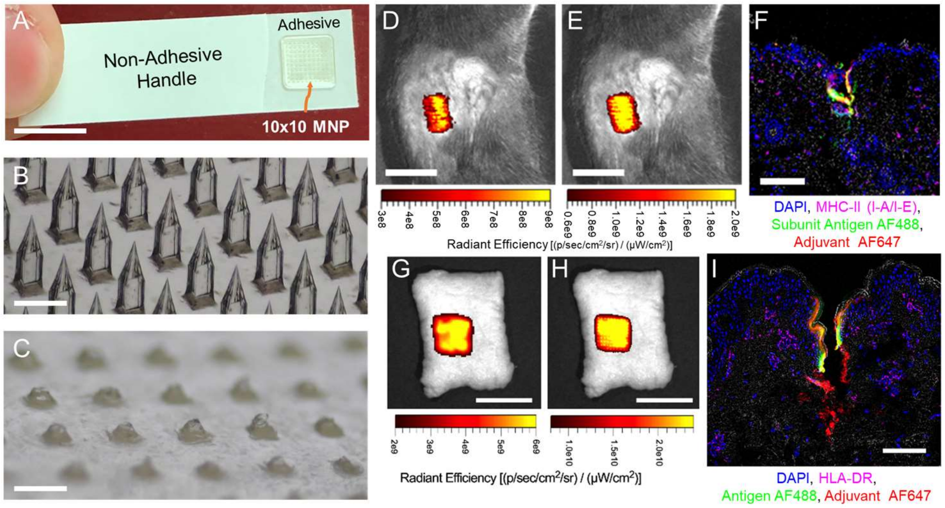

Fig. 2. Engineering dissolvable MNPs for cutaneous vaccination. **A)** Patient-friendly skin-targeted vaccine delivery platform with dissolvable MNPs. <u>Scale bar (SB) = 10 mm</u>. Microscopy images of MNPs **B)** before and **C)** after application to the skin demonstrate efficient dissolution of microneedles. <u>SBs = 500 μm</u>. Effective delivery of fluorescent labeled vaccine components (subunit antigen AF488 and adjuvant AF647) to **(D-E)** murine skin *in vivo* and **(G-H)** human skin *ex vivo* using MNPs. <u>SBs = 10 mm</u>. MNP-based precise delivery of these fluorescent labeled vaccine components to the immunologically rich milieu of the **F)** murine skin *in vivo* and **I)** human skin *ex vivo*. <u>SBs = 100 μm</u>.

Our published data have consistently demonstrated that vaccine delivery via our high-quality MNPs is effective in eliciting robust, potent, and durable antigen-specific systemic adaptive immune responses that are often superior to those generated by traditional intramuscular and subcutaneous vaccination approaches(16, 34, 35). Importantly, as part of our ongoing collaboration with Dr. Broder, Dr. Geisbert, and Dr. Andrianov, we have initiated the development of PPZ-MNP vaccine candidates against henipahviruses. The research team of Dr. Andrianov has pioneered the design and development of PPZ adjuvants to boost vaccine-induced immunity, and has validated the successful formulation of PPZ with soluble recombinant G glycoprotein of Hendra Virus (HeV-sG). *<u>In the MNP Core, we will exploit this expertise to develop PPZ vaccine complexes with RP-specific viral antigens based on the established guidelines and inspection checklists of PPZ adjuvants.</u>* We have then formulated the antigen HeV-sG, the PPZ adjuvant PCEP, the two water-soluble biomaterials, carboxymethyl cellulose and trehalose (GRAS), into dissolvable MNPs, and have tested the immunogenicity of these MNPs incorporating PCEP and HeV-sG. **Fig.3** demonstrates our unpublished representative data with regard to the ***immunogenicity*** [<u>*Humoral:*</u> (Antigen-specific binding antibody levels by ELISA and virus-specific neutralization activity *in vitro* by the FRNT assay) and <u>*Cellular:*</u> (Polyfunctional T-cell responses by *in vitro* stimulation of isolated cells with antigenic peptides, followed by intracellular cytokine staining (ICS) and flow cytometry) responses] and ***temperature stability*** [Ag-specific

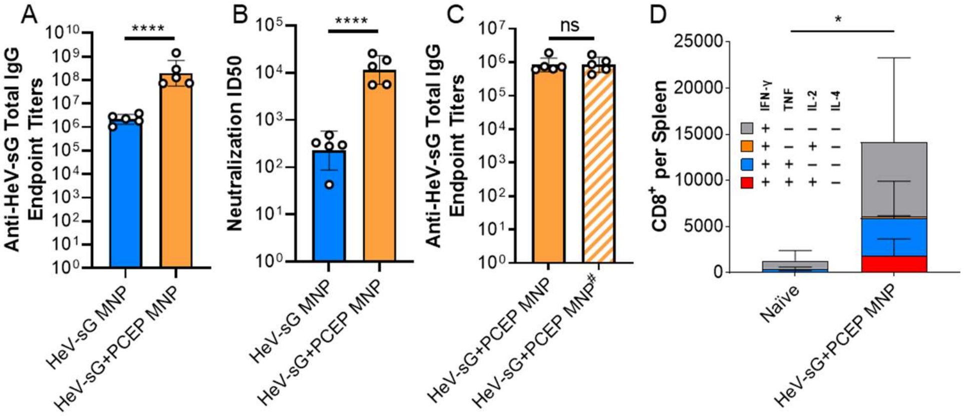

Fig.3. Antigen-specific humoral and cellular responses elicited by PPZ MNP vaccines. Mice were immunized using MNPs loaded with HeV-sG (5 μg)±PCEP (50 μg) MNPs on days 0 and 21. **A)** Anti-HeV-sG total IgG endpoint titers at Week 5 after primary vaccination. **B)** HeV-sG-specific neutralizing antibody titers (ID_{50}) from mice 5 weeks after primary immunization. **C)** Mice were immunized by HeV-sG±PCEP MNPs that were freshly prepared, or stored for 1 month at room temperature after fabrication(HeV-sG±PCEP MNP$^{\#}$), and serum anti-HeV-sG total IgG levels were measured 2 weeks later as an indicator of the retention of immunogenicity. **D)** Mice were immunized by HeV-sG±PCEP MNPs in multiple times and 5 days after the boost, splenocytes were obtained via single-cell suspension and stimulated with an antigenic pool of peptides, followed by intracellular cytokine staining and flow cytometry, to assess systemic cellular responses in terms frequencies of HeV-sG-specific $CD8^+$ T cells in spleen.

binding antibody levels by ELISA after storage of vaccine MNPs for an extended period of time without refrigeration] assessment of PPZ MNP platforms with HeV-sG. These results strongly support the key features of our innovative skin-targeted vaccination strategy and demonstrate that ***1)*** our dissolvable MNPs can effectively deliver the HeV-sG antigen, with or without the PPZ adjuvant PCEP, to immunoresponsive skin microenvironments to induce robust, durable antigen-specific protective immune responses; ***2)*** co-delivery of the rationally selected adjuvant PCEP with HeV-sG in MNPs significantly improves the immunogenicity of HeV-sG; and ***3)*** Dissolvable MNP-embedded vaccines retain the immunogenicity for an extended period of time (at least a month) without a need for expensive "cold-chain". _More comprehensive data demonstrating the feasibility of PPZ MNP platform with HeV-sG are presented in the RP2._ Importantly, we have demonstrated the broad applicability of our PPZ MNP platforms with different viral antigens, such as HeV-sG, Sudan ebolavirus glycoprotein (SUDV GP), Influenza Hemagglutinin (HA) protein, and SARS-CoV-2 Spike (S) protein through animal studies. **Fig.4** shows the relevant representative data that further demonstrate our experience with PPZ MNP platforms, as well as support that skin immunization with PPZ MNPs results in potent immune responses that are superior to those elicited by vaccination with traditional intramuscular (IM) injection of the traditional adjuvant Alum-complexed antigens. _In the MNP Core, we will exploit this expertise to validate the immunogenicity of PPZ MNP platforms with RP-specific antigens in mice._

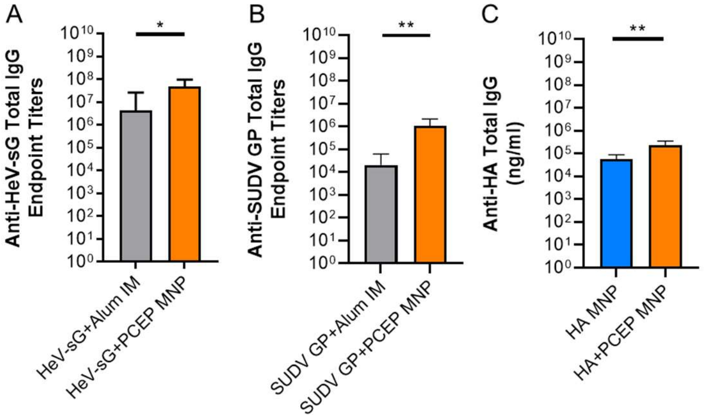

Fig.4. Antibody levels induced by PPZ MNP vaccine platforms incorporating different viral antigens in mice after prime-boost immunization. A) Anti-HeV-sG, **B)** Anti-SUDV GP, and **C)** Anti-HA total IgG levels.

Collectively, the MNP Core will exploit the expertise of our investigators at the UPITT and UMD in PPZ adjuvants, dissolvable MNPs, skin-targeted vaccines, and immunogenicity studies in murine models to develop and batch release the PPZ MNP platforms (drug products) with RP-specific antigens (drug substances from Core D).

3.2. Experimental Design and Methods

Our cohesive research support plan involves two Specific Aims in the MNP Core to provide the RP1 and RP2 with the physicochemically and immunologically validated PPZ MNP platforms, with RP-specific antigens.

Specific Aim 1: To Develop And Characterize PPZ MNP Vaccines. The ***objective*** of *AIM 1* is to fabricate and characterize dissolvable MNPs integrating PPZ-complexed project-specific antigens ***(drug substances will be released from Core D)*** using the standard operating procedures and analysis methods developed at the UMD and UPITT for PPZ-complexed antigens and vaccine-loaded dissolvable MNPs, respectively.

The research team of Dr. Andrianov will formulate project-specific viral antigens with PPZ immunoadjuvants and validate the physicochemical properties of PPZ-complexed antigens for successful incorporation into dissolvable MNPs and for subsequent animal studies. PPZ adjuvants, ***PCPP and PCEP***, will be formulated with project-specific antigens by mixing them in aqueous solutions in the absence of agglomeration, which results in formation of stable supramolecular nanoassemblies **(Fig.5A),** effectively displaying multiple copies of antigen. To validate the physicochemical properties of PPZ-complexed antigens, _antigen-PPZ binding_ [asymmetric field flow fractionation (AF4) method and isothermal titration calorimetry (ITC)], _hydrodynamic diameter_ [dynamic light scattering (DLS)], _short-term stability_ [DLS], and _antigenic integrity and stability_ [dose-dependent ELISA with specific antibodies from RPs.] will be tested and PPZ-antigen formulations will be released based on the pass criteria: **1) Antigen-PPZ binding strength:** <10% unbound antigen and similar Kd values to viral antigen alone, **2) Hydrodynamic diameter:** Z-average diameter of 60-100 nm, **3) Biological integrity:** <1% loss in antigenicity after PPZ formulation, **4) Stability**: <2% aggregation of PPZ complex by volume and <5% loss in antigenic integrity after incubation at temperatures simulating storage conditions.

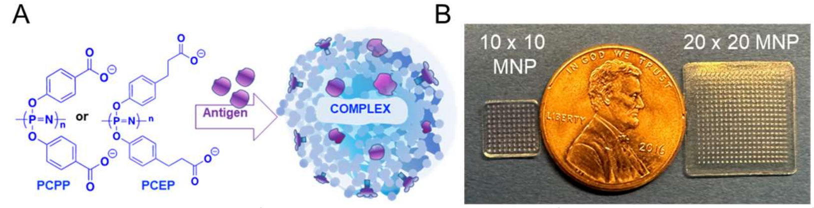

Fig.5. Development of PPZ MNP vaccine platforms. A) Antigen self-assembles with PPZ into supramolecular nanocomplexes (~60-100 nm), and **B)** Different patch sizes of MNPs for small animal (10 x 10 MNP)- and large animal (20 x 20 MNP)-studies.

The research teams of Dr. Korkmaz and Dr. Falo will manufacture PPZ MNP platforms with project-specific antigens and validate the physicochemical properties of these PPZ MNP vaccines for subsequent animal studies. Dissolvable MNPs integrating PPZ-complexed RP-specific antigens released from Core D will be fabricated in the Bioengineering Laboratory of the Department of Dermatology in the University of Pittsburgh School of Medicine (UPSOM) using the established methods well described in our publications. This Bioengineering Laboratory is fully equipped for reproducible manufacturing of high-quality MNPs up to 10,000 MNPs per day. Notably, the manufacturing strategy is flexible to allow precisely and reproducibly dictate needle geometries and their spatial distribution, as well as patch sizes **(Fig.5B)** to achieve different doses of integrated biocargos to enable various studies with small animals, large animals, and humans. Importantly, we have devised standard manufacturing and QC procedures for biocargo-loaded MNPs and outlined these SOPs in our FDA-approved IND (Falo-IND#122488), which we have been using for production and QC of MNPs that are being tested in our investigator-initiated clinical trials (*NCT02192021, NCT05377905*). In the MNP Core, PPZ-MNP platforms, with our clinically validated obelisk-shaped microneedle designs, will be manufactured using our scalable spin-casing strategy with high-precision MNP molds to incorporate RP-specific viral antigens in strict compliance with clinical protocols and will be batch released with our well-established pass criteria. Two different patch sizes of MNPs **(Fig.5B)** will be considered for animal studies. While small MNPs (10x10 obelisk-shaped microneedles – *the total needle volume of 2.4 µl*) will be utilized for small animal studies, large MNPs (20x20 microneedles with the same needle design and same spatial distribution in a larger patch area – *the total needle volume of 9.6 µl*) will be released with the validated properties to facilitate the large-animal studies. To validate the physicochemical properties of PPZ MNP vaccines, *geometric integrity* [advanced microscopy], *mechanical integrity* [standard insertion tests on a force measurement setup (Mark-100 ESM 303)], *vaccine loading accuracy and reproducibility* [Fluorescence spectrometry of MNPs integrating fluorescent-labeled vaccine], *biological integrity* [DLS, AF4 or ITC for antigen-adjuvant complex and antigen-specific ELISA for antigen], *endotoxin content* [the limulus lysate test], *vaccine stability* [DLS, AF4 or ITC for antigen-adjuvant complex and antigen-specific ELISA for antigen after storage], *and skin delivery performance* [Fluorescence spectrometry and advanced microscopy with the skin applied MNPs to quantify the remaining vaccine components and the dissolution volume of microneedles] of PPZ MNP vaccines will be evaluated and PPZ MNP vaccine platforms with project-specific vaccines will then be batch released based on the quantitative pass criteria: **1) Geometric integrity and accuracy:** >95% high-quality microneedles across a PPZ MNP vaccine and <10% deviation from the target geometric values, **2) Structural integrity:** Factor of safety (FOS) > 2 (FOS = Failure forces/penetration forces), **3) Vaccine loading accuracy/reproducibility:** 10% deviation from the mean of theoretical amounts and <10% standard deviation of the mean value. **4) Biological integrity:** No significant ($p>0.05$) aggregation of adjuvant-antigen complexes and no significant loss ($p>0.05$) of antigenicity with respect to those of fresh antigen and fresh PPZ-antigen complexes, **5) Endotoxin content:** < 1 endotoxin unit per PPZ MNP vaccine, provided that the endotoxin content of RP-specific antigens is relatively low, **6) Thermostability:** No significant ($p>0.05$) aggregation of PPZ-antigen complexes and no significant loss ($p>0.05$) of antigenicity after storage at elevated temperatures. **7) Skin delivery performance**: <10% of remaining materials in terms volume and <15% of remaining materials in terms of vaccine after 20 min murine skin application *in vivo* and 20 min the RP-specific animal-skin application *ex vivo*.

Completion of AIM 1 for each batch of PPZ MNP vaccines will be achieved when PPZ-antigen complexes and PPZ MNP vaccine platforms, with project-specific antigens, meet with the aforementioned QC pass criteria.

Specific Aim 2: *To Validate The Correlates Of Protection Of PPZ MNP Vaccines In Mice*. The ***objective*** of *AIM 2* is to evaluate the humoral and cellular responses induced by physicochemically validated PPZ MNP vaccines from *AIM 1* in mice to immunologically validate these novel vaccine technologies with project-specific antigens before advancing them to RP1 and RP2 for the translational development of highly protective vaccines against several prototype viral pathogens.

The research teams of Dr. Falo and Dr. Korkmaz have comprehensive experience with the assessment of the immunogenicity of MNP vaccines through murine studies(16). We will leverage this expertise to determine the immunogenicity of ***PCPP (50 and 100 µg per MNP)*** and ***PCEP (50 and 100 µg per MNP)*** MNP platforms with ***project-specific viral antigens (5 and 10 µg per MNP)*** in mice. These antigen and adjuvant doses were chosen based on our preliminary studies with PPZ MNP HeV-sG vaccine in which the selected doses were found to elicit potent and robust immune responses. We will optimize the doses of antigens and adjuvants for the lead PPZ MNP vaccines to advance them to RPs by using different MNP patch sizes. ***C57BL/6 mice (both female and male)*** will be immunized in ***two different immunization regimens (single dose or prime-boost fashion with a three-week interval between two doses)***. Naïve or unimmunized mice will serve as negative controls. Mice immunized with the intramuscular injection of Alum-complexed RP-specific antigens will be our positive controls. To complete the initial immunogenicity evaluation of physicochemically validated PPZ MNP vaccines, effector immune responses to PPZ MNP vaccines will be determined to rapidly advance the physicochemically and immunologically validated PPZ MNP vaccines to RP1 and RP2. _To investigate humoral responses_, blood samples will be collected on Week 2 and Week 5 after primary immunization. Serum antigen-specific antibody levels (IgM, total IgG, IgG1, IgG2c, and IgA) will be measured by ELISA and virus-specific neutralization capacity of these antibodies will be determined by an *in vitro* neutralization assay (**Fig.3)**. _To evaluate cellular responses,_ mice will be sacrificed five days after the final vaccine dose, vaccine-draining lymph nodes and spleens will be isolated and their single cell suspensions will be obtained. These cells will be stimulated with a pool of antigenic peptides (custom-synthesized PepTivators for RP-specific antigens), followed by intracellular cytokine staining and by multi-color flow cytometry to determine antigen-specific polyfunctional cellular responses (IFN-γ, TNF-α, IL-2, IL-4, IL-17: $CD4^+$ and $CD8^+$ T-cell responses) **(Fig.3)**. _Upon this initial evaluation, both physicochemically and immunologically validated PPZ-MNP vaccines, with RP-specific antigens, will be advanced to RPs, for which if necessary larger size MNPs will be used to achieve the target antigen and adjuvant doses._

The durability of vaccine-induced immune responses and the temperature stability of PPZ MNP vaccines will also be tested in the MNP Core through murine studies using the lead PPZ MNP vaccine platforms that will be advanced to RPs. _To evaluate the longevity of humoral responses_, blood samples will be collected on Week 2, Week 5, Week 8, and then monthly for at least a year. Serum antigen-specific antibody levels and virus-specific neutralization capacity of these antibodies will be determined as described above. _To determine the memory T-cell responses_, mice will be boosted 6 months and 1 year after the primary immunization and then sacrificed five days after the booster dose, vaccine-draining lymph nodes, spleens, and other relevant tissues will be isolated and antigen-specific polyfunctional T-cell responses will be assessed as described above. _To test the stability of PPZ MNP vaccines_, the afromentioned physicochemical and immunological evaluation of PPZ MNP vaccines will be repeated with the lead PPZ MNP vaccine platforms stored at room temperature and 40°C for an extended period of time (1-, 3-, 6-, and 12-month storage), and the results will be compared with those obtained with fresh PPZ MNP platforms. These long-term validation studies of our novel vaccine technology will facilitate the clinical translation of PPZ MNP vaccines, with the further support from Core D, Emergent Biosolutions and Moderna.

Completion of AIM 2 will be marked when both physicochemically and immunologically validated PPZ MNP platforms with RP-specific antigens are advanced to RPs, as well as when the durability of PPZ MNP vaccine-induced immune responses and the thermostability of PPZ MNP vaccines are comprehensively characterized.

Expectations, Potential Issues, and Alternative Strategies. Based on our supporting data, we expect that our established PPZ formulation and MNP manufacturing methods will result in high-quality PPZ MNP vaccine platforms with project-specific viral antigens, which will meet all of our QC pass criteria. In the unlikely event that PPZ MNP vaccines fail to pass the physicochemical validation tests, PPZ and MNP formulations, and processing conditions will be altered to further optimize the PPZ MNP vaccine formulations. Based on our strong preliminary data, we also expect that PPZ MNP vaccines will induce robust and potent antigen-specific immune responses. We expect no insurmountable technical issues with our immunological assays since all of the proposed assays has been validated in our laboratories. One potential issue is the availability of antigen-specific peptide pools to pulse cells for assaying T-cell responses. In the event that custom-synthesized PepTivators may take too long to obtain, we will pulse these cells with the whole protein or use stably transduced dendritic cells (DC2.4) for restimulation. To accommodate the potential dose variations between mice and other animals that will be used in RPs, we will modify the patch size and/or number (if necessary, also the frequency of MNP applications).

Statistical Considerations. To demonstrate the reproducibility of our results with PPZ-MNP vaccines in both sexes, experiments in *AIM 2* will be independently repeated with *female and male* mice. If treatment effects and trends differ significantly between sexes, experiments will be repeated to confirm sex-based differences and reproducibility. To eliminate age as a confounding variable, young adult aged-matched mice (8-14 weeks) will be used for all experiments, and mice will be randomly assigned to masked groups. The aforementioned established QC procedures will eliminate confounding effects of batch-to-batch variability of PPZ MNP vaccine platforms. The statistical aspects will be considered for all aspects of the project. For the experiments involving multiple groups with outcomes at a single time point, results will be analyzed by one-way ANOVA followed by Tukey's multiple comparisons. For time-course experiments, results will be analyzed by two-way mixed ANOVA, followed by one-way ANOVA on treatment effect and Tukey's multiple comparisons. Power analyses were conducted using our preliminary data with PCEP MNP HeV-sG vaccine with significance level $\alpha<0.05$, power $(1-\beta) = 0.8$, and $k=3$-6 pairwise comparisons (for 3-4 groups), minimum sample sizes for statistical significance were determined to be $N = 5$ mice and $N = 6$ mice per group for each independent experiment (at least two independent experiments) for humoral and cellular responses, respectively.

REFERENCES

1. Excler J-L, Saville M, Berkley S, Kim JH. Vaccine development for emerging infectious diseases. Nature Medicine. 2021;27(4):591-600.
2. Roth GA, Picece VC, Ou BS, Luo W, Pulendran B, Appel EA. Designing spatial and temporal control of vaccine responses. Nature Reviews Materials. 2022;7(3):174-95.
3. Monrad JT, Sandbrink JB, Cherian NG. Promoting versatile vaccine development for emerging pandemics. npj Vaccines. 2021;6(1):26.
4. Shin MD, Shukla S, Chung YH, Beiss V, Chan SK, Ortega-Rivera OA, Wirth DM, Chen A, Sack M, Pokorski JK. COVID-19 vaccine development and a potential nanomaterial path forward. Nature Nanotechnology. 2020;15(8):646-55.
5. Labouta HI, Langer R, Cullis PR, Merkel OM, Prausnitz MR, Gomaa Y, Nogueira SS, Kumeria T. Role of drug delivery technologies in the success of COVID-19 vaccines: a perspective. Drug Delivery and Translational Research. 2022;12(11):2581-8.
6. Pulendran B, S. Arunachalam P, O’Hagan DT. Emerging concepts in the science of vaccine adjuvants. Nature Reviews Drug Discovery. 2021;20(6):454-75.
7. Rotrosen E, Kupper TS. Assessing the generation of tissue resident memory T cells by vaccines. Nature Reviews Immunology. 2023:1-11.
8. Glenn GM, Taylor DN, Li X, Frankel S, Montemarano A, Alving CR. Transcutaneous immunization: a human vaccine delivery strategy using a patch. Nature Medicine. 2000;6(12):1403-6.
9. Belyakov IM, Hammond SA, Ahlers JD, Glenn GM, Berzofsky JA. Transcutaneous immunization induces mucosal CTLs and protective immunity by migration of primed skin dendritic cells. The Journal of Clinical Investigation. 2004;113(7):998-1007.
10. Zhu W, Wei L, Dong C, Wang Y, Kim J, Ma Y, Gonzalez GX, Wang B-Z. cGAMP-adjuvanted multivalent influenza mRNA vaccines induce broadly protective immunity through cutaneous vaccination in mice. Molecular Therapy-Nucleic Acids. 2022;30:421-37.
11. Kenney RT, Frech SA, Muenz LR, Villar CP, Glenn GM. Dose sparing with intradermal injection of influenza vaccine. New England Journal of Medicine. 2004;351(22):2295-301.
12. Korkmaz E, Balmert SC, Sumpter TL, Carey CD, Erdos G, Falo Jr LD. Microarray patches enable the development of skin-targeted vaccines against COVID-19. Advanced Drug Delivery Reviews. 2021;171:164-86.
13. Korkmaz E, Balmert SC, Carey CD, Erdos G, Falo Jr LD. Emerging skin-targeted drug delivery strategies to engineer immunity: A focus on infectious diseases. Expert Opinion On Drug Delivery. 2021;18(2):151-67.
14. Wang J, Shah D, Chen X, Anderson RR, Wu MX. A micro-sterile inflammation array as an adjuvant for influenza vaccines. Nature Communications. 2014;5(1):4447.
15. Wang J, Li B, Wu MX. Effective and lesion-free cutaneous influenza vaccination. Proceedings of the National Academy of Sciences. 2015;112(16):5005-10.
16. Balmert SC, Ghozloujeh ZG, Carey CD, Li’an HW, Zhang J, Shahi P, Amer M, Sumpter TL, Erdos G, Korkmaz E. A microarray patch SARS-CoV-2 vaccine induces sustained antibody responses and polyfunctional cellular immunity. Iscience. 2022;25(10).
17. Mishra D, Mishra PK, Dubey V, Nahar M, Dabadghao S, Jain N. Systemic and mucosal immune response induced by transcutaneous immunization using Hepatitis B surface antigen-loaded modified liposomes. European Journal of Pharmaceutical Sciences. 2008;33(4-5):424-33.
18. Pan Y, Liu L, Tian T, Zhao J, Park CO, Lofftus SY, Stingley CA, Yan Y, Mei S, Liu X. Epicutaneous immunization with modified vaccinia Ankara viral vectors generates superior T cell immunity against a respiratory viral challenge. npj Vaccines. 2021;6(1):1.
19. Irvine DJ, Aung A, Silva M. Controlling timing and location in vaccines. Advanced Drug Delivery Reviews. 2020;158:91-115.
20. Irvine DJ, Read BJ. Shaping humoral immunity to vaccines through antigen-displaying nanoparticles. Current Opinion In Immunology. 2020;65:1-6.
21. Anderluzzi G, Schmidt ST, Cunliffe R, Woods S, Roberts CW, Veggi D, Ferlenghi I, O'Hagan DT, Baudner BC, Perrie Y. Rational design of adjuvants for subunit vaccines: The format of cationic adjuvants affects the induction of antigen-specific antibody responses. Journal of Controlled Release. 2021;330:933-44.
22. Andrianov AK, Langer R. Polyphosphazene immunoadjuvants: Historical perspective and recent advances. Journal of Controlled Release. 2021;329:299-315.
23. Chand DJ, Magiri RB, Wilson HL, Mutwiri GK. Polyphosphazenes as adjuvants for animal vaccines and other medical applications. Frontiers in Bioengineering and Biotechnology. 2021;9:625482.

24. Eng NF, Garlapati S, Gerdts V, Potter A, Babiuk LA, Mutwiri GK. The potential of polyphosphazenes for delivery of vaccine antigens and immunotherapeutic agents. Current drug delivery. 2010;7(1):13-20.
25. Andrianov AK, Marin A, Fuerst TR. Molecular-level interactions of polyphosphazene immunoadjuvants and their potential role in antigen presentation and cell stimulation. Biomacromolecules. 2016;17(11):3732-42.
26. Andrianov AK, Marin A, Wang R, Chowdhury A, Agnihotri P, Yunus AS, Pierce BG, Mariuzza RA, Fuerst TR. In vivo and in vitro potency of polyphosphazene immunoadjuvants with hepatitis C virus antigen and the role of their supramolecular assembly. Molecular Pharmaceutics. 2020;18(2):726-34.
27. Andrianov AK, DeCollibus DP, Gillis HA, Kha HH, Marin A, Prausnitz MR, Babiuk LA, Townsend H, Mutwiri G. Poly [di (carboxylatophenoxy) phosphazene] is a potent adjuvant for intradermal immunization. Proceedings of The National Academy of Sciences. 2009;106(45):18936-41.
28. Valencia SM, Zacharia A, Marin A, Matthews RL, Wu C-K, Myers B, Sanders C, Difilippantonio S, Kirnbauer R, Roden RB. Improvement of RG1-VLP vaccine performance in BALB/c mice by substitution of alhydrogel with the next generation polyphosphazene adjuvant PCEP. Human Vaccines & Immunotherapeutics. 2021;17(8):2748-61.
29. Magiri RB, Lai KJ, Mutwiri GK, Wilson HL. Experimental PCEP-adjuvanted swine influenza H1N1 vaccine induced strong immune responses but did not protect piglets against heterologous H3N2 virus challenge. Vaccines. 2020;8(2):235.
30. Magiri R, Lai K, Chaffey A, Zhou Y, Pyo H-M, Gerdts V, Wilson HL, Mutwiri G. Intradermal immunization with inactivated swine influenza virus and adjuvant polydi (sodium carboxylatoethylphenoxy) phosphazene (PCEP) induced humoral and cell-mediated immunity and reduced lung viral titres in pigs. Vaccine. 2018;36(12):1606-13.
31. Bal SM, Ding Z, van Riet E, Jiskoot W, Bouwstra JA. Advances in transcutaneous vaccine delivery: do all ways lead to Rome? Journal of Controlled Release. 2010;148(3):266-82.
32. O'Shea J, Prausnitz MR, Rouphael N. Dissolvable microneedle patches to enable increased access to vaccines against SARS-CoV-2 and future pandemic outbreaks. Vaccines. 2021;9(4):320.
33. Balmert SC, Ghozloujeh ZG, Carey CD, Akilov OE, Korkmaz E, Falo Jr LD. Research techniques made simple: Skin-targeted drug and vaccine delivery using dissolvable microneedle arrays. Journal of Investigative Dermatology. 2021;141(11):2549-57.
34. Balmert SC, Carey CD, Falo GD, Sethi SK, Erdos G, Korkmaz E, Falo Jr LD. Dissolving undercut microneedle arrays for multicomponent cutaneous vaccination. Journal of Controlled Release. 2020;317:336-46.
35. Erdos G, Balmert SC, Carey CD, Falo GD, Patel NA, Zhang J, Gambotto A, Korkmaz E, Falo Jr LD. Improved cutaneous genetic immunization by microneedle array delivery of an adjuvanted adenovirus vaccine. The Journal of Investigative Dermatology. 2020;140(12):2528.

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Subject: Re: UTMB/USUHS ReVAMPP Specific Aims draft - DUE Friday 5/12
Date: Thursday, May 11, 2023 10:12:22 AM

CORE D (Abelson, Geisbert, Gunn and Zeitlin)

EARLY AND IND-ENABLING TRANSLATIONAL RESEARCH FOR VACCINES AND MABS

Core D will leverage the relationship between vaccines and antibodies to optimize the translational activities performed by the Janus team (just a placeholder – *i.e.* duality of vaccines and mAbs). Vaccines and antibodies are inextricably linked:

1. mAbs can help define protective epitopes for a vaccine
2. mAbs can help establish correlates of protection for vaccines
3. Vaccine antigens can be used to discover mAbs
4. mAbs can provide an alternative to a vaccine in immunocompromised populations and in emergency situations where immediate protection is required (*i.e*. there isn’t sufficient time to allow natural host immunity to develop from vaccination)

Developing mAb countermeasures and vaccines simultaneously will allow us to take advantage of natural synergies between the two types of interventions and more efficiently advance superior prototypes (Table 1).

Table 1: Vaccine and mAb translational efforts

Family	Prototype (Y1-3)	Test case (Y4-5)
Arenaviruses	Lassa (Old world), Junin (New world)	Lujo (Old world), Machupo (New world)
Nairoviruses	CCHF	Kasokero, Dugbe
Paramyxoviruses	Nipah, Hendra	Langya, Angavokely and/or Mojiang

Aim 1: Manufacture vaccine antigens and mAbs in support of the RPs and Cores

Small and larger scale (*e.g.* sufficient for stability testing and NHP dosing) will be performed by Mapp Biopharmaceutical and Geneovac to support the efforts of the other Scientific Cores and the Research Projects.

Aim 2: Lead optimization, stability, manufacturability testing, and early process development of subunit vaccines.

Optimization of the lead subunit vaccines (and adjuvants) will result in stable subunit vaccines with favorable manufacturability characteristics. Small scale manufacturing and early-stage process development will be performed. As vaccines are regulated by CBER, all activities will be performed consistent with regulatory expectations for this branch of the

FDA. The Core's vaccine industrial partners' input (Emergent and Moderna) will be integrated into the translational development efforts.

***Aim 3: Identification of correlates of protection, lead optimization, stability and manufacturability testing of mAbs*.**
Together with Dr. Gunn, engineered mAbs (altered N-glycans, Fc mutations for effector functions and extended half-life, multimeric formats and nanobodies from RPx) will be evaluated to correlate mechanisms of action with maximal efficacy. This work, in conjunction with stability and manufacturability assessment, will identify a lead prototype format to transition to early process development. The goal will be to identify a single prototype for long-term intramuscular prophylaxis as well as for intravenous post-exposure prophylaxis and therapy. As mAbs are regulated by CDER, performance of all activities will be as per regulatory expectations.

The PIs of Core D bring decades of experience with preclinical translational efforts on vaccines and mAb countermeasures and are long time collaborators with the other scientists involved in the project.

Core D will work closely with Cores C and E and the Research Projects to coordinate and integrate the translational research being performed in development of the Janus prototypes.

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Date: Tuesday, May 9, 2023 at 1:58 PM
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Subject: UTMB/USUHS ReVAMPP Specific Aims draft - DUE Friday 5/12

Good afternoon,

Drs. Geisbert and Broder have requested a one-page rough draft of the Specific Aims page for each Research Project and Core to help with their development of the Overall Research Strategy. Please send your draft by 12pm CST this Friday (5/12).

Thank you,
Sonja

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ANIMAL MODEL DEVELOPMENT AND PRECLINICAL EVALUATION (SCIENTIFIC CORE E)

1. SPECIFIC AIMS

The main objectives of the Animal Model Development and Preclinical Evaluation Core are to optimize and/or develop animal models for Paramyxoviridae and Bunyavirales and to evaluate vaccines and treatments identified in RP1, RP2, RP4, RP5, and Cores C and D against Paramyxoviridae and Bunyavirales under BSL-3 and/or BSL-4 containment.

The Specific Aims of the Animal Model Development and Preclinical Evaluation Core are to:

Aim 1: Provide resources including a secure repository of well characterized seed stocks of henipaviruses, arenaviruses, and nairoviruses and develop and rescue recombinant viruses.

Aim 2: Develop and/or optimize animal models of henipavirus, arenavirus, and nairovirus infection that faithfully reproduce human disease.

Aim 3: Provide technical expertise and conduct *in vitro* assays.

Aim 4: Provide technical expertise and perform vaccinations, animal challenges, treatments, clinical pathology assays, virology assays, necropsies, and histopathological analysis of tissues.

The Aims will be achieved by the following specific functions to be carried out by the Animal Model Development and Preclinical Evaluation Core PI and personnel:

1. Maintain records of well characterized Paramyxovirus and Bunyavirales seed stocks including background history, passage history, genomic information, periodic monitoring of titer, mycoplasma testing, endotoxin testing, and sterility testing.
2. In collaboration with RP2 develop a reverse genetics system to rescue recombinant Langya virus (do we also propose other viruses?).
3. Perform *in vitro* virus neutralization assays; optimize assays for new viruses.
4. Develop and/or optimize animal models for strains or variants of henipaviruses, arenaviruses, and nairoviruses to fill gaps where no models exist; make these models available to Center Research Projects and Scientific Cores as well as external ReVAMPP Centers.
5. Generate tools including inactivated samples from infected animals and immune reagents obtained from animal studies and provide to Center Research Projects and Scientific Cores as well as external ReVAMPP Centers.
6. Assist Center Investigators in designing animal studies, prepare animal protocols, and obtain IACUC approval of animal studies.
7. Perform animal challenges using well characterized henipavirus, arenavirus, and nairovirus seed stocks to support RP1, RP2, RP4, RP5, and Cores C and D.
8. Perform vaccination and treatment of animals with vaccines and treatments provided by RP1, RP2, RP4, RP5, and Cores C and D.
9. Perform all duties associated with animal challenge studies including monitoring of animals, collection of samples, and necropsies.
10. Perform clinical pathology assays including hematology and clinical chemistry assays.
11. Perform virology assays on blood and/or tissue samples collected from infected animals including viral load by plaque assay and RT-PCR.
12. Perform gross pathology analysis on all animals at necropsy.
13. Perform histopathologic and immunohistochemical analysis on tissues from animals collected at necropsy.
14. Provide all data generated in Core E to Data Management Core B.

From: Stutz, Sonja J. on behalf of Stutz, Sonja J. <sjstutz@utmb.edu>
To: Haller, Sherry; Geisbert, Thomas W.; CHRISTOPHER BRODER; Cross, Robert W.; Larry Zeitlin; Dafna Abelson; brian.schaefer@usuhs.edu; antony.dimitrov.ctr@usuhs.edu; Boxley, Kimberly; Haseltine, Barb; Golovko, Georgiy; Moushimi Amaya; lof2@pitt.edu; aandrianov@ibbr.umd.edu; EK001@pitt.edu; Woolsey, Courtney B. (Micro); jianliang.xu@gmail.com; xu.4692@osu.edu; brian.walters@genovac.com; troy.brendemuhl@genovac.com; pete.leland@genovac.com; Mitchell, Janee T; James E. Crowe - Vanderbilt University Medical Center (james.crowe@vumc.org); ldf2ldf2@gmail.com; Iwona Budnicki; Andreas Weise; Beril Kromer; Hubertus Schleer; lhornsby@gsu.edu; bronwyn.gunn@wsu.edu; Jamie L Robinson; workman.45@osu.edu
Subject: RE: UTMB/USUHS ReVAMPP Specific Aims draft - DUE Friday 5/12
Date: Thursday, May 18, 2023 10:50:30 AM
Attachments: 17MAY23_RP1-BUNYAVIRALES VACCINES-RWC.docx
RP2_Specific Aims-UTMB-USU-05102023-v3.docx
ReVAMPP RP3 Specific Aims 5-12-2023.pdf
RP5-Specific Aim_U19-UTMB_USU_JX1_XuK1_2023y05m12d.docx
Specific_Aims_Core_A.docx
Preliminary AIMS DMC core.docx
ReVAMPP U19 - Geisbert-Broder CORE C - 05-13-2023.docx
Mapp Specific Aims draft.pdf
Specific Aims Core E.docx

Hello everyone,

The Specific Aims draft for Research Projects 1-3, 5, and all Cores are attached for your review.

Thank you,
Sonja

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THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 24, pp. 21678–21686, June 17, 2011

Structural Characterization of the Crimean-Congo Hemorrhagic Fever Virus Gn Tail Provides Insight into Virus Assembly*[S]

Received for publication, December 25, 2010, and in revised form, April 6, 2011 Published, JBC Papers in Press, April 20, 2011, DOI 10.1074/jbc.M110.216515

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The RNA virus that causes the Crimean Congo Hemorrhagic Fever (CCHF) is a tick-borne pathogen of the *Nairovirus* genus, family *Bunyaviridae*. Unlike many zoonotic viruses that are only passed between animals and humans, the CCHF virus can also be transmitted from human to human with an overall mortality rate approaching 30%. Currently, there are no atomic structures for any CCHF virus proteins or for any *Nairovirus* proteins. A critical component of the virus is the envelope Gn glycoprotein, which contains a C-terminal cytoplasmic tail. In other *Bunyaviridae* viruses, the Gn tail has been implicated in host-pathogen interaction and viral assembly. Here we report the NMR structure of the CCHF virus Gn cytoplasmic tail, residues 729–805. The structure contains a pair of tightly arranged dual $\beta\beta\alpha$ zinc fingers similar to those found in the *Hantavirus* genus, with which it shares about 12% sequence identity. Unlike Hantavirus zinc fingers, however, the CCHF virus zinc fingers bind viral RNA and contain contiguous clusters of conserved surface electrostatics. Our results provide insight into a likely role of the CCHF virus Gn zinc fingers in *Nairovirus* assembly.

Recent outbreaks of the Crimean Congo Hemorrhagic Fever (CCHF)[2] virus along with the reported ability of the virus to transfer between humans have raised concerns of a widespread pandemic (1). The virus is transmitted to humans by tick bite or by direct handling of infected animal meat or blood (1, 2). Infection causes a hemorrhagic fever and myalgia resulting in mortality rates approaching 30% (1–3). The virus contains an antisense RNA genome divided into three segments, and named according to lengths as the S, M, and L (for Small, Medium, and Large) segments (4). The viral proteins are the nucleocapsid protein, two membrane glycoproteins Gn and Gc (also referred to as G1 and G2 in other *Bunyaviridae*) (5, 6), a nonstructural protein (NSm) (7), and an RNA polymerase (4). In the mature virion, the Gn glycoprotein contains a 176 residue ectodomain followed by a 24 residue transmembrane region and terminates in a long cytoplasmic tail consisting of ~100 residues (5, 7).

Recent results from other related *Bunyaviridae* viruses suggest the role of the Gn tail in viral assembly. For example, alanine mutagenesis of the cytoplasmic tails of Uukuniemi virus (genus *Phlebovirus*) (8) and Bunyamwera virus (genus *Orthobunyavirus*) (9) affect the ability of virus-like particles (VLPs) to effectively incorporate ribonucleoproteins, thus intimating a role for Gn tails in genome packaging. More recently, the Gn tail of Puumala virus (genus *Hantavirus*) was shown to co-immunoprecipitate with the Puumala nucleocapsid protein (10). These results suggest that the CCHF virus Gn tail plays an equally important role in viral assembly of genus *Nairovirus*.

The sequence of the CCHF virus cytoplasmic tail is somewhat variable in Nairoviruses (~24% identity) and even more so when compared with other Bunyaviruses (12% identity with Hantavirus Gn tails). However, one characteristic feature present in four of the five genera of *Bunyaviridae* is a conserved dual **C**-X-**C**-X-**H**-X-**C** motifs of cysteine and histidine residues with X representing any amino acid (Fig. 1). Others have suggested that the high cysteine content of the CCHF virus Gn tail could be due to extensive disulfide bonding (5). Recently, we reported that the cysteines in the Andes hantavirus Gn tail fold into a novel arrangement of back-to-back classical $\beta\beta\alpha$ zinc fingers (11). Despite low sequence identity between the Gn tail of Nairoviruses and Hantaviruses, the spacing of the dual CCHC motif in the CCHF virus most closely resembles that of Hantaviruses, suggesting the presence of a similar dual zinc finger structure. To test this hypothesis, we determined the NMR structure of the CCHF virus Gn cytoplasmic tail from residues 729–805. We report here the first known atomic structure of any protein component of the CCHF virus and demonstrate that the high cysteine content of the Gn cytoplasmic tail is partly due to the presence of dual, back to back $\beta\beta\alpha$-type zinc fingers similar to those found in Hantaviruses. Unlike Hantaviral zinc fingers, however, the electrostatic surface of the CCHF virus zinc finger reveals a clear distribution of conserved electrostatic charges. Moreover, we demonstrate using electrophoretic mobility shift assays (EMSA) that these conserved electrostatics may play a role in forming a surface for binding viral

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* This work was supported in part by AHA 0755724Z, K-INBRE, and NIH P20 RR016475 (to R. N. D.) and the Madison and Lila Self Graduate Fellowship (to D. F. E.).

The atomic coordinates and structure factors (code 2L7X) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

NMR assignments were deposited at the Biological Magnetic Resonance Bank (BMRB ID 17383).

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

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[2] The abbreviations used are: CCHF, Crimean Congo Hemorrhagic Fever; EMSA, electrophoretic mobility shift assay; GB1, the B1 domain of *Streptococcus* protein G; HSQC, heteronuclear single-quantum coherence spectroscopy; NOE, nuclear Overhauser effect; R_1, longitudinal or spin-lattice relaxation rate; R_2, transverse or spin-spin relaxation rate; ZF1, first CCHC zinc binding array, residues 736–756; ZF2, second CCHC zinc binding array, residues 761–780.

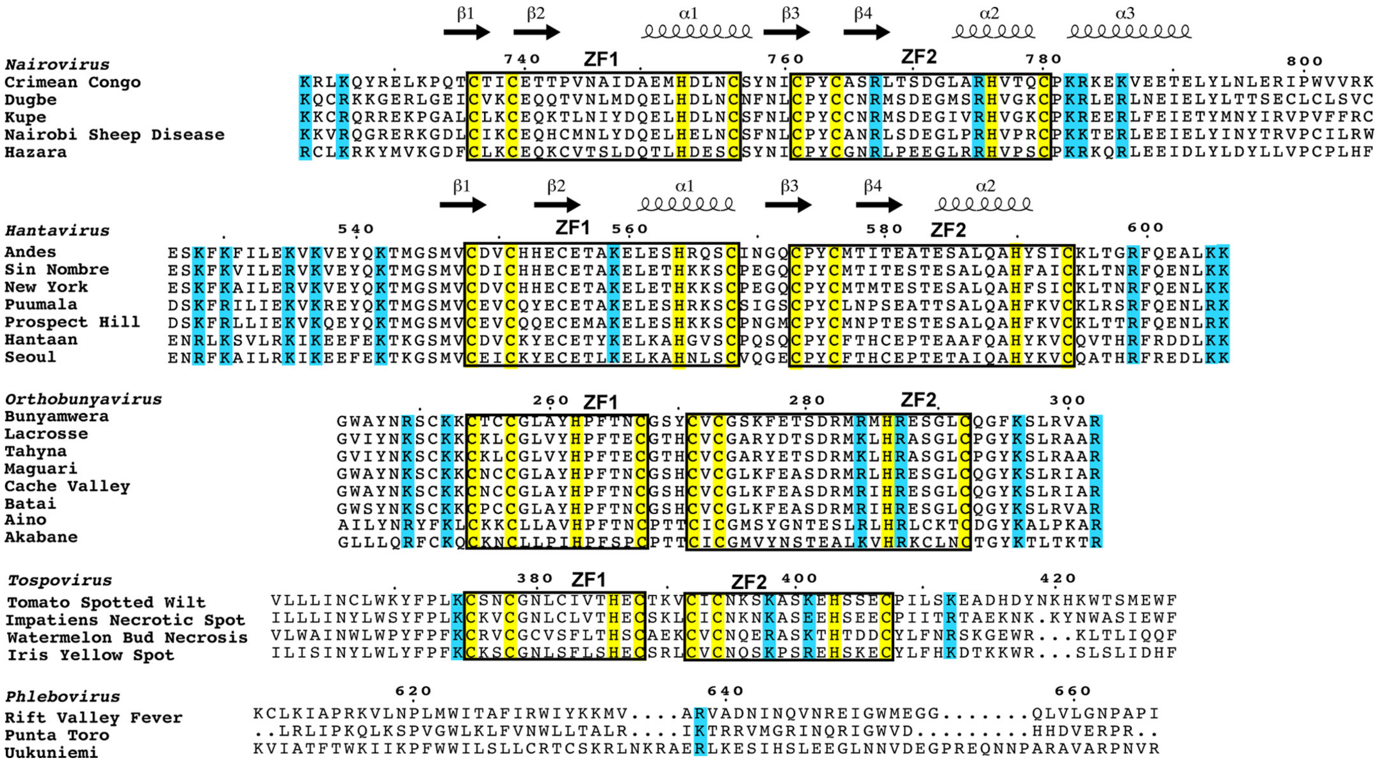

FIGURE 1. **Sequence alignment of the Gn tails of representative members of family *Bunyaviridae*.** *Bunyaviridae* is comprised of five genera: Nairovirus, Hantavirus, Orthobunyavirus, Tospovirus, and Phlebovirus. The conserved CCHC-zinc finger motifs (*boxed*) are present in four of the five genera, with Phlebovirus the lone exception. Another recurring feature is the clustering of conserved basic residues (*in blue*) in the vicinity of the CCHC-motifs. Notably, these basic residues overlap with ZF2 in Nairovirus, Orthobunyavirus, and Tospovirus, but are located outside ZF2 in Hantavirus.

RNA. Together, these data provide insight into the role of the Gn tail in *Nairovirus* assembly.

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—Various constructs of the CCHF virus (strain SPU103/87) Gn cytoplasmic region (spanning residues 719–819) were subcloned from a synthetic gene (GenScript) into the expression vectors pDZ1 and pDZ3 (12), which expressed His$_6$-tagged GB1 fusion proteins with TEV protease cleavage sites. For NMR structure determination, the soluble Gn construct spanning residues 729–805 (Gn$^{729-805}$) was expressed and purified under native conditions following the method reported previously for the Andes hantavirus zinc finger domain (11). Briefly, ^{15}N- and ^{15}N/^{13}C-labeled proteins were expressed in *Escherichia coli* BL21(DE3) grown in 1 liter M9 minimal media supplemented with 0.1 mM $ZnSO_4$ before and after induction. Cells were grown at 37 °C, induced with 1 mM isopropyl-β-D-thiogalactopyranoside at A_{600} ~0.8, and cell growth was continued in a 15 °C shaker incubator overnight (to a final A_{600} ~2.0). Cells were harvested by centrifugation, resuspended in buffer A (20 mM Tris-HCl pH 8.0, 20 mM NaCl, 1 mM DTT, 0.1 mM $ZnSO_4$), and lysed by sonication. Cellular debris was removed by centrifugation, and to the supernatant was added one-tenth volume of 1% polyethyleneimine (pH 8) to precipitate the nucleic acids. Following centrifugation, the supernatant was bound to a 40 ml of Q column (GE Healthcare) and eluted with a 280 ml linear gradient of buffer B (20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 1 mM DTT, 1 mM $ZnSO_4$). For TEV protease digestion, fractions containing the fusion protein were pooled and dialyzed at 25 °C overnight in buffer (50 mM Tris-HCl pH 8.0, 20 mM NaCl, 1 mM DTT, 1 mM $ZnSO_4$) with 0.16 mg recombinant TEV protease (13) per 10 ml of fusion protein. The TEV digestion mixture was dialyzed back into buffer A and passed again through a 40 ml Q column (GE Healthcare). The GB1 tag (theoretical pI of 5.6) was retained on the column while Gn$^{729-805}$ (theoretical pI of 8.6) was present in the flow-through. The 50 ml flow-through fraction was concentrated using Ultra-15 centrifugal filters (Amicon) and dialyzed in NMR buffer (10 mM $NaPO_4$ pH 7.0, 10 mM NaCl, 1 mM DTT, 0.1 mM $ZnSO_4$). The Gn$^{729-805}$ construct retained three residues (Gly-His-Met) cloning artifacts at the N terminus.

NMR Spectroscopy—NMR data were acquired at 25 °C using a Bruker Avance 800 MHz spectrometer equipped with a cryoprobe, processed with NMRPipe (14), and analyzed with NMRView (15). Backbone assignments were obtained from two-dimensional ^{1}H-^{15}N HSQC (16) and three-dimensional HNCA (17), CBCA(CO)NH (17), and HNCACB (18). Secondary structures were identified from the Cα, Cβ, and Hα chemical shifts (19). Side chain assignments were obtained from two-dimensional ^{1}H-^{13}C HMQC (20), three-dimensional HBHA(CO)NH (21), and three-dimensional ^{13}C-edited HMQC-NOESY (22) (t_{mix} = 120 ms). The histidine ring nitrogen atoms coordinated to Zn^{2+} ions were identified from two-dimensional ^{15}N HMQC (23) using a nitrogen sweep width of 160–230 ppm. NOE (nuclear Overhauser effect) crosspeaks were identified from three-dimensional ^{15}N-edited NOESY-

HSQC (24) (t_{mix} = 120 ms) and three-dimensional ^{13}C-edited HMQC-NOESY (22) (t_{mix} = 120 ms).

Backbone ^{15}N relaxation parameters were acquired on a 0.5 mM ^{15}N-labeled sample in NMR buffer. The steady-state heteronuclear {^1H}-^{15}N NOE was acquired as a pair of two-dimensional datasets in an interleaved manner (where portions of each two-dimensional spectrum were acquired sequentially until both datasets were completed) (25). The first two-dimensional dataset contained a 3-s proton saturation (achieved with a series of 120° pulses) whereas the second two-dimensional dataset contained a 3-s delay. The heteronuclear {^1H}-^{15}N NOE was calculated as the ratio of the intensities for each peak in the two datasets. Each two-dimensional dataset was acquired with 2048 (^1H) × 128 (^{15}N) complex points, 32 scans per point, and a 5 s recycle delay. Error bars were estimated using the standard deviation of the background signal of each spectrum. The ^{15}N backbone relaxation rates R_1 and R_2 were acquired as described (26). The time delays used to determine R_1 were 10, 60, 120, 240*, 400, 900, and 1100 ms, and the time delays used to determine R_2 were 20, 40*, 50, 60, 70, 90, 100, 120, and 150 ms (asterisk denotes spectra acquired in duplicate to estimate reproducibility). Peak intensities were obtained from NMRView (15) and fitted using GNUPLOT (27). Deviations from fitting were reported as error bars. Because of peak overlap, residues 749, 787, 791, and 796 were not used in the analysis.

Structure Calculation—The protocol used for NMR structure calculation has been described previously (11). Briefly, unique NOE distance restraints were classified into upper bounds of 2.7, 3.5, 4.5, and 5.5 Å and lower bound of 1.8 Å based on peak volumes. Backbone dihedral angles in the α-helical regions identified by the secondary Cα, Cβ, and Hα chemical shifts (19) were restrained to φ (−60 ± 20°) and ψ (−40 ± 20°). Initial structures were generated using CYANA (28), followed by molecular dynamics and simulated annealing in AMBER7 (29); first *in vacuo*, then with the generalized Born (GB) potential. Initial structural calculations were performed in CYANA without the Zn^{2+} restraints to confirm that the zinc finger domain will fold from NOE-derived restraints only. Once the topology of the Zn^{2+}-coordinated residues were confirmed, subsequent CYANA structure calculations used distance restraints that imposed tetrahedral Zn^{2+}-coordination to Cys and His residues (22). Iterative cycles of AMBER calculations followed by refinement of NMR-derived restraints were performed until the structures converged with low restraint violations and good statistics in the Ramachandran plot. A family of twenty lowest energy structures were analyzed using PROCHECK (30) and molecular graphics were generated using PYMOL (31). The surface electrostatic potentials were calculated using APBS (32) and visualized in PYMOL (31).

In Vitro Transcription—A DNA oligonucleotide representing the M genomic segment panhandle was assembled by PCR primer extension and used for *in vitro* transcription. *In vitro* transcription was carried out following manufacturer's protocol (MAXIscript Kit, Ambion). Briefly, a 20-μl reaction was carried out for 1.5 h (37°) and terminated by adding 2 μl 0.25 M EDTA and heating to 90° followed by rapid cooling on ice. Reaction mixtures were then treated with DNase I and subjected to ethanol precipitation. The RNA transcripts were resuspended in RNase-free ddH_2O and analyzed for purity on a native 12% acrylamide gel stained with SYBR Green II dye (Invitrogen).

RNA Binding Assays—To assess protein-RNA binding by gel electrophoresis, RNA transcripts were incubated on ice for 15 min with increasing amounts of either CCHF virus $Gn^{729-805}$ or Andes hantavirus $G1^{543-599}$ in binding buffer (30 mM $NaPO_4$, 30 mM NaCl, pH 7.4). Samples were mixed with one-half volume 50% glycerol and loaded onto a native 12% acrylamide Tris borate gel. The gel was run in a cooling water bath at 90 V for 1 h in Tris borate buffer, pH 8.3 and visualized by staining with SYBR Green II dye. For nucleic acid size determination, each gel included a 100 bp DNA ladder (NEB, NO467S).

CD Spectroscopy—CD spectra were collected in triplicate at 25° on a JASCO J-815 Spectro-polarimeter using a scanning speed of 50 nm/min. Protein concentrations were kept at 1 μM in buffer (10 μM $NaPO_4$, 10 μM NaCl, 0.1 mM $ZnSO_4$). EDTA and $ZnSO_4$ titrations were applied to the same sample.

RESULTS

Protein Expression and Purification—Our previous work with Hantavirus glycoprotein cytoplasmic tails indicates expression of the tail is toxic to *E. coli* (11). Therefore, all constructs of the CCHF virus Gn cytoplasmic tail were expressed as GB1 fusion proteins. The GB1 tag contained His_6 for nickel affinity purification and a TEV protease cleavage site to recover the native Gn zinc finger domain. The fusion protein was expressed in soluble form in *E. coli*, purified under native conditions, and digested with TEV protease to obtain the Gn zinc finger domain. Longer constructs comprising the entire predicted cytoplasmic tail ($Gn^{719-819}$) expressed as insoluble inclusion bodies. $Gn^{729-819}$, which was missing the first ten residues following the transmembrane region, expressed as soluble protein but with low yield. $Gn^{729-805}$ represented the longest construct containing the conserved $\mathbf{C}$-X_2-$\mathbf{C}$-X_{11-12}-$\mathbf{H}$-X_3-$\mathbf{C}$ (where X is any amino acid) that also expressed in high enough yield to give high resolution NMR data.

Zn^{2+} Is Required for Proper Folding—To examine the reliance of Zn^{2+}-coordination on the proper folding of the CCHF virus Gn tail, we recorded the two-dimensional ^{15}N HSQC of the $Gn^{729-805}$ in the presence of 4 mM EDTA (Fig. 2*A*). The spectrum in the presence of EDTA is collapsed between ppm values of 6.5 and 8.6, whereas the folded spectrum in the absence of EDTA is well dispersed between 6.5 and 9.3 ppm. Narrowing of the spectrum suggests a loss of tertiary structure upon removal of Zn^{2+}, indicating the requirement for Zn^{2+} binding in folding of the domain. A similar titration using circular dichroism (CD) spectroscopy demonstrates that the presence of EDTA causes a downward spectral shift, indicating a transition toward an unfolded protein (Fig. 2*B*). Here we also demonstrate that the addition of Zn^{2+} ion back into the sample recovers the trace of the original native spectrum. Therefore, Zn^{2+} is required for proper folding of the CCHF virus Gn tail.

NMR Structure Determination—CCHF virus $Gn^{729-805}$ showed a well dispersed two-dimensional ^1H-^{15}N HSQC (Fig. 3*A*). Complete backbone assignments were obtained from three-dimensional HNCA, CBCA(CO)NH, HNCACB, and ^{15}N-edited NOESY-HSQC. The C^{α}, H^{α}, and C^{β} secondary chemical shifts (Fig. 3*B*) showed the presence of three short

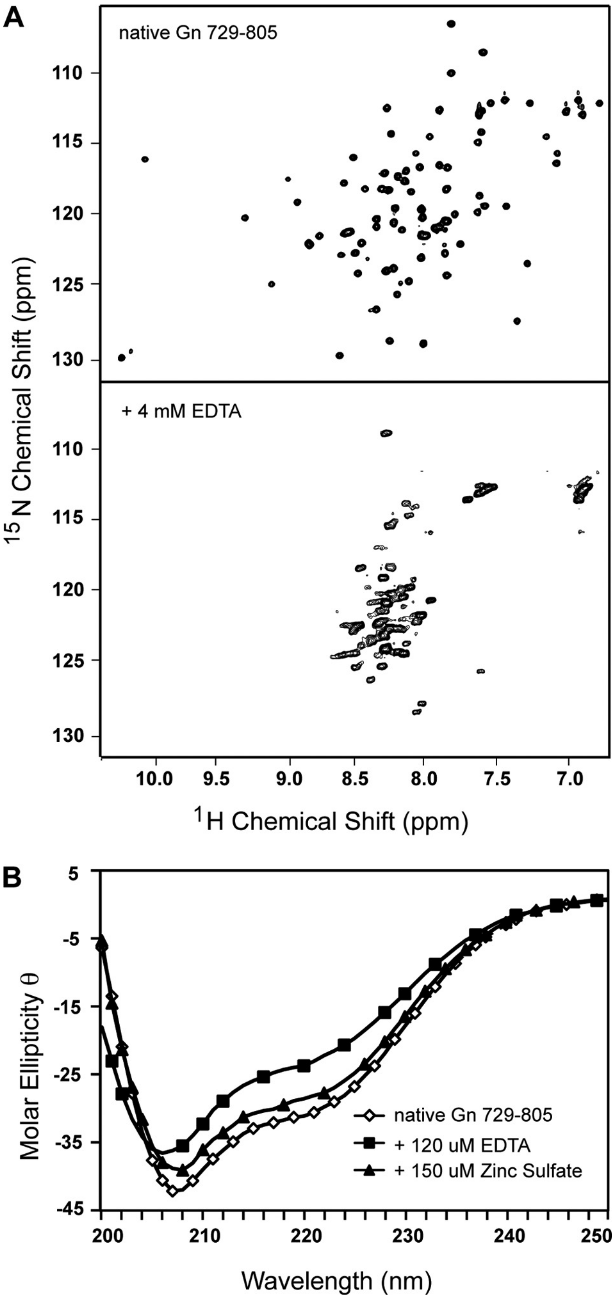

FIGURE 2. **CCHF virus Gn zinc finger domain (residues 729–805) relies on Zn^{2+} for proper folding.** Addition of 4 mM EDTA to a sample of ^{15}N-labeled $Gn^{729-805}$ effectively narrows the HSQC spectrum into a characteristic of an unfolded protein (*A*). Likewise, addition of a metal chelator causes a downward shift at 208 nm in the CD spectra toward random coil (Y axis: molar ellipticity θ per residue, deg·cm^2 dmol^{-1}residue^{-1} × 10^4) (*B*). Titration of zinc sulfate back into the sample recovers the original CD trace (*B*).

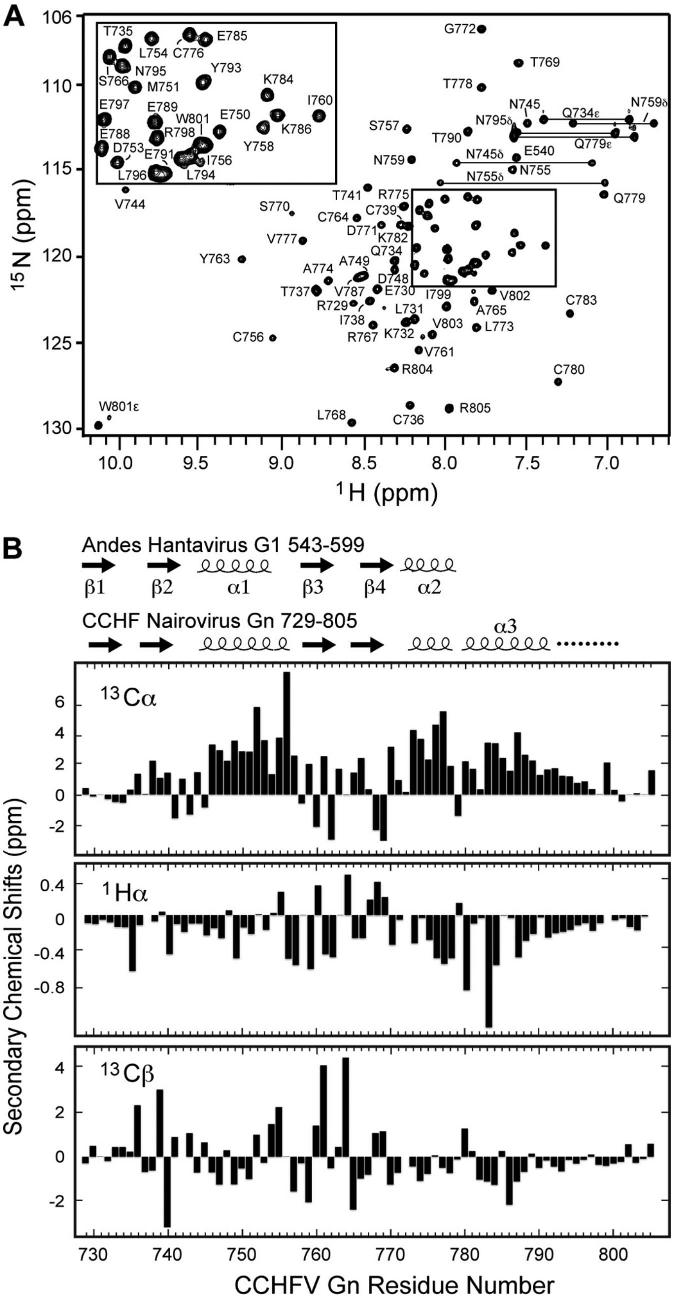

FIGURE 3. The CCHF virus Gn zinc finger yielded a well dispersed two-dimensional ^{1}H-^{15}N HSQC spectrum (*A*). The smaller peak in the tryptophan (*W801*) side-chain suggested a minor conformation of the tryptophan ring possibly due to ring flip-flop. Secondary chemical shifts for ^{13}Cα, ^{1}Hα, and ^{13}Cβ suggest the presence of three short α helices interspersed with two short β hairpins (*B*).

α-helices with an intervening random coil region between the second and third helix. Two more regions in random coil orientations flanked the central sequence of the domain as indicated by the heteronuclear {^{1}H}-^{15}N NOE (Fig. 4*C*). Side chain assignments were completed using two-dimensional ^{1}H-^{13}C HMQC, three-dimensional HBHA(CO)NH, and three-dimensional ^{13}C edited HMQC-NOESY. There were six invariant cysteine and two histidine residues (His-752 and His-776) in $Gn^{729-805}$ (Fig. 1), all of which were involved in Zn^{2+} coordination. Long distance NOE's confirmed that His-752 and His-776 were involved in Zn^{2+} coordination. Notably, His-752 Hϵ1 shares an NOE with Cys^{736} Hβ′s. Likewise, His-776 Hϵ1 and Hδ2 share NOEs with Cys-761 and Cys-780 Hβ′s. A two-dimensional ^{15}N HMQC (23) spectrum showed that His-752 and His-776 coordinated Zn^{2+} through the Nδ1 and Nϵ2 atoms (supplemental Fig. S1), respectively. Manual analysis of three-dimensional ^{15}N- and ^{13}C-edited NOESY spectra identified 1193 unambiguous interproton NOE distance restraints. The NOE restraints together with 26 φ and 26 ψ dihedral angle restraints and zinc coordination restrains (Table 1) were used in structure calculation and refinement in CYANA and

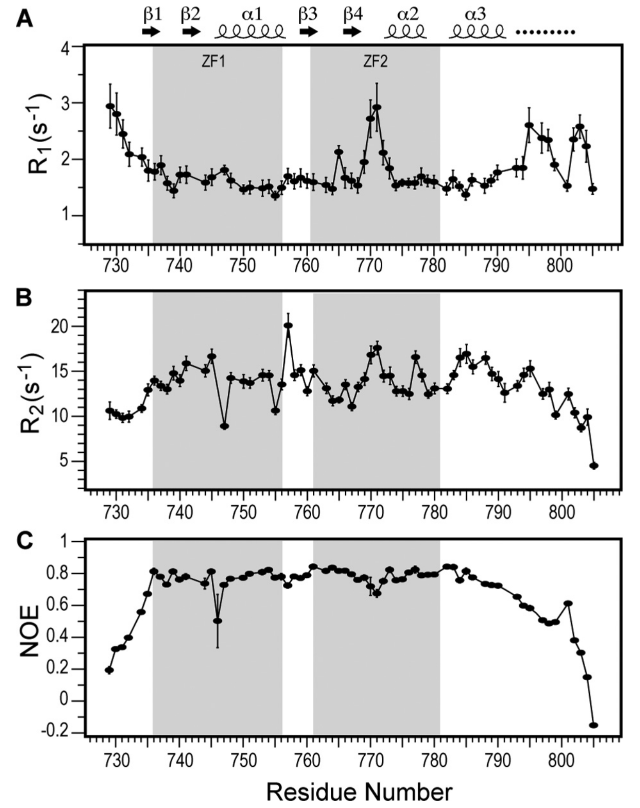

FIGURE 4. **Amide backbone relaxation rates R_1 (*A*), R_2 (*B*), and heteronuclear {^{1}H}-^{15}N NOE (*C*) of the CCHF virus Gn zinc finger.**

TABLE 1

NMR restraints and structural statistics for the 20 refined NMR structures

Total distance restraints	1193
Intraresidue (i, i)	246
Sequential (i, i +1)	407
Long Range (i-j) > 4)	307
Total dihedral angle restraints	52
Phi	26
Psi	26
RMS deviation from mean structure	
Backbone atoms (N, Cα, C′) (Å)	0.25
All heavy atoms (C, N, O) (Å)	0.76
NOE violations	
Max distance violation (Å)	0.47
Max dihedral angle violation (°)	5.3
Energies (kcal/mol)	
Mean GB[a]-AMBER energy	−3359
Mean restraint energy	79
Ramachandran plot	
Most favorable region (%)	79.2
Additionally allowed regions (%)	20.1
Generously allowed regions (%)	0.6
Disallowed regions (%)	0.2

[a] Generalized Born potential.

AMBER. The 20 low energy NMR structures of Gn$^{729-805}$ converged into a family of structures (Fig. 5) with low restraint violations and good Ramachandran plot statistics (Table 1).

Structure of CCHF Virus Zinc Finger—The NMR structure of Gn$^{729-805}$ reveals a rigid, compact three-helix structure with four short β-strands (Fig. 5). The structure contains a pair of tightly associated, back to back ββα zinc fingers connected by a short four residue linker (Ser^{757}-Ile^{760}) (Fig. 5). The first CCHC-zinc finger array (ZF1) consists of a Zn^{2+} ion coordinated to residues Cys-736, Cys-739, His-752, and Cys-756 and forms the classical ββα zinc finger fold. Cys-736 and Cys-739 form part of a short β-hairpin. Thr-737 and Ile-738 form a loop with Cys-736 and Cys-739 on either side of the hairpin. The structure contains helix α1 formed by Ile-747 to Ser-757 that folds back toward the β-hairpin, forming the ββα zinc finger fold. Cys-756 forms the fourth Zn^{2+}-coordinating residue and is located on the same surface of helix α3 with His-752.

Likewise, the second CCHC-zinc finger array (ZF2) consists of a second Zn^{2+} ion coordinated to residues Cys-761, Cys-764, His-776, and Cys-780 into a classical ββα zinc finger fold. Cys-761 and Cys-764 are positioned on either side of a short β-hairpin. Pro-762 and Tyr-763 form a loop between Cys-761 and Cys-764. The structure is followed by a short helix α2 formed by Leu773-Cys-780 and folded back toward the β-hairpin, forming the ββα zinc finger fold. His-776 is located toward the middle of helix α3, and the final coordinating cysteine (Cys-780) is located at the end of helix α3. Although ZF2 also resembles the classical ββα fold, a minor difference exists when compared with ZF1. The helix α2 of ZF2 is shorter than helix α1 by three residues. This is due to the presence of helix breakers Gly-772 and Pro-781 located at either end of helix α2.

Unlike many classical ββα zinc fingers which form independently folded domains like "beads-on-a-string," the two CCHF virus zinc fingers were tightly stuck together, with over 65 NOEs observed between ZF1 and ZF2. These NOEs fix the relative orientation of ZF1 with respect to ZF2. Among these NOEs, the strongest were observed between Met-751 (ZF1) and Tyr-763 (ZF2), Cys-739 (ZF1) and Ala-774 (ZF2), His-752 (ZF1) and Val-777 (ZF2), and Thr-741 (ZF1) and Val-777 (ZF2).

In addition to the two classical ββα fold zinc fingers, the structure contains an additional helix, helix α3, formed by Lys-782 to Glu-791 that packs against the dual zinc finger fold. A hydrophobic interaction between Val-744 of ZF1 and Val-787 keeps helix α3 pinned to the core structure. The orientation of helix α3 to ZF2 is partially determined by the helix breaker Pro-781, the residue immediately following ZF2. Pro-781 is 100% identical among Nairoviruses (Fig. 1) and serves as a kink between helix α2 and α3. Strong Cys Cα to Pro-718 Cδ NOEs indicated a trans proline isomer. The C-terminal 13 residues (Leu-792 to Lys-805) are primarily unstructured. NOEs between Ile-799 Cγ2 and Met-751 Cγ indicate the unstructured tail is pinned to the rest of the structure.

The ^{15}N backbone relaxation rates (R_1 and R_2) as well as the heteronuclear {^{1}H}-^{15}N NOE (Fig. 4) showed that the ZF1, linker (residue 757–760), and ZF2 regions behave with nearly similar amide backbone dynamics. The average R_1 values for ZF1, ZF2 and linker regions were well within each other, with values of 1.60 (+ 0.15), 1.81 (+ 0.40), and 1.65 (+ 0.04) s^{-1}, respectively (Fig. 4*A*). ZF1 and the linker had essentially similar R_1 values, however, within ZF2, the R_1 values increased for residues 770 and 771 of the loop connecting β4 and α2, indicating increased mobility of this region. Likewise, the average R_2 values for ZF1, ZF2, and linker regions were similar to each other, with values of 13.8 (+ 1.8), 13.8 (+ 1.8), and 15.6 (+ 3.1) s^{-1}, respectively. Interestingly, the first linker residue, Ser-756, showed increased R_2 without a corresponding increase in R_1, which suggested chemical exchange on the μs-ms timescale for

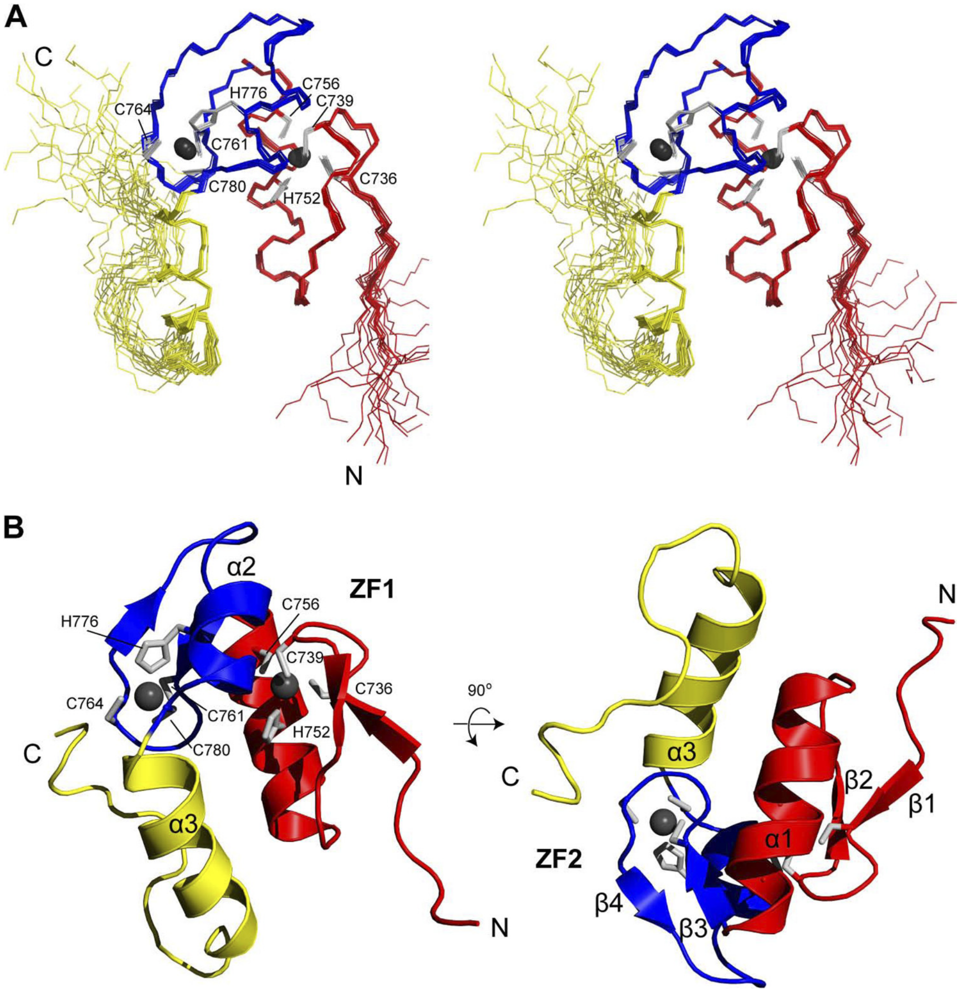

FIGURE 5. **NMR structure of CCHF virus Gn tail zinc finger.** Stereoview of the superposition of 20 lowest energy NMR structures of CCHF virus Gn zinc finger (*A*). CCHF virus Gn zinc finger domain folds into a compact three-helix structure consisting of two back-to-back $\beta\beta\alpha$ zinc fingers with helix $\alpha3$ pinned underneath the core zinc finger structure (*B*).

Ser-756. The average heteronuclear $\{^1H\}$-^{15}N NOE for the ZF1, linker and ZF2 regions was 0.8 (Fig. 4*C*), indicating reduced flexibility for the dual zinc finger domain including the linker region. In brief, the NMR amide backbone relaxation parameters (Fig. 4) confirmed that the two zinc fingers essentially tumble as one entity, that the linker between the two zinc fingers was rigid and tumble at the same rate as the zinc fingers, and that the loops and tails were flexible.

CCHF Virus Zinc Finger Contains Conserved Electrostatic Surfaces—Analysis of the surface electrostatics of the CCHF virus $Gn^{729-805}$ reveals clustering of positive and negatively charged surfaces on opposite faces of the structure (Fig. 6). Surface residues Glu-740, Glu-750, and Asp-753 of ZF1 converge with surface residues Glu-789 and Glu-791 of helix $\alpha3$ and Glu-797 of the C-terminal unstructured region to form a large, nearly contiguous negatively charged surface. Similarly, Arg-767 and Arg-775 of ZF2 converge with Lys-782, Lys-784, and Lys-786 of helix $\alpha3$ and Arg-798 of the C-terminal unstructured region to form a large contiguous positively charged surface. Of the charged surface residues, only Glu-750, Glu-797, Lys-784, and Arg-798 are not conserved in Nairoviruses. Most of the surface electrostatics, therefore, is a conserved feature of the Nairoviruses zinc finger domain.

CCHF Virus Gn Tail Binds RNA—RNA electrophoretic mobility shift binding assays (EMSA) were carried out using two different proteins: the zinc finger domain of CCHF virus consisting of $Gn^{729-805}$, and the zinc finger domain $G1^{534-599}$ of the related Andes hantavirus. The RNA sequences used in the EMSA were a 58-mer RNA of the Andes hantavirus and a 51-mer RNA of the CCHF virus (Fig. 7*A*). Both RNA sequences contain 23–26 nucleotides at the 5′ and 3′ termini of the M genomic segments of the Andes and CCHF viruses, and these 5′ and 3′ strands are complementary to each other and are expected to form into hairpin-like panhandle structures (Fig. 7*A*). On a 12% native acrylamide gel, the Andes hantavirus RNA traveled as a single band consistent with a 58-mer hairpin (Fig. 7*B*), however, the CCHF virus 51-mer RNA migrated as two bands, a higher molecular weight form and a lower band migrating at a size consistent with a 51-mer hairpin (Fig. 7*B*). Incubation of the Andes hantavirus protein with the 58-mer RNA failed to affect the migration of RNA (Fig. 7*B*). The RNA bands in the presence of the Andes hantavirus protein were similar to the free RNA band (Fig. 7*B*). However, incubation of the CCHF virus zinc finger protein notably affected the migration of the CCHF virus RNA, as demonstrated by the appearance of an additional band (marked with *asterisk*, Fig. 7*B*)

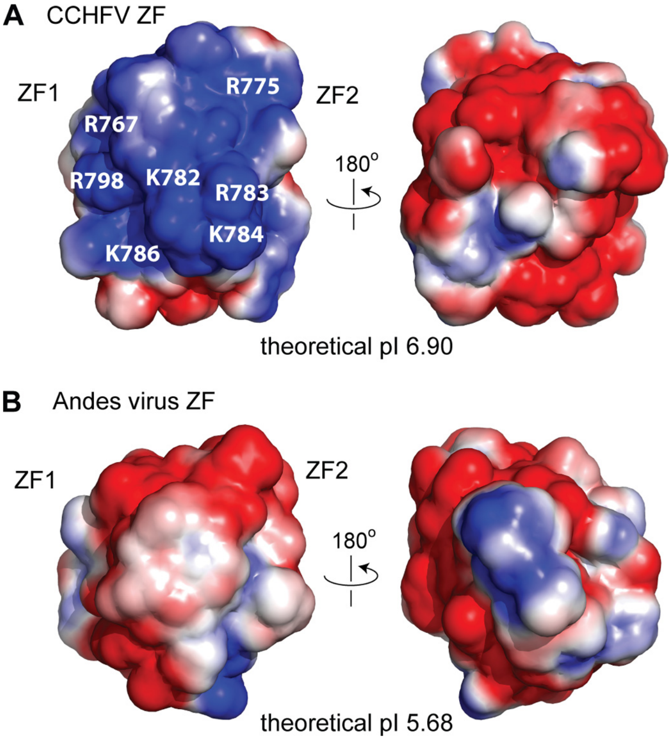

FIGURE 6. **Comparison of the surface electrostatics of the CCHF virus (*A*) and Andes hantavirus (*B*) zinc fingers.** Analysis of the surface electrostatics of the CCHF virus Gn zinc finger reveals a contiguous cluster of basic charges (colored *blue*) that cover the entire half of the structure (*A*). Rotating the structure 180° reveals an equally large cluster of acidic charges (colored *red*) on the opposite face (*A*). Surface electrostatics of the Andes hantavirus zinc fingers (PDB 2K9H) in the same orientation as the CCHF virus structure (*B*). Notably, the clustering of conserved basic surface in Nairovirus (*A*) is absent in Hantavirus (*B*).

migrating between the hairpin and the higher MW form of the CCHF virus RNA. The protein-RNA complex band was most likely formed between the zinc finger protein and the RNA hairpin. Because the 51-mer CCHFV RNA existed in two forms (the hairpin and the higher MW form), the relative amounts of each form must be in equilibrium and binding of the CCHFV zinc finger protein to the hairpin RNA will also affect the amount of the RNA B form as seen in Fig. 7*B*. We are designing new RNA sequences that will only form hairpins for future studies of the protein-RNA interaction of the CCHF virus zinc finger. Nevertheless, the main conclusion from the EMSA results demonstrated that the CCHF Nairovirus Gn tail (residues 729–805) interacted with RNA whereas the Andes hantavirus G1 tail (residues 534–599) did not.

To test our hypothesis that conserved basic residues in the CCHF virus zinc finger protein may be involved in RNA binding, point mutations were introduced in conserved lysine and arginine residues into aspartic acid and the mutant proteins were used in EMSA. The point mutants K783D, K786D, and R767D retained the ability to bind RNA suggesting these basic residues may not be critical in RNA binding (supplemental Fig. S2*A*). However, the K782D mutation disrupted the protein-RNA interaction, as its shift pattern resembles that of RNA alone. Coincidentally, Lys-782 is located at the kink between helix $\alpha 2$ and $\alpha 3$, and is pointed away from the zinc finger domain (supplemental Fig. S2*B*), suggesting that the kink between helix $\alpha 2$ and $\alpha 3$ may be important for RNA binding interaction of the CCHF virus Gn zinc finger.

DISCUSSION

We report here that an envelope glycoprotein of a Nairovirus contains a dual CCHC-type zinc finger (Figs. 2–5) domain that

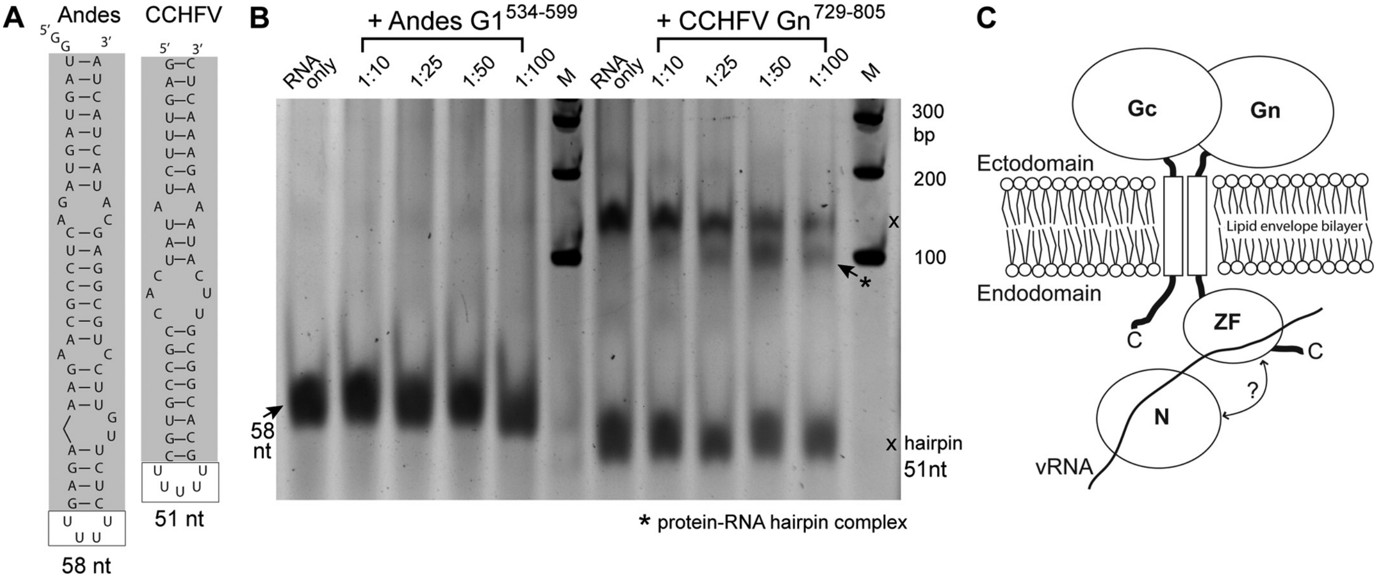

FIGURE 7. **RNA electrophoretic mobility shift assay comparing the Andes Hantavirus and the CCHF virus zinc fingers.** RNA sequences used in the EMSA: Andes Hantavirus RNA (58 nt) and CCHF virus RNA (51 nt) (*A*). CCHF virus RNA traveled in two forms (marked with ×), lower band consistent with a 51-nt hairpin form, and a higher molecular weight band above 100 bp (*B*). While the Andes hantavirus zinc finger fails to alter the mobility of Andes hantavirus RNA, increasing the amount of CCHF virus zinc finger causes the appearance of an additional band (marked with *) for the CCHF virus RNA, thus suggesting a protein-RNA complex (*B*). Each lane contained 0.24 μmol RNA, proteins came from 0.4 mM stock diluted accordingly (*B*). Surface electrostatics combined with EMSA results of the Gn tail provide mechanistic insight into RNP packaging (*C*). We propose a model in which packaging consists of a zinc finger-RNA complex. Studies in related viruses suggest a nucleocapsid, Gn tail interaction, presented here as a possible additional packaging site (*C*).

binds RNA. Although zinc fingers have been known in viruses, in particular, the HIV-1 nucleocapsid protein zinc fingers (33) are critical in RNA packaging and viral assembly, zinc fingers are rarely found in viral envelope glycoproteins. Including the zinc finger in this report, there are currently only three known structures of viral envelope glycoprotein zinc fingers. First is the zinc finger of the Hantavirus G1 envelope glycoprotein (11), and second is the zinc finger of the Junin virus envelope glycoprotein (34) (also an RNA virus, of family *Arenaviridae*). In all three cases, the cytoplasmic tails of the envelope glycoproteins contain the zinc finger domains. The Junin virus zinc fingers (34) form a unique fold that do not show any structural nor sequence similarity with the Nairovirus and Hantavirus zinc fingers. Although the Nairovirus and the Hantavirus zinc fingers (11), which both belong to family *Bunyaviridae* (Fig. 1), show an overall similar global fold (supplemental Fig. S3), they also have major structural differences (Fig. 6) and properties (Fig. 7).

Key Differences between Nairovirus and Hantavirus Gn Zinc Fingers—Examination of the surface electrostatics of the CCHF virus Gn tail reveals key differences when compared with the Andes hantavirus structure (Fig. 6). Whereas the CCHF Gn tail displays sharp clustering of conserved charges that form a large contiguous swath on the protein surface, the Andes hantavirus zinc fingers display charges that are predominately negative and apparently randomly dispersed (Fig. 6). The variation in charge conservation is also evident in the sequence analysis (supplemental Fig. S3). Whereas the spacing of CCHC motif is mostly conserved, the spacing between conserved charges is highly variable. The CCHF virus displays conserved negative charges on ZF1 and conserved positive charges on ZF2. The Andes hantavirus sequence, however, displays clustering of conserved positive charges on the sequences flanking the negatively charged core zinc finger structure.

Moreover, the CCHF virus Gn tail contains a structural motif that is absent in the Andes hantavirus structure. Helices $\alpha 2$ and $\alpha 3$ (Fig. 3*B*), residues Leu-773 to Leu-792, form a helix-kink-helix motif due the positioning of the conserved helix breaker Pro-781. While not an uncommon motif, this structural aspect in the CCHF Gn tail forms the core scaffold for a large positively charged surface partly composed of the conserved charges at Arg-775, Lys-782, and Lys-786 (Fig. 6*A*). By contrast, the Andes hantavirus structure contains neither the corresponding proline nor the charges to support a similar motif (Fig. 1). Instead, it contains the non-conserved helix breaker Gly-598 followed by conserved charges exclusively on the C-terminal end of ZF2 (Fig. 1). In this respect, the surface electrostatics of the CCHF virus Gn tail may more closely resemble that of Orthobunyaviruses, with conserved helix breakers flanked by conserved basic charges (Fig. 1). Perhaps not coincidentally, Nairoviruses and Orthobunyaviruses are both arthropod-borne, whereas Hantaviruses are rodent-borne (3). Overall, the general preservation of the fold indicates that the dual zinc finger motif plays a general but important role in the life cycle of both Nairoviruses and Hantaviruses. However, the major differences in the surface electrostatics of the CCHF virus and Hantavirus cytoplasmic tail structures (Fig. 6) also suggests that while general, the function of the tail may be species specific.

Proposed Role in Viral Assembly—Given the data available regarding the *Bunyaviridae* Gn role in viral assembly (8–10), it is likely that the surface electrostatics play an important role in assembly of the CCHF virus, presumably via direct interaction with some component of the ribonucleoprotein. The large positively charged surface of the Gn tail would suggest RNA binding, as is the traditional role for zinc fingers in retroviruses (33, 35). Our EMSA results (Fig. 7) suggest that this may in fact be the case. Using increasing amounts of $\mathrm{Gn}^{729-805}$ revealed the migration of an additional RNA band (Fig. 7*B*), which likely represents a protein-RNA complex consisting of $\mathrm{Gn}^{729-805}$ and the hairpin-like M segment panhandle. While the observed complex is weakly bound and therefore likely to be nonspecific, these results suggest RNA interaction mediated by some of the conserved residues in the Gn tail. Additionally, these results suggest the possibility of an interaction between the Gn tail and the RNA component of the ribonucleoproteins (Fig. 7*C*). A reverse genetics system for studying the CCHF virus has only recently been developed (36), thus allowing testing of this model in the future.

In summary, we present the NMR structure of a zinc finger domain in the Gn tail of the CCHF virus. Currently, this is the only available atomic structure for a protein component of the Nairovirus genus. The global fold of this zinc finger is similar to that of the Hantavirus zinc finger, which represents a unique fold of two classical-type $\beta\beta\alpha$-zinc fingers that are stuck together (in contrast, individual classical $\beta\beta\alpha$-zinc fingers behave as independent domains, like beads-on-a-string). We also demonstrated that the CCHF virus Gn tail binds RNA *in vitro*, thus suggesting the possibility of an interaction between the Gn tail with the viral RNA. Taken together, our results contribute novel mechanistic insight toward understanding the CCHF virus life cycle.

Acknowledgments—We thank Lou Altimura (United States Army Medical Research Institute of Infectious Diseases), Gaya Amarasinghe (Iowa State University), Sunhwan Jo (University of Kansas), Gerard Kroon (Scripps Research Institute), Dan McElheny (University of Illinois at Chicago), and Asokan Anbanandam (University of Kansas) for helpful discussions.

REFERENCES

1. Ergönül, O. (2006) *Lancet Infect. Dis.* **6,** 203–214
2. Whitehouse, C. A. (2004) *Antiviral. Res.* **64,** 145–160
3. Elliott, R. M., Bouloy, M., Calisher, C. H., Goldbach, R., Moyer, J. T., Nichol, S. T., Pettersson, R., Plyusnin, A., and Schmaljohn, C. (2000) in *Bunyaviridae.* in *Virus Taxonomy: The classification and Nomenclature of Viruses. The Seventh Report of the International Committee on Taxonomy of Viruses.* (Van Regenmortel, M. H. V., Fauquet, C. M., Bishop, D. H. L., Carsten, E. B., Estes, M. K., Lemon, S. M., Maniloff, J., Mayo, M. A., McGeoch, D. J., Pringle, C. R., and Wickner, R. B., eds.), Academic Press, San Diego
4. Schmaljohn, C. S., and Hooper, J. W. (2001) in *Bunyaviridae: the Viruses and Their Replication*, 4th Ed. *Fields Virology* (Knipe, D. M., and Howley, P. M. eds.), Lippincott, Williams & Wilkins, Philadelphia
5. Sanchez, A. J., Vincent, M. J., and Nichol, S. T. (2002) *J. Virol.* **76,** 7263–7275
6. Sanchez, A. J., Vincent, M. J., Erickson, B. R., and Nichol, S. T. (2006) *J. Virol.* **80,** 514–525
7. Altamura, L. A., Bertolotti-Ciarlet, A., Teigler, J., Paragas, J., Schmaljohn,

C. S., and Doms, R. W. (2007) *J. Virol.* **81,** 6632–6642
8. Overby, A. K., Pettersson, R. F., and Neve, E. P. (2007) *J. Virol.* **81,** 3198–3205
9. Shi, X., Kohl, A., Li, P., and Elliott, R. M. (2007) *J. Virol.* **81,** 10151–10160
10. Hepojoki, J. M., Strandin, T., Wang, H., Vapalahti, O., Vaheri, A., and Lankinen, H. (2010) *J. Gen. Virol.* **91,** 2341–2350
11. Estrada, D. F., Boudreaux, D. M., Zhong, D., St Jeor, S. C., and De Guzman, R. N. (2009) *J. Biol. Chem.* **284,** 8654–8660
12. Chatterjee, S., Zhong, D., Nordhues, B. A., Battaile, K. P., Lovell, S., and De Guzman, R. N. (2011) *Protein Sci.* **20,** 75–86
13. Geisbrecht, B. V., Bouyain, S., and Pop, M. (2006) *Protein Expr. Purif.* **46,** 23–32
14. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A. (1995) *J. Biomol. NMR* **6,** 277–293
15. Johnson, B. A. (2004) *Methods Mol. Biol.* **278,** 313–352
16. Grzesiek, S., and Bax, A. (1993) *J. Am. Chem. Soc.* **115,** 12593–12594
17. Grzesiek, S., Döbeli, H., Gentz, R., Garotta, G., Labhardt, A. M., and Bax, A. (1992) *Biochemistry* **31,** 8180–8190
18. Wittekind, M., and Mueller, L. (1993) *J. Magn. Reson.* **101,** 201–205
19. Wishart, D. S., and Nip, A. M. (1998) *Biochem. Cell Biol.* **76,** 153–163
20. Tolman, J. R., Chung, J., and Prestegard, J. H. (1992) *J. Magn. Reson.* **98,** 462–467
21. Grzesiek, S., and Bax, A. (1993) *J. Biomol. NMR* **3,** 185–204
22. Hoffman, R. C., Xu, R. X., Klevit, R. E., and Herriott, J. R. (1993) *J. Magn. Reson. Ser. B* **102,** 61–72
23. Pelton, J. G., Torchia, D. A., Meadow, N. D., and Roseman, S. (1993) *Protein Sci.* **2,** 543–558
24. Marion, D., Driscoll, P. C., Kay, L. E., Wingfield, P. T., Bax, A., Gronenborn, A. M., and Clore, G. M. (1989) *Biochemistry* **28,** 6150–6156
25. Stone, M. J., Fairbrother, W. J., Palmer, A. G., 3rd, Reizer, J., Saier, M. H., Jr., and Wright, P. E. (1992) *Biochemistry* **31,** 4394–4406
26. Farrow, N. A., Muhandiram, R., Singer, A. U., Pascal, S. M., Kay, C. M., Gish, G., Shoelson, S. E., Pawson, T., Forman-Kay, J. D., and Kay, L. E. (1994) *Biochemistry* **33,** 5984–6003
27. Janert, P. K. (2009) *Gnuplot in Action: Understanding Data with Graphs*, Manning Publication Co., Greenwich, CT
28. Güntert, P. (2004) *Methods Mol. Biol.* **278,** 353–378
29. Case, D. A., Pearlman, D. A., Caldwell, J. W., Cheatham Iii, T. E., Wang, J., Ross, W. S., Simmerling, C. L., Darden, T. A., Merz, K. M., Stanton, R. V., Cheng, A. L., Vincent, J. J., Crowley, M., Tsui, V., Gohlke, H., Radmer, R. J., Duan, Y., Pitera, J., Massova, I., Seibel, G. L., Singh, U. C., Weiner, P. K., and Kollman, P. A. (2002) *AMBER7*, University of California, San Francisco
30. Laskowski, R. A., Rullmannn, J. A., MacArthur, M. W., Kaptein, R., and Thornton, J. M. (1996) *J. Biomol. NMR* **8,** 477–486
31. DeLano, W. L. (2002) *The PyMOL Molecular Graphics System*, DeLano Scientific, San Carlos, CA
32. Baker, N. A., Sept, D., Joseph, S., Holst, M. J., and McCammon, J. A. (2001) *Proc. Natl. Acad. Sci. U.S.A.* **98,** 10037–10041
33. De Guzman, R. N., Wu, Z. R., Stalling, C. C., Pappalardo, L., Borer, P. N., and Summers, M. F. (1998) *Science* **279,** 384–388
34. Briknarová, K., Thomas, C. J., York, J., and Nunberg, J. H. (2011) *J. Biol. Chem.* **286,** 1528–1536
35. D'Souza, V., and Summers, M. F. (2005) *Nat. Rev. Microbiol.* **3,** 643–655
36. Flick, R., Flick, K., Feldmann, H., and Elgh, F. (2003) *J. Virol.* **77,** 5997–6006

JOURNAL OF VIROLOGY, June 2006, p. 5189–5198 Vol. 80, No. 11
0022-538X/06/$08.00+0 doi:10.1128/JVI.00208-06

Role of the Stable Signal Peptide and Cytoplasmic Domain of G2 in Regulating Intracellular Transport of the Junín Virus Envelope Glycoprotein Complex

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Received 28 January 2006/Accepted 20 March 2006

Enveloped viruses utilize the membranous compartments of the host cell for the assembly and budding of new virion particles. In this report, we have investigated the biogenesis and trafficking of the envelope glycoprotein (GP-C) of the Junín arenavirus. The mature GP-C complex is unusual in that it retains a stable signal peptide (SSP) as an essential component in association with the typical receptor-binding (G1) and transmembrane fusion (G2) subunits. We demonstrate that, in the absence of SSP, the G1-G2 precursor is restricted to the endoplasmic reticulum (ER). This constraint is relieved by coexpression of SSP in *trans*, allowing transit of the assembled GP-C complex through the Golgi and to the cell surface, the site of arenavirus budding. Transport of a chimeric CD4 glycoprotein bearing the transmembrane and cytoplasmic domains of G2 is similarly regulated by SSP association. Truncations to the cytoplasmic domain of G2 abrogate SSP association yet now permit transport of the G1-G2 precursor to the cell surface. Thus, the cytoplasmic domain of G2 is an important determinant for both ER localization and its control through SSP binding. Alanine mutations to either of two dibasic amino acid motifs in the G2 cytoplasmic domain can also mobilize the G1-G2 precursor for transit through the Golgi. Taken together, our results suggest that SSP binding masks endogenous ER localization signals in the cytoplasmic domain of G2 to ensure that only the fully assembled, tripartite GP-C complex is transported for virion assembly. This quality control process points to an important role of SSP in the structure and function of the arenavirus envelope glycoprotein.

Arenaviruses are endemic in rodent populations worldwide (53), and infection can be transmitted to humans to cause severe acute hemorrhagic fevers (44, 51). Recurring outbreaks are common in regions of arenavirus endemicity, and therapeutic options to combat arenavirus infection are limited. Phylogenetic analyses divide the arenaviruses into the Old World species, such as Lassa fever and lymphocytic choriomeningitis (LCM) viruses, and the New World species, such as Junín and Machupo viruses. Up to 300,000 infections with Lassa fever virus occur annually in Africa (45), and outbreaks of New World viruses in the Americas are sporadic but routine (51). Recently, infections by LCM virus in transplant recipients have been reported (8). In the absence of effective prophylaxis and treatment, the hemorrhagic fever arenaviruses remain an urgent public health concern.

The arenaviruses are enveloped viruses whose genomes consist of two single-stranded RNA molecules, each of which encodes the ambisense expression of two of the four viral proteins (5, 9). The viral envelope glycoprotein (GP-C) is translated from a genomic-sense mRNA generated from the short (S) genomic RNA, whereas the nucleocapsid protein is translated from the antigenomic-sense mRNA. Similarly, the viral matrix protein (Z) and RNA-dependent RNA polymerase are encoded in an ambisense orientation by the long (L) RNA. During biogenesis, arenaviral particles assemble and bud at the plasma membrane (49, 60). Viral entry into target cells is initiated by GP-C binding to cell surface receptors followed by endocytosis of the virion into smooth vesicles (2). Although α-dystroglycan serves as a binding receptor for the Old World arenaviruses (6), the receptor utilized by the major New World group of arenaviruses is unknown (59). GP-C-mediated membrane fusion is activated upon acidification of the maturing endosome (2, 7, 13, 14) to deposit the virion core into the cell cytoplasm and initiate replication.

The arenavirus envelope glycoprotein complex consists of three noncovalently associated subunits derived from the GP-C precursor: in addition to the typical receptor-binding (G1) and transmembrane fusion (G2) subunits, the complex contains a stable signal peptide (SSP) subunit (4, 18, 65) (Fig. 1). The 58-amino-acid SSP is generated by the cellular signal peptidase and subsequently myristoylated (65). The mature G1 and G2 subunits are generated upon cleavage by the cellular SKI-1/S1P protease (1, 35, 38) in the early Golgi compartment (3). This proteolytic maturation event is essential for membrane fusion activity. The arenavirus G2 is a member of the class I group of viral fusion proteins (25, 64) that orchestrate membrane fusion through the triggered formation of a stable six-helix bundle core (references 16, 17, 32, and 63 and references therein).

A tripartite envelope glycoprotein complex is unusual among viral envelope glycoproteins, and the role of the unique arenavirus SSP subunit has not been fully defined. In the GP-C complex, SSP exists as a transmembrane protein, likely in a type II topology with an extended luminal C terminus (19, 23). The N terminus is modified by myristoylation, which is important for efficient membrane fusion activity (65). Recombinant GP-C constructs in which SSP is replaced by a conventional

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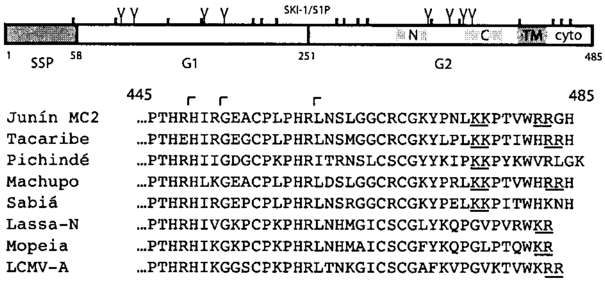

Junín MC2	…PTHRHIRGEACPLPHRLNSLGGCRCGKYPNLKKPTVWRRGH
Tacaribe	…PTHEHIRGEGCPLPHRLNSMGGCRCGKYLPLKKPTIWHRRH
Pichindé	…PTHRHIIGDGCPKPHRITRNSLCSCGYYKIPKKPYKWVRLGK
Machupo	…PTHRHLKGEACPLPHRLDSLGGCRCGKYPRLKKPTVWHRRH
Sabiá	…PTHRHIRGEPCPLPHRLNSRGGCRCGKYPELKKPITWHKNH
Lassa-N	…PTHRHIVGKPCPKPHRLNHMGICSCGLYKQPGVPVRWKR
Mopeia	…PTHRHIKGKPCPKPHRLNHMAICSCGFYKQPGLPTQWKR
LCMV-A	…PTHRHIKGGSCPKPHRLTNKGICSCGAFKVPGVKTVWKRR

FIG. 1. Schematic representation of the Junín virus GP-C glycoprotein and G2 cytoplasmic domain sequences. Amino acids of the Junín virus envelope glycoprotein are numbered from the initiating methionine, and cysteine residues (|) and potential glycosylation sites (Y) are marked. The SSP and SKI-1/S1P cleavage sites and the resulting SSP, G1, and G2 subunits are indicated. Within G2, the C-terminal transmembrane (TM) and cytoplasmic (cyto) domains are shown, as are the N- and C-terminal heptad repeat regions (light-gray shading). A comparison of G2 cytoplasmic domain sequences among arenavirus species is detailed below the schematic. Sequences include the New World isolates Junín (D10072), Tacaribe (M20304), Pichindé (*U*77601), Machupo (AY129248), and Sabiá (YP_089665) and Old World isolates Lassa-Nigeria (X52400), Mopeia (M33879), and LCMV-Armstrong (M20869). The sites used to generate truncations in the Junín virus cytoplasmic tail are indicated by angle brackets and dibasic amino acid sequences are underlined.

signal peptide do not undergo significant proteolytic maturation by the SKI-1/S1P protease (18, 65). In the Old World Lassa fever arenavirus, this defect can be rescued by coexpression of SSP in *trans* (18).

In the present report, we examine the biogenesis of the GP-C complex of the Junín virus, a member of the New World Tacaribe complex of arenaviruses that is responsible for recurring outbreaks of hemorrhagic fever in the pampas grasslands of Argentina. We show that SSP association is required for transport of the G1-G2 precursor from the endoplasmic reticulum (ER) and thereby for proteolytic maturation in the Golgi. In the absence of SSP, the G1-G2 precursor is constrained to the ER by dibasic amino acid sequences in the cytoplasmic domain of G2. Association with SSP overcomes this block to permit transit of the fully assembled complex through the Golgi and to the cell surface. Moreover, our studies suggest that, in addition to modulating trafficking of GP-C, SSP association may also be important for the membrane fusion activity of the GP-C complex. The unique roles for SSP in the arenavirus life cycle may suggest novel strategies towards the prevention and treatment of arenaviral disease.

MATERIALS AND METHODS

Molecular reagents, recombinant vaccinia viruses, and monoclonal antibodies. The GP-C coding region from the pathogenic Junín virus strain MC2 (28) was provided by Victor Romanowski (Universidad Nacional de La Plata, Argentina) and introduced into the mammalian expression vector pcDNA3.1+ as described previously (65). For *trans*-complementation studies (18), the CD4sp-GPC construct in which SSP was replaced by the conventional signal peptide of CD4 (65) was coexpressed with an SSP construct in which a stop codon was introduced following the C-terminal SSP amino acid T58 (SSP-term). A chimeric glycoprotein (CD4ecto) bearing the CD4 signal peptide and ectodomain fused to the transmembrane and cytoplasmic domains of G2 was constructed using the human CD4 cDNA (41) obtained through the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program. Mutations were introduced by QuikChange mutagenesis (Stratagene), and PCR was used to generate truncations and chimeric plasmids. For the cytoplasmic-domain truncation series and in a control cleavage-defective GP-C plasmid (cd-GPC) (65), a C-terminal 15-amino-acid S-peptide (Spep) affinity tag (34) was introduced to facilitate biochemical analysis (65). All constructs were verified by DNA sequencing, and three independent clones typically were tested to ensure consistent phenotypes.

Optimal expression of the Junín virus GP-C gene and its derivatives in Vero 76 cells was achieved using the bacteriophage T7 promoter of the pcDNA3.1 vector and infection by a recombinant vaccinia virus expressing the T7 polymerase (vTF7-3) (24). The vaccinia virus vCB21R-lacZ expressing the β-galactosidase gene under the control of the T7 promoter was used in our analysis of cell-cell fusion (47). These recombinant vaccinia virus reagents were provided by T. Fuerst and B. Moss and C. Broder, P. Kennedy, and E. Berger, respectively, through the NIH AIDS Research and Reference Reagent Program.

Mouse monoclonal antibodies (MAbs) QC03-BF11 (BF11) and GB03-BE08 (BE08) (54), directed against the G1 subunit of GP-C, were kindly provided by Tom Ksiasek and Tony Sanchez (Special Pathogens Branch, CDC, Atlanta, Georgia). The anti-CD4 ectodomain MAb SIM.2 (43, 48) was obtained through the NIH AIDS Research and Reference Reagent Program.

Expression of GP-C and its derivatives. The glycoproteins were expressed and characterized as previously described (64, 65). Briefly, Vero 76 cells were infected with the recombinant vaccinia virus vTF7-3 (24) at a multiplicity of 2 in Dulbecco's minimal essential medium containing 2% fetal bovine serum (FBS) and 10 μM cytosine arabinoside (araC) (31). After 30 min, the cells were washed and transfected with the GP-C expression plasmid using Lipofectamine 2000 reagent (Invitrogen). Metabolic labeling using 32 to 50 μCi/ml of ^{35}S-ProMix (Amersham Pharmacia Biotech) was initiated 6 h posttransfection in methionine- and cysteine-free medium containing 10% dialyzed FBS and 10 μM araC and was continued for 12 to 16 h. Cultures were then washed in physiological buffered saline (PBS) and lysed using cold Tris-saline buffer (50 mM Tris-HCl and 150 mM NaCl, pH 7.5) containing 1% Triton X-100 nonionic detergent and protease inhibitors (1 μg/ml each of aprotinin, leupeptin, and pepstatin). The expressed glycoproteins were isolated from cleared lysates by immunoprecipitation using either the G1-directed MAbs or the CD4-directed MAb SIM.2 and protein A-Sepharose (Sigma). In some experiments, glycoproteins containing the C-terminal Spep affinity tag were isolated using S-protein agarose (Novagen). Isolated glycoproteins were deglycosylated using peptide:*N*-glycosidase F (PNGase F; New England Biolabs). Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using NuPAGE 4 to 12% bis-Tris gels (Invitrogen) and the recommended sample buffer containing lithium dodecyl sulfate and reducing agent. Molecular size markers included ^{14}C-methylated Rainbow proteins (Amersham Pharmacia Biotech). Radiolabeled proteins were imaged using a Fuji FLA-3000G imager and analyzed using ImageGauge software (Fuji).

For immunoprecipitation of cell surface glycoproteins, monolayers of metabolically labeled cells were incubated with MAb BE08 or SIM.2 in ice-cold PBS containing 2% FBS and 0.1% NaN_3 for 2 h. Following extensive washing, cells were resuspended by scraping in PBS and lysed as described above. Immune complexes were isolated from cleared lysates using protein A-Sepharose.

Flow cytometry. Vero 76 cells expressing GP-C or its derivatives were labeled using the G1-specific MAb BE08 (54) and a secondary fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Jackson ImmunoResearch). CD4 was detected using a fluorescein isothiocyanate-conjugated mouse anti-CD4 MAb (BD Biosciences). Cells were subsequently stained using propidium iodide (1 μg/ml) and then fixed in 2% formaldehyde (64). Populations were analyzed using a FACSCalibur flow cytometer and CellQuest software (BD Biosciences).

GP-C-mediated cell-cell fusion. The β-galactosidase fusion reporter assay (47) was used to characterize the ability of the envelope glycoproteins to mediate pH-dependent cell-cell fusion (64, 65). Briefly, Vero cells infected with vTF7-3 and expressing the envelope glycoprotein were cocultured with reporter cells infected with vCB21R-lacZ, a recombinant vaccinia virus expressing β-galactosidase under the control of the T7 promoter. The reporter cells were obtained by incubating Vero 76 cells with vCB21R-lacZ at a multiplicity of 2 and allowing the infection to proceed overnight in the presence of 100 μg/ml rifampin (31). The GP-C-expressing cells and reporter cells were cocultured in medium containing both araC and rifampin for 5 h and then subjected to a 30-min pulse of neutral or acidic (pH 5.0) medium. β-Galactosidase expression is induced upon fusion of the effector and reporter cells and was detected, after 5 h of continued cultivation at neutral pH, in cell lysates (Tropix) using the chemiluminescent substrate GalactoLite Plus (Tropix). Cell-cell fusion was quantified using a Tropix TR717 microplate luminometer.

Confocal microscopy. Cells expressing GP-C glycoproteins were harvested by trypsinization 6 h after transfection and reseeded to 8-well chambered cover glasses (Lab Tek II) in medium containing 10 μM araC. After 18 h, cultures were washed in PBS and fixed with 4% formaldehyde for 10 min at room temperature. Following washing and quenching with 50 mM Tris (pH 7.4) in PBS, cultures were either permeabilized in PBS containing 0.1% Triton X-100 and blocked in the same buffer containing 5% FBS (for intracellular staining) or simply blocked in the absence of detergent (for cell surface staining). GP-C glycoproteins were detected using the G1-directed MAb BF11 and an Alexa Fluor 488-conjugated anti-mouse antibody (Molecular Probes) in the appropriate blocking buffer. The Golgi marker giantin was detected using a rabbit polyclonal antiserum (Covance Research Products) and an Alexa Fluor 568-conjugated anti-rabbit antibody (Molecular Probes). Chambers were covered with Slow Fade Gold (Molecular Probes) and visualized using an inverted Nikon TE-300 microscope. Fluorescence was examined using a Bio-Rad Radiance 2000 confocal laser scanning microscope and images were merged using Lasersharp software (Bio-Rad).

RESULTS

SSP association is required for proteolytic maturation. The arenavirus SSP is distinct from conventional signal peptides in that it is retained as an essential subunit of the mature GP-C envelope glycoprotein complex and mediates functions beyond translocation of the nascent polypeptide to the ER (18, 20, 65). We previously showed that a recombinant Junín virus GP-C glycoprotein in which SSP was replaced by the conventional signal peptide of human CD4 (CD4sp-GPC) was unable to undergo efficient maturation by the SKI-1/S1P protease (65), extending similar observations with GP-C of the Old World Lassa fever virus (18). In this Old World virus, the deficiency in proteolytic cleavage in the absence of SSP was reversed by coexpression of SSP in *trans* (18).

To investigate the role of SSP in the proteolytic maturation of the Junín virus GP-C, we determined whether the coexpression of SSP in *trans* could likewise rescue cleavage. In these studies, the Junín virus CD4sp-GPC construct was cotransfected with the SSP-term plasmid encoding the 58-amino-acid SSP. Optimal expression in Vero cells was dependent on T7 RNA polymerase provided by the recombinant vaccinia virus vTF7-3 (24). Cells were metabolically labeled, and GP-C glycoproteins were immunoprecipitated using the G1-directed MAb BE08 (54). Baseline studies were performed using the native GP-C glycoprotein that included its endogenous SSP. Expression of the native glycoprotein resulted in the isolation of a 60-kDa G1-G2 precursor glycoprotein and a heterodisperse smear of G1 and G2 subunits (30 to 35 kDa) (Fig. 2A, top panel). These mature subunits are best resolved following deglycosylation by PNGase F to yield 22- and 27-kDa polypeptides, respectively (bottom panel). The G1 and G2 subunits were absent upon expression of an SKI-1/S1P cleavage-defective glycoprotein (cd-GPC) (65). A GP-C precursor glycoprotein bearing SSP is often detected as a minor species, suggesting incomplete signal peptidase cleavage in transfected cells (20, 23, 65). As previously reported (18, 20, 23, 65), SSP was coprecipitated as part of the wild-type and cleavage-defective GP-C complexes (Fig. 2A, top panel).

Expression of the CD4sp-GPC glycoprotein in the absence of SSP generated the 60-kDa G1-G2 precursor (Fig. 2A, top panel, −SSP) and considerably lesser amounts of the cleaved glycoproteins (bottom panel). By contrast, expression of SSP in *trans* (+SSP) enabled efficient cleavage of the G1-G2 precursor glycoprotein to produce mature G1 and G2 subunits (bottom panel). The relative efficiency of proteolytic maturation of CD4sp-GPC in *trans* was similar to that of the native GP-C glycoprotein. Furthermore, SSP was coprecipitated with the CD4sp-GPC complex (top panel). Thus, coexpression of SSP appears to rescue wild-type assembly and proteolytic processing in the New World Junín virus CD4sp-GPC complex.

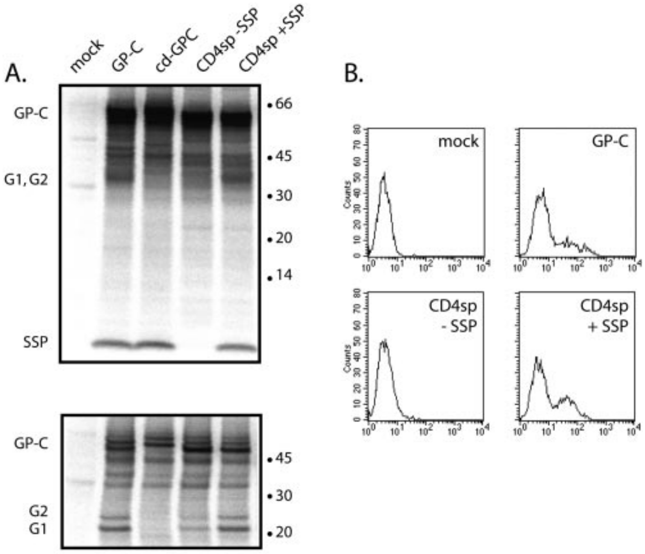

FIG. 2. Coexpression of SSP in *trans* rescues SKI-1/S1P cleavage and cell surface expression of the G1-G2 precursor. (A) Metabolically labeled glycoproteins were immunoprecipitated using the G1-specific MAb BE08 and separated on NuPAGE 4-to-12% bis-Tris gels. The wild-type (GP-C) and SKI-1/S1P cleavage-defective (cd-GPC) glycoproteins are shown for comparison with the CD4sp-GPC construct encoding the conventional signal peptide of human CD4. CD4sp-GPC was expressed alone (−SSP) or with SSP (+SSP). In the bottom panel, the glycoproteins have been treated with PNGase F to resolve G1 and G2 polypeptides. The deglycosylated GP-C polypeptides reveal both the G1-G2 precursor and, in SSP-containing constructs, the pre-GP-C precursor (65); additional species that migrate more slowly than the G1-G2 precursor and with the pre-GP-C precursor are likely products of incomplete deglycosylation. cd-GPC contains a C-terminal S-peptide affinity tag and migrates slightly slower than the other G1-G2 precursors. Known GP-C species are labeled at left; minor unidentified bands are also present. The ^{14}C-labeled protein markers (Amersham Biosciences) are indicated (in kilodaltons). (B) Cell surface expression of GP-C in Vero cells was determined by flow cytometry using the G1-specific MAb BE08 (54). The cell population was subsequently stained using propidium iodide (1 μg/ml) to exclude dead cells. Cells were fixed using 2% formaldehyde and analyzed using a FACSCalibur flow cytometer (BD Biosciences). The histograms plot cell number (counts) versus the fluorescence intensity of MAb binding. Background staining of mock-transfected cells is shown to identify nonexpressing cells in the transfected cell populations.

SSP rescues cell-cell fusion activity in *trans*. To determine whether the *trans*-complemented complex was also able to mediate pH-dependent membrane fusion, we cocultured cells expressing GP-C glycoproteins with Vero target cells infected with the fusion reporter vaccinia virus vCB21R-LacZ expressing the β-galactosidase gene under control of the T7 promoter (47). In this assay, activation of GP-C-mediated membrane fusion by acidic pH (5.0) results in syncytium formation between the effector and reporter cells and expression of β-galactosidase; the enzymatic activity is then monitored using a chemiluminescent substrate (64). As shown in Fig. 3, pH-dependent cell-cell fusion is readily detected using the native GP-C glycoprotein and absent in the cleavage-defective cd-GPC mutant. Cells expressing the CD4sp-GPC glycoprotein in the absence of SSP were unable to mediate cell-cell fusion

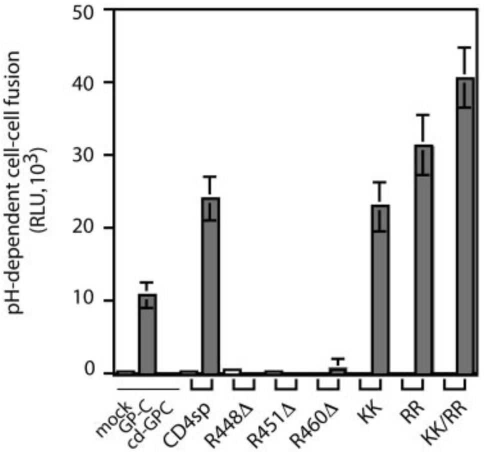

FIG. 3. pH-dependent cell-cell fusion activity. pH-dependent fusion was detected using the recombinant vaccinia virus-based β-galactosidase reporter assay (47) as previously described (64, 65). β-Galactosidase activity was quantitated using the chemiluminescent substrate GalactoLite Plus (Tropix). Relative light unit (RLU) measurements from cultures treated at pH 5.0 are shown after subtraction of background levels from neutral-pH cultures (average background, 1,500 RLU). Control conditions are shown in the underlined bars at left (mock, wild-type GP-C, and cd-GPC). Note that CD4sp-GPC constructs are bracketed in pairs (below the axis) representing the absence (open bars) and presence (gray bars) of SSP. Some bars are not discernible on the scale of the graph. All conclusions were replicated using X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining of parallel cocultures.

(Fig. 3, CD4sp, first of the bracketed pairs of bars). By contrast, coexpression of SSP reconstituted pH-dependent cell-cell fusion activity in the *trans*-complemented CD4sp-GPC complex (second of bracketed pairs of bars) to levels greater than those seen with the native GP-C glycoprotein. Thus, expression of SSP in *trans* can fully restore membrane fusion activity to the Junín virus G1-G2 precursor glycoprotein.

SSP association is required for exit from the ER. To investigate the role of SSP in the biogenesis of GP-C, we examined the intracellular localization of the complex by confocal microscopy. In these experiments, Vero cells expressing the wild-type and CD4sp-GPC glycoproteins were fixed, permeabilized, and immunochemically stained using the anti-G1 MAb BF11 (54) and an Alexa Fluor 488-conjugated secondary antibody. Nonpermeabilized cells were similarly stained to detect GP-C accumulation on the cell surface. As shown in Fig. 4, the native GP-C glycoprotein accumulated in the ER and Golgi-like perinuclear structures (GP-C, permeabilized) and on the cell surface (GP-C, surface). Localization to the Golgi apparatus was confirmed using a rabbit polyclonal antibody directed against an integral Golgi membrane protein, giantin (40), and a secondary Alexa Fluor 568-conjugated antibody. Colocalization of GP-C with the Golgi marker is visualized in yellow in the merged images. Expression of CD4sp-GPC in the presence of SSP resulted in a pattern of localization and transport to the cell surface similar to that of native GP-C (Fig. 4, CD4sp, +SSP). These findings highlight the reconstitution of the GP-C complex upon *trans* complementation with SSP.

In the absence of SSP, however, the G1-G2 precursor of CD4sp-GPC exhibited a diffuse reticulate pattern of intracellular expression consistent with retention in the ER (Fig. 4, CD4sp, −SSP). Notably absent was any concentration of GP-C staining to a morphologically defined Golgi apparatus or specific colocalization with the antigiantin MAb (merged image). The orange in the merged image likely reflects the spatial coincidence of green and red fluorescence rather than specific colocalization to a definable Golgi structure. Also absent was any staining of CD4sp-GPC on the cell surface (surface). The lack of transport to the cell surface is not due to the absence of proteolytic cleavage per se, because the cleavage-site-defective cd-GPC mutant is transported to the cell surface as the wild-type glycoprotein (not shown) (1, 35). Nor did we detect punctate staining in the ER that might suggest misfolding of the G1-G2 precursor in the absence of SSP. The difference in trafficking of the G1-G2 precursor to the Golgi in the presence or absence of SSP likely accounts for the effect of *trans* complementation on proteolytic cleavage (Fig. 2A), consistent with the activation of SKI-1/S1P protease in the *cis*-medial Golgi compartment (10, 21).

Next, we examined the role of SSP in the transport of the GP-C complex to the cell surface by using flow cytometry and the G1-specific MAb BE08. In cell cultures transiently expressing the wild-type GP-C glycoprotein, a clear population of GP-C-expressing cells was evident (Fig. 2B, top right). A comparison of cells expressing CD4sp-GPC in the presence or absence of SSP revealed that the GP-C glycoproteins were present on the cell surface only upon coexpression of SSP (bottom panels). Cell surface accumulation of the *trans*-complemented CD4sp-GPC glycoprotein was comparable to that of the native GP-C glycoprotein. Taken together, these results demonstrate that SSP is essential for GP-C transport to the Golgi and the cell surface. In the absence of SSP, the G1-G2 precursor is localized to the ER.

Transit of a CD4 chimera bearing G2 sequences. To further investigate the role of the G2 subunit in ER localization and the role of SSP in regulating transit to the cell surface, we determined whether control by SSP and the G2 subunit might be transferable to a heterologous cell surface protein. Because the ectodomain of human CD4 forms a soluble and secreted protein (11, 58), we fused the CD4 signal peptide and ectodomain to the transmembrane and cytoplasmic regions of G2. In the CD4ecto construct, the C terminus of soluble CD4 (TPV_{372}) (11) was spliced at the G2 ectodomain sequence TPL_{420}, three residues upstream of D_{424}, that nominally defines the junction with the transmembrane domain.

Cells expressing the CD4ecto chimera or native CD4 were metabolically labeled, and cell lysates were immunoprecipitated using the anti-CD4 ectodomain MAb SIM.2. The CD4ecto chimera was expressed as a 55-kDa glycoprotein that comigrated with native CD4 (Fig. 5A, left panel). Upon coexpression, SSP was found to coprecipitate with CD4ecto (Fig. 5A, left panel). This association was specific to G2 sequences in the CD4ecto glycoprotein; SSP did not bind to native CD4 (when coexpressed) (not shown). Thus, the transmembrane and cytoplasmic domains of G2 are sufficient for SSP binding.

Importantly, transport of the CD4ecto chimera through the Golgi apparatus and to the cell surface was dependent on coexpression of SSP. As shown by immunochemical staining using SIM.2 MAb and confocal microscopy (Fig. 4, CD4ecto, permeabilized), the chimeric glycoprotein was largely con-

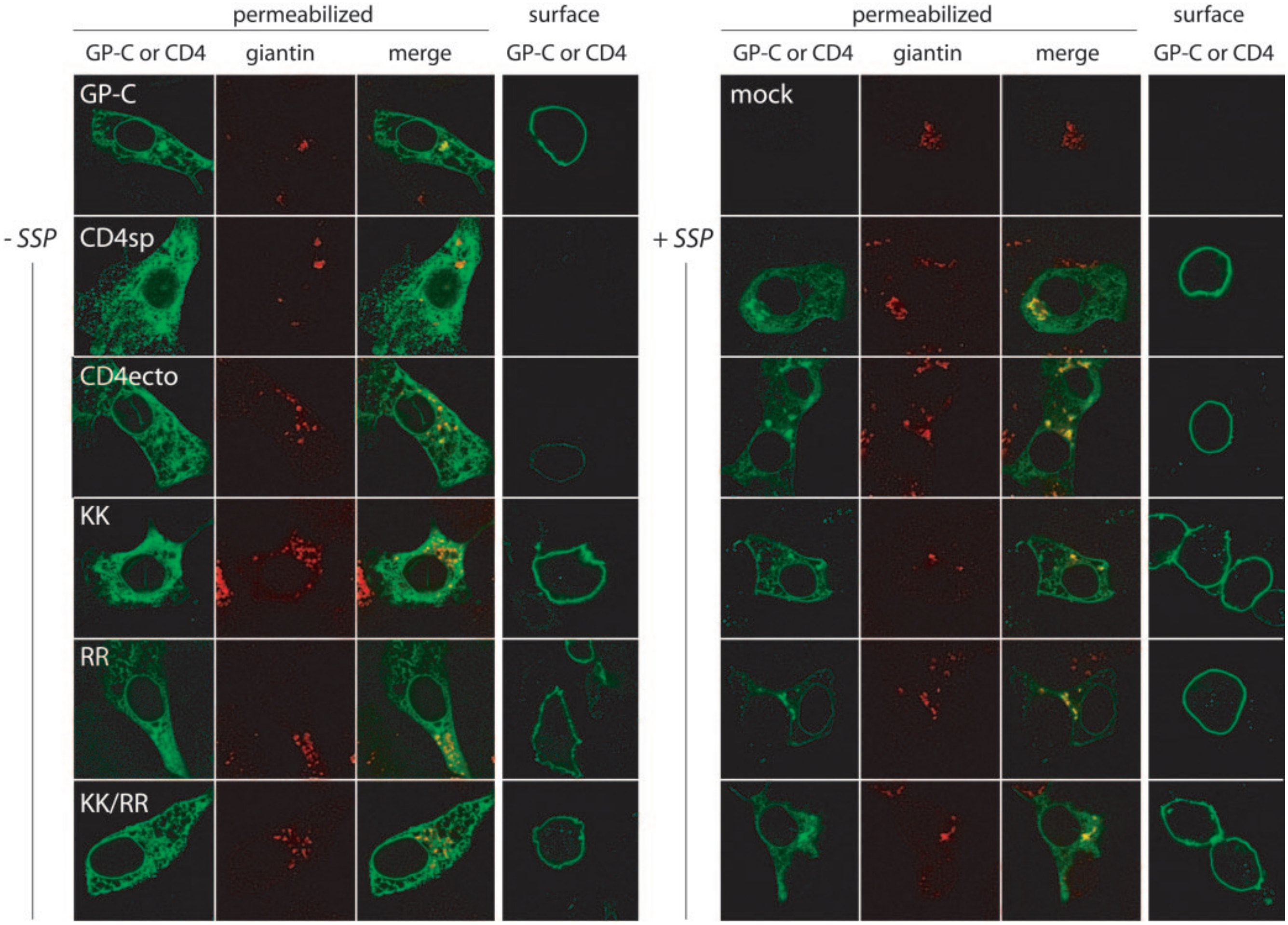

FIG. 4. Intracellular and cell surface visualization of glycoproteins. Confocal images were obtained as described in Materials and Methods. Permeabilized cells were stained in green using either the MAb BF11 (GP-C) or, for CD4ecto, SIM.2 (CD4). Golgi structures were identified using a rabbit polyclonal antiserum and stained in red. Merged images (merge) were created using Lasersharp software. Nonpermeabilized cells (surface) were stained in green using either MAb BF11 or SIM.2. The expressed glycoproteins are indicated in white letters superimposed on the leftmost images. The top row depicts cells expressing native GP-C or mock-transfected cells (all infected with the recombinant vaccinia virus vTF7-3). In subsequent rows, the glycoproteins were expressed either in the absence (−SSP) or presence (+SSP) of SSP. In some images, the Golgi apparatus is vesiculated and dispersed, perhaps due to infection of the cells by vaccinia virus.

strained to the ER in the absence of SSP and failed to colocalize with the Golgi apparatus (−SSP). In addition, only trace amounts of the CD4ecto glycoprotein were detected in the absence of SSP on the cell surface, either through confocal microscopy (Fig. 4, surface) or flow cytometry (Fig. 5B, −SSP). Thus, fusion to the G2 transmembrane and cytoplasmic domains prevented transport of the CD4 ectodomain from the ER.

By contrast, coexpression with SSP resulted in significant localization of CD4ecto in the Golgi (Fig. 4, +SSP) and expression on the cell surface (surface). Mobilization of the chimeric glycoprotein by SSP was confirmed by flow cytometry (Fig. 5B, +SSP). Furthermore, immunoprecipitation studies of CD4ecto expression on the cell surface (Fig. 5A, right panel) identified the surface moiety as the complex of CD4ecto and SSP. Together, these findings demonstrated that the essential elements of ER localization and its control by SSP binding can be recapitulated in a chimeric CD4ecto glycoprotein bearing the transmembrane and cytoplasmic domains of G2.

Analysis of C-terminal truncations in the G2 cytoplasmic domain. Among transmembrane proteins that are retained in the ER, specific localization signals are often encoded within the cytoplasmic domain (references 22, 37, and 62 and references therein). In order to define the determinants in G2 that are required for ER localization, we constructed a series of C-terminal truncations in the cytoplasmic domain of G2. Three arginine residues, spaced 4, 7, and 17 amino acids from the nominal transmembrane domain, were used as endpoints in the truncations (Fig. 1). These positively charged termini were chosen to facilitate anchoring of the truncated CD4sp-GPC glycoprotein in the membrane. The arginine codons were fused to those encoding an S-peptide affinity tag (34) to facilitate analysis of the G2 moiety (65). Metabolically labeled glycoprotein was isolated using the Spep affinity tag and S-protein agarose (Novagen). The truncated CD4sp-GPC glycoproteins (R448Δ, R451Δ, and R460Δ) were well expressed in Vero cells yet failed to coprecipitate significant amounts of SSP (Fig. 6A, top panel). Nonetheless, all three truncated glycoproteins were

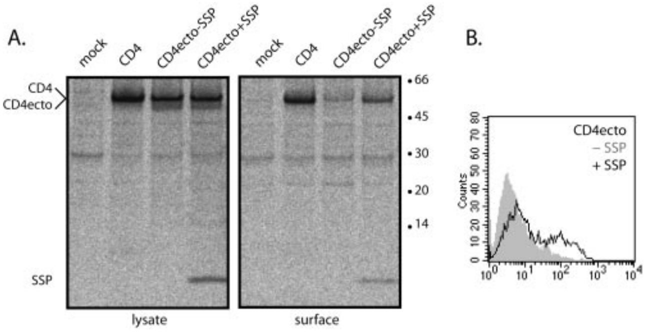

FIG. 5. The chimeric CD4 glycoprotein bearing the transmembrane and cytoplasmic domains of G2 requires SSP for transport to the cell surface. (A) The chimeric CD4ecto construct was expressed alone (−SSP) or with SSP (+SSP) and metabolically labeled. Intact cells were incubated with the anti-CD4 MAb SIM.2 (43, 48) and the cell surface glycoproteins were subsequently isolated from cleared cell lysates using protein A-Sepharose (surface). Intracellular CD4ecto glycoprotein was immunoprecipitated from the post-protein A-Sepharose supernatant using additional SIM.2 MAb (lysate). Mock- and human CD4-transfected cells served as controls. Molecular size markers (in kilodaltons) are as described in the legend to Fig. 2A. (B) Flow cytometry using SIM.2 MAb was otherwise performed as described in the legend to Fig. 2B, and results are plotted similarly. The filled gray (−SSP) and open (+SSP) histograms are overlaid.

subjected to SKI-1/S1P cleavage, in the presence or absence of SSP, to produce truncated and affinity-tagged G2 moieties (Fig. 6A, bottom panel). The relative migrations of the truncated G2 polypeptides correspond to their expected molecular weights but cause them to overlap with the intact G1 polypeptide. The association between G1 and the truncated G2 subunits was separately confirmed by coimmunoprecipitation using a MAb directed to G1 (not shown). By contrast, similar truncations in G2 of the Old World LCM virus were reported to prevent SKI-1/S1P cleavage (35).

Flow cytometry was used to determine whether the truncated Junín virus glycoproteins were also transported to the cell surface without SSP. As shown in Fig. 6B, all three truncation mutants were expressed on the cell surface in the absence of SSP, at levels comparable to the *trans*-complemented CD4sp-GPC glycoprotein (Fig. 2B). Truncations in the context of CD4ecto likewise enabled transport from the ER (not shown). In the LCM virus (35), the truncated GP-C was also expressed on the cell surface. Taken together, these results suggest that amino acid sequences within the cytoplasmic domain of G2 are important in constraining the G1-G2 precursor to the ER. The cytoplasmic region is also important for SSP association.

We have demonstrated that GP-C glycoproteins bearing truncations in the cytoplasmic domain of G2 can be proteolytically processed and transported to the cell surface in the absence of SSP. Surprisingly, however, none of the truncated complexes was able to mediate pH-dependent cell-cell fusion (Fig. 3). It is possible that this failure may be due to insufficient cleavage or transport of the truncated glycoproteins. Alternatively, the failure may reflect a requirement for either SSP or the cytoplasmic domain of G2 for membrane fusion activity.

Dibasic amino acid sequences participate in ER localization. Sequence analysis of the G2 cytoplasmic domain revealed conserved motifs that may be involved in protein trafficking and ER localization. In particular, dibasic amino acid sequences such as the canonical KKXX and RXR motifs are widely utilized in the retrieval of transmembrane proteins to the ER (see references 22, 37, and 62 and references therein). The cytoplasmic domain of Junín virus G2 contains two related dibasic sequences: KKPT$_{479}$ and a C-terminal RRGH$_{485}$. Variants of these sequences appear in other arenavirus G2 proteins (Fig. 1). To assess the potential role of these sequences in ER localization, we mutated the two basic amino acids at each site to alanines, both individually (KK and RR glycoproteins) and as the double mutant (KK/RR).

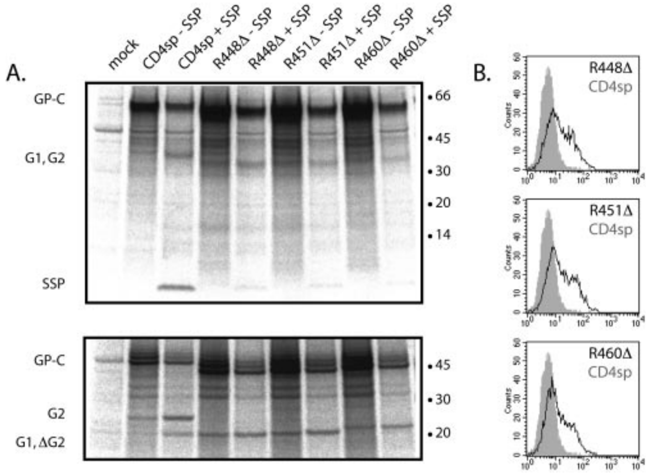

FIG. 6. Truncations to the cytoplasmic domain of G2 ablate SSP binding yet enable transport to the cell surface. (A) The wild-type and truncated CD4sp-GPC glycoproteins (R448Δ, R451Δ, and R460Δ) were expressed alone (−SSP) or with SSP (+SSP). Metabolically labeled glycoproteins were precipitated using the C-terminal Spep affinity tag and S-protein agarose (Novagen) and analyzed as described in the legend to Fig. 2. The G1 and G2 glycoproteins are best resolved following deglycosylation with PNGase F (bottom). Note that the truncated G2 moieties (ΔG2) migrate near the wild-type G1 polypeptide; coassociation between G1 and ΔG2 was formally demonstrated by immunoprecipitation using anti-G1 MAb BF11, which coprecipitated ΔG2 (not shown). Although coprecipitation of SSP was markedly reduced with the truncated glycoproteins, trace amounts could be discerned upon darkening of the image (not shown). This low level of SSP association is judged to be insignificant, as the properties of the truncated glycoproteins are independent of SSP coexpression. Molecular size markers (in kilodaltons) are as described in the legend to Fig. 2A. (B) Cell surface expression of the truncated glycoproteins was determined by flow cytometry as described in the legend to Fig. 2B, and results are plotted similarly. Note that expression of the wild-type CD4sp-GPC glycoprotein (gray histograms in all three panels) is compared with that of the truncations (open histograms), all in the absence of SSP.

Immunoprecipitation studies of metabolically labeled whole-cell lysates revealed that all of the mutant CD4sp-GPC glycoproteins were able to associate with SSP (Fig. 7A, top panel). Neither of the dibasic sequences was essential for SSP binding. *trans* complementation with SSP enabled wild-type levels of cell surface expression (Fig. 7B, +SSP) and efficient pH-dependent cell-cell fusion (Fig. 3), arguing against significant adverse effects of the mutations on overall protein folding.

In the absence of SSP, importantly, both the single and double mutants were now capable of transport to the cell

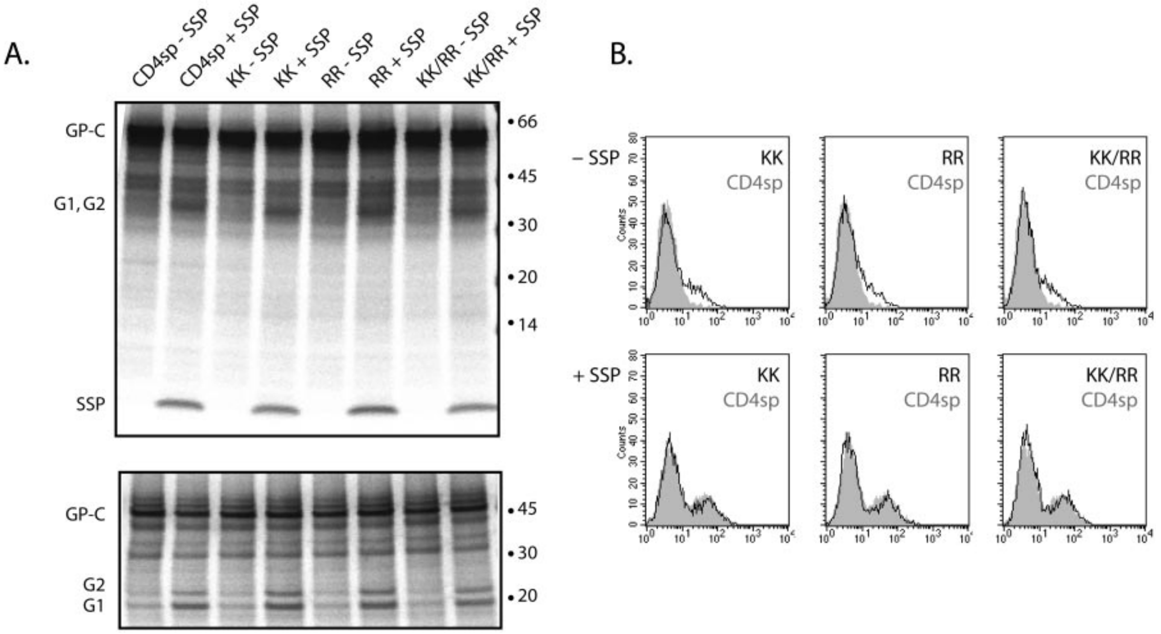

FIG. 7. Alanine mutations to dibasic amino acid motifs enable transport from the ER. (A) CD4sp-GPC constructs containing KK, RR, and double KK/RR mutations were expressed alone (−SSP) or with SSP (+SSP). Metabolically labeled glycoproteins were immunoprecipitated and analyzed as described in the legend to Fig. 2A. Molecular size markers (in kilodaltons) are as described in the legend to Fig. 2A. (B) Flow cytometry was performed as described in the legend to Fig. 2B, and results are plotted similarly. Note that the top panels compare expression of the CD4sp-GPC glycoprotein (gray histograms in all panels) with that of the mutants (open histograms) in the absence of SSP. Expression with SSP is shown in the bottom panels.

surface. This phenotype was evident upon confocal microscopic analysis of nonpermeabilized cells (Fig. 4, surface), although specific localization in the Golgi was difficult to discern (green and merged images). Flow-cytometric studies of cell surface expression indicated that both the single and double mutations provided modest, albeit significant, relief of ER retention (Fig. 7B, −SSP). Evidence for enhanced SKI-1/S1P cleavage of the mutant glycoproteins was, however, difficult to discern in whole-cell lysates, above the residual level of cleavage in the wild-type glycoprotein (Fig. 7A, bottom panel). It is possible that the cleaved species in the wild-type G1-G2 glycoprotein reflect transient residence in the Golgi, prior to retrieval to the ER. In the Old World Lassa fever virus glycoprotein, where cleaved products are not observed in the absence of SSP (18), retrieval of the G1-G2 precursor may be more rapid. Nonetheless, mobilization of the mutant glycoproteins to the cell surface was consistently observed and distinct from the strict intracellular retention seen with the wild-type glycoprotein. Both KK and RR mutations appeared to be comparably efficacious, and no synergy was observed in the double KK/RR mutant. However, none of the mutant glycoproteins was able to mediate cell-cell fusion in the absence of SSP (Fig. 3). This defect is not attributable to the amino acid substitutions per se, as wild-type levels of fusion were restored upon *trans* complementation with SSP.

To confirm that the mutations are sufficient for significant mobilization of the G1-G2 precursor in the absence of SSP, we examined the glycoprotein by immunoprecipitation from the cell surface (Fig. 8). These experiments confirmed significant expression of the dibasic sequence mutants on the cell surface and demonstrated a preponderance of the proteolytically processed G1-G2 complex, reflecting access to the SKI-1/S1P protease in the Golgi. The efficiency of cleavage in the mutant glycoproteins was relatively unaffected by the presence or absence of SSP (60% cleaved versus 40% cleaved, respectively).

Taken together, these studies identify the two dibasic amino

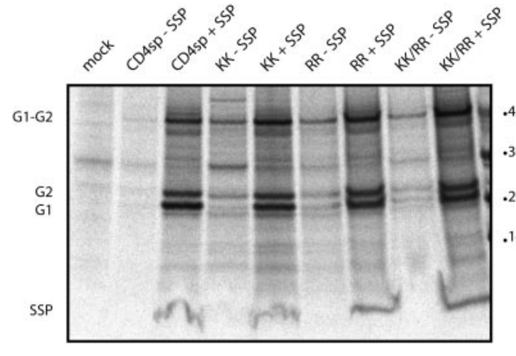

FIG. 8. Cell surface expression of dibasic amino acid motif CD4sp-GPC mutants. Intact cells expressing the constructs shown in Fig. 7 were incubated with the G1-specific MAb BE08, and the cell surface GP-C glycoproteins were isolated from cleared cell lysates using protein A-Sepharose and deglycosylated. The relative amounts of G1, G2, and G1-G2 precursor in each lane were quantitated from the phosphorimage using Image Gauge software (Fuji), and the efficiency of cleavage was determined as the sum of G1 plus G2 relative to total of all forms. Distortion of the SSP band is due to the detergents used in PNGase F treatment. Molecular size markers (in kilodaltons) are as described in the legend to Fig. 2A.

acid sequences (KKPT$_{479}$ and RRGH$_{485}$) as important determinants of ER localization in the absence of SSP. Alanine mutations at either or both of these sites result in partial relief from ER retention and enable transport to the cell surface in the absence of SSP. On the other hand, these mutations do not completely obviate the requirement for SSP association in transport of the G1-G2 precursor (Fig. 8). Quantitative analysis of the glycoproteins indicated that, whereas the mutations were able to increase cell surface expression at least 10-fold, coexpression of SSP resulted in an additional 10-fold increase in all mutants, to the levels of the wild-type glycoprotein. These findings are consistent with our results from confocal microscopy and flow cytometry studies (Fig. 4 and 7). Thus, constraints on the trafficking of the G1-G2 precursor include the dibasic sequence motifs in the cytoplasmic domain of G2 but also involve additional structural elements provided upon full assembly with SSP.

DISCUSSION

The regulation of trafficking through intracellular membranous compartments is central to the biogenesis of membrane glycoproteins (15, 22). Quality control mechanisms for protein folding and assembly are proposed to operate through checkpoints on exit from the ER and through bidirectional transport to and from the Golgi apparatus. Viruses make use of these cellular pathways in the biosynthesis, assembly, and release of new virion particles (15). In our studies, we have characterized the biogenesis of the arenavirus envelope glycoprotein and the requirement for tripartite assembly to enable transport of the GP-C complex from the ER. Without the association of SSP, the wild-type G1-G2 precursor remains localized to the ER. We show that localization is mediated by the cytoplasmic domain of G2 and that the control of trafficking by SSP association is transferable to a chimeric CD4 molecule bearing the G2 transmembrane and cytoplasmic domains. Conversely, regulation of intracellular transport of the GP-C complex does not require G1 or the ectodomain of G2.

Our studies demonstrate that ER localization is mediated in part through dibasic amino acid sequences in the cytoplasmic domain of G2. Alanine mutations to either of two dibasic motifs provide partial relief from ER localization and enable expression of the proteolytically cleaved G1-G2 complex on the cell surface. Upon exit from the ER and transit through the Golgi, the mutant G1-G2 precursor is now fully susceptible to proteolytic maturation by SKI-1/S1P protease. Thus, absent ER localization signals, the arenavirus GP-C precursor can undergo proteolytic maturation much as do the precursor glycoproteins of other class I viral fusion proteins.

Dibasic amino acid sequences are known to mediate ER localization through retrograde transport (retrieval) from the Golgi (references 22, 37, and 62 and references therein). The specific dibasic sequences we have identified as important for ER localization in the Junín virus G2 glycoprotein do not match precisely either of the canonical ER retrieval motifs: the C-terminal KKXX or internal RXR sequences. Although the internal KK sequence studied here is conserved among the New World arenaviruses, the C-terminal RRXX sequence shows considerable variation (Fig. 1). Among the Old World viruses, only the C-terminal motif is identifiable. However, variants to the canonical motifs are also common in other ER-localized transmembrane proteins (46, 55, 62) and the efficiency of retention by these sequences is often highly context dependent (26, 57, 66). Many details regarding the mechanisms and molecular determinants involved in ER-Golgi trafficking remain unresolved.

It is noteworthy that a viral envelope glycoprotein destined for the cell surface should encode an ER localization signal. For cellular transmembrane proteins that traverse the Golgi and beyond, dibasic ER localization motifs are commonly found to control the assembly and trafficking of heteromultimeric membrane protein complexes (12, 33, 39, 42, 67; reviewed in references 22 and 46). These endogenous signals prevent transport of the individual subunits and are overcome upon assembly of the multimeric complex. This quality control mechanism ensures that only the fully and properly assembled complex is transported from the ER. In the biogenesis of the Junín virus GP-C complex, we propose an analogous role for SSP association—namely, to mask endogenous ER localization signals in the cytoplasmic domain of G2 and thus enable transport of only the fully assembled tripartite complex.

This strategy for assembly-dependent control of viral envelope glycoprotein trafficking is likely not unique to the arenaviruses. The bunyavirus G$_C$ glycoprotein also contains a noncanonical basic amino acid cluster that may be involved in ER localization (29). In these viruses, transport of G$_C$ from the ER requires association with a second envelope glycoprotein, G$_N$ (30, 36), which in turn retains the G$_C$-G$_N$ complex in the Golgi (27, 29, 56), the site of virus budding. Together, these observations highlight the use of cellular ER-Golgi trafficking mechanisms during the viral life cycle to control the assembly and transport of multimeric envelope glycoprotein complexes.

Despite mutations that enable the transport of the G1-G2 complex in the absence of SSP, wild-type levels of trafficking were not restored by point mutations to the dibasic amino acid sequences or by truncations in the cytoplasmic domain (not shown). It is possible that additional constraints on GP-C transport lie within the transmembrane domain of G2. Moreover, it is likely that the association with SSP remains essential for the integrity of the GP-C complex. The SSP subunit has uniquely evolved within the arenaviruses for purposes other than simply to relieve ER retention of an envelope glycoprotein precursor. It is telling, then, that despite the accumulation of cleaved G1-G2 complex on the cell surface, none of the glycoproteins lacking SSP is able to mediate membrane fusion (Fig. 3). Notably, GP-C glycoproteins bearing mutations at the dibasic amino acid motifs are unable to promote fusion in the absence of SSP yet are restored to full activity by coexpression of SSP. This defect in fusion is likely not due to the lower levels of cell surface glycoprotein in the absence of SSP, as robust fusion is observed with comparably low levels of cleaved wild-type glycoprotein (see Fig. 6 of reference 64). Rather, we suggest that SSP may be directly involved in modulating pH-dependent membrane fusion by the GP-C complex.

In addition, the G1-G2 complex lacking SSP is not myristoylated. GP-C complexes in which myristoylation is blocked by a G2A mutation are less able to mediate cell-cell fusion than the wild-type glycoprotein (65), perhaps due to alterations in trafficking to specific membrane microdomains (52, 61). The G2A glycoprotein, however, retains 30% of the wild-type fusion activity, significantly more than the present G1-G2 complexes in the absence of SSP. This comparison suggests defects beyond the lack of acylation in G1-G2 complexes lacking SSP. Separately, myristoylation may also be important during virion assembly in facilitating the colocalization of GP-C with the myristoylated Z matrix protein (50).

Further studies will no doubt delineate the additional roles of the unique SSP subunit in the arenavirus life cycle. Unique solutions embodied in the assembly, trafficking, and membrane fusion activity of the arenavirus GP-C complex may suggest novel approaches for intervention towards the prevention and treatment of arenavirus hemorrhagic fevers.

ACKNOWLEDGMENTS

We are grateful to Victor Romanowski (Universidad Nacional de La Plata, Argentina) for his interest and expertise in initiating our collaborative studies of the Junín virus and to Tony Sanchez and Tom Ksiazek (Centers for Disease Control and Prevention, Atlanta, Georgia) for providing Junín virus GP-C MAbs. Additional reagents were obtained from the NIH AIDS Research and Reference Reagent Program through the contributions of T. Fuerst, B. Moss, C. Broder, P. Kennedy, E. Berger, R. Axel, and J. Hildreth. We also thank Lou Herritt for her technical assistance in confocal microscopy at the Molecular Histology and Fluorescence Imaging Core (D. Lurie, Director). Finally, we thank Meg Trahey for useful discussions during the course of the work and for editorial comments.

Confocal microscopy was supported by NIH COBRE grants RR01760 and RR15583. The work was supported by NIH research grant AI059355.

REFERENCES

1. **Beyer, W. R., D. Pöpplau, W. Garten, D. von Laer, and O. Lenz.** 2003. Endoproteolytic processing of the lymphocytic choriomeningitis virus glycoprotein by the subtilase SKI-1/S1P. J. Virol. **77:**2866–2872.
2. **Borrow, P., and M. B. A. Oldstone.** 1994. Mechanism of lymphocytic choriomeningitis virus entry into cells. Virology **198:**1–9.
3. **Brown, M. S., and J. L. Goldstein.** 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc. Natl. Acad. Sci. USA **96:**11041–11048.
4. **Buchmeier, M. J.** 2002. Arenaviruses: protein structure and function. Curr. Top. Microbiol. Immunol. **262:**159–173.
5. **Buchmeier, M. J., M. D. Bowen, and C. J. Peters.** 2001. Arenaviruses and their replication, p. 1635–1668. *In* D. M. Knipe and P. M. Howley (ed.), Fields virology, vol. 2. Lippincott Williams & Wilkins, Philadelphia, Pa.
6. **Cao, W., M. D. Henry, P. Borrow, H. Yamada, J. H. Elder, E. V. Ravkov, S. T. Nichol, R. W. Compans, K. P. Campbell, and M. B. A. Oldstone.** 1998. Identification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus. Science **282:**2079–2081.
7. **Castilla, V., S. E. Mersich, N. A. Candurra, and E. B. Damonte.** 1994. The entry of Junin virus into Vero cells. Arch. Virol. **136:**363–374.
8. **Centers for Disease Control and Prevention.** 2005. Lymphocytic choriomeningitis virus infection in organ transplant recipients–Massachusetts, Rhode Island, 2005. Morb. Mortal. Wkly. Rep. **54:**537–539.
9. **Clegg, J. C. S., M. D. Bowen, M. J. Buchmeier, J.-P. Gonzalez, I. S. Lukashevich, C. J. Peters, R. Rico-Hesse, and V. Romanowski.** 2000. Arenaviridae, p. 633–640. *In* M. H. V. van Regenmortel, C. M. Fauquet, D. H. L. Bishop, E. B. Carstens, M. K. Estes, S. M. Lemon, J. Maniloff, M. A. Mayo, D. J. McGeoch, C. R. Pringle, and R. B. Wickner (ed.), Virus taxonomy: seventh report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, Calif.
10. **DeBose-Boyd, R. A., M. S. Brown, W. P. Li, A. Nohturfft, J. L. Goldstein, and P. J. Espenshade.** 1999. Transport-dependent proteolysis of SREBP: relocation of site-1 protease from Golgi to ER obviates the need for SREBP transport to Golgi. Cell **99:**703–712.
11. **Deen, K. C., J. S. McDougal, R. Inacker, G. Folena-Wasserman, J. Arthos, J. Rosenberg, P. J. Maddon, R. Axel, and R. W. Sweet.** 1988. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. Nature **331:**82–84.
12. **Delgado, P., and B. Alarcon.** 2005. An orderly inactivation of intracellular retention signals controls surface expression of the T cell antigen receptor. J. Exp. Med. **201:**555–566.
13. **Di Simone, C., and M. J. Buchmeier.** 1995. Kinetics and pH dependence of acid-induced structural changes in the lymphocytic choriomeningitis virus glycoprotein complex. Virology **209:**3–9.
14. **Di Simone, C., M. A. Zandonatti, and M. J. Buchmeier.** 1994. Acidic pH triggers LCMV membrane fusion activity and conformational change in the glycoprotein spike. Virology **198:**455–465.
15. **Doms, R. W., R. A. Lamb, J. K. Rose, and A. Helenius.** 1993. Folding and assembly of viral membrane proteins. Virology **193:**545–546.
16. **Earp, L. J., S. E. Delos, H. E. Park, and J. M. White.** 2005. The many mechanisms of viral membrane fusion proteins, p. 25–66. *In* M. Marsh (ed.), Membrane trafficking in viral replication, vol. 285. Springer Verlag, New York, N.Y.
17. **Eckert, D. M., and P. S. Kim.** 2001. Mechanisms of viral membrane fusion and its inhibition. Annu. Rev. Biochem. **70:**777–810.
18. **Eichler, R., O. Lenz, T. Strecker, M. Eickmann, H. D. Klenk, and W. Garten.** 2003. Identification of Lassa virus glycoprotein signal peptide as a trans-acting maturation factor. EMBO Rep. **4:**1084–1088.
19. **Eichler, R., O. Lenz, T. Strecker, M. Eickmann, H. D. Klenk, and W. Garten.** 2004. Lassa virus glycoprotein signal peptide displays a novel topology with an extended ER-luminal region. J. Biol. Chem. **279:**12293–12299.
20. **Eichler, R., O. Lenz, T. Strecker, and W. Garten.** 2003. Signal peptide of Lassa virus glycoprotein GP-C exhibits an unusual length. FEBS Lett. **538:**203–206.
21. **Elagoz, A., S. Benjannet, A. Mammarbassi, L. Wickham, and N. G. Seidah.** 2002. Biosynthesis and cellular trafficking of the convertase SKI-1/S1P: ectodomain shedding requires SKI-1 activity. J. Biol. Chem. **277:**11265–11275.
22. **Ellgaard, L., and A. Helenius.** 2003. Quality control in the endoplasmic reticulum. Nat. Rev. Mol. Cell Biol. **4:**181–191.
23. **Froeschke, M., M. Basler, M. Groettrup, and B. Dobberstein.** 2003. Long-lived signal peptide of lymphocytic choriomeningitis virus glycoprotein pGP-C. J. Biol. Chem. **278:**41914–41920.
24. **Fuerst, T. R., E. G. Niles, F. W. Studier, and B. Moss.** 1986. Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. Proc. Natl. Acad. Sci. USA **83:**8122–8126.
25. **Gallaher, W. R., C. DiSimone, and M. J. Buchmeier.** 2001. The viral transmembrane superfamily: possible divergence of Arenavirus and Filovirus glycoproteins from a common RNA virus ancestor. BMC Microbiol. **1:**1.
26. **Gassmann, M., C. Haller, Y. Stoll, S. A. Aziz, B. Biermann, J. Mosbacher, K. Kaupmann, and B. Bettler.** 2005. The RXR-type endoplasmic reticulum-retention/retrieval signal of GABAB1 requires distant spacing from the membrane to function. Mol. Pharmacol. **68:**137–144.
27. **Gerrard, S. R., and S. T. Nichol.** 2002. Characterization of the Golgi retention motif of Rift Valley fever virus G_N glycoprotein. J. Virol. **76:**12200–12210.
28. **Ghiringhelli, P. D., R. V. Rivera-Pomar, M. E. Lozano, O. Grau, and V. Romanowski.** 1991. Molecular organization of Junin virus S RNA: complete nucleotide sequence, relationship with other members of the Arenaviridae and unusual secondary structures. J. Gen. Virol. **72:**2129–2141.
29. **Haferkamp, S., L. Fernando, T. F. Schwarz, H. Feldmann, and R. Flick.** 2005. Intracellular localization of Crimean-Congo Hemorrhagic Fever (CCHF) virus glycoproteins. Virol. J. **2:**42.
30. **Hobman, T. C., L. Woodward, and M. G. Farquhar.** 1992. The rubella virus E1 glycoprotein is arrested in a novel post-ER, pre-Golgi compartment. J. Cell Biol. **118:**795–811.
31. **Hruby, D. E., D. L. Lynn, and J. R. Kates.** 1980. Identification of a virus-specified protein in the nucleus of vaccinia virus-infected cells. J. Gen. Virol. **47:**293–299.
32. **Hughson, F. M.** 1997. Enveloped viruses: a common mode of membrane fusion? Curr. Biol. **7:**R565–R569.
33. **Khalil, H., A. Brunet, I. Saba, R. Terra, R. P. Sekaly, and J. Thibodeau.** 2003. The MHC class II beta chain cytoplasmic tail overcomes the invariant chain p35-encoded endoplasmic reticulum retention signal. Int. Immunol. **15:**1249–1263.
34. **Kim, J.-S., and R. T. Raines.** 1993. Ribonuclease S-peptide as a carrier in fusion proteins. Protein Sci. **2:**348–356.
35. **Kunz, S., K. H. Edelmann, J.-C. de la Torre, R. Gorney, and M. B. A. Oldstone.** 2003. Mechanisms for lymphocytic choriomeningitis virus glycoprotein cleavage, transport, and incorporation into virions. Virology **314:**168–178.
36. **Lappin, D. F., G. W. Nakitare, J. W. Palfreyman, and R. M. Elliott.** 1994. Localization of Bunyamwera bunyavirus G1 glycoprotein to the Golgi requires association with G2 but not with NSm. J. Gen. Virol. **75:**3441–3451.
37. **Lee, M. C., E. A. Miller, J. Goldberg, L. Orci, and R. Schekman.** 2004. Bi-directional protein transport between the ER and Golgi. Annu. Rev. Cell Dev. Biol. **20:**87–123.
38. **Lenz, O., J. ter Meulen, H. Feldmann, H.-D. Klenk, and W. Garten.** 2000. Identification of a novel consensus sequence at the cleavage site of the Lassa virus glycoprotein. J. Virol. **74:**11418–11421.
39. **Letourneur, F., S. Hennecke, C. Demolliere, and P. Cosson.** 1995. Steric masking of a dilysine endoplasmic reticulum retention motif during assembly of the human high affinity receptor for immunoglobulin E. J. Cell Biol. **129:**971–978.
40. **Linstedt, A. D., and H. P. Hauri.** 1993. Giantin, a novel conserved Golgi

membrane protein containing a cytoplasmic domain of at least 350 kDa. Mol. Biol. Cell **4:**679–693.

41. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47:**333–348.
42. **Margeta-Mitrovic, M., Y. N. Jan, and L. Y. Jan.** 2000. A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron **27:**97–106.
43. **McCallus, D. E., K. E. Ugen, A. I. Sato, W. V. Williams, and D. B. Weiner.** 1992. Construction of a recombinant bacterial human CD4 expression system producing a bioactive CD4 molecule. Viral Immunol. **5:**163–172.
44. **McCormick, J. B., and S. P. Fisher-Hoch.** 2002. Lassa fever. Curr. Top. Microbiol. Immunol. **262:**75–109.
45. **McCormick, J. B., P. A. Webb, J. W. Krebs, K. M. Johnson, and E. S. Smith.** 1987. A prospective study of the epidemiology and ecology of Lassa fever. J. Infect. Dis. **155:**437–444.
46. **Michelsen, K., H. Yuan, and B. Schwappach.** 2005. Hide and run. Arginine-based endoplasmic-reticulum-sorting motifs in the assembly of heteromultimeric membrane proteins. EMBO Rep. **6:**717–722.
47. **Nussbaum, O., C. C. Broder, and E. A. Berger.** 1994. Fusogenic mechanisms of enveloped-virus glycoproteins analyzed by a novel recombinant vaccinia virus-based assay quantitating cell fusion-dependent reporter gene activation. J. Virol. **68:**5411–5422.
48. **Oravecz, T., and M. A. Norcross.** 1993. Costimulatory properties of the human CD4 molecule: enhancement of CD3-induced T cell activation by human immunodeficiency virus type 1 through viral envelope glycoprotein gp120. AIDS Res. Hum. Retrovir. **9:**945–955.
49. **Perez, M., R. C. Craven, and J. C. de la Torre.** 2003. The small RING finger protein Z drives arenavirus budding: implications for antiviral strategies. Proc. Natl. Acad. Sci. USA **100:**12978–12983.
50. **Perez, M., D. L. Greenwald, and J. C. de La Torre.** 2004. Myristoylation of the RING finger Z protein is essential for arenavirus budding. J. Virol. **78:**11443–11448.
51. **Peters, C. J.** 2002. Human infection with arenaviruses in the Americas. Curr. Top. Microbiol. Immunol. **262:**65–74.
52. **Resh, M. D.** 1999. Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim. Biophys. Acta **1451:**1–16.
53. **Salazar-Bravo, J., L. A. Ruedas, and T. L. Yates.** 2002. Mammalian reservoirs of arenaviruses. Curr. Top. Microbiol. Immunol. **262:**25–63.
54. **Sanchez, A., D. Y. Pifat, R. H. Kenyon, C. J. Peters, J. B. McCormick, and M. P. Kiley.** 1989. Junin virus monoclonal antibodies: characterization and cross-reactivity with other arenaviruses. J. Gen. Virol. **70:**1125–1132.
55. **Scott, M., G. Lu, M. Hallett, and D. Y. Thomas.** 2004. The Hera database and its use in the characterization of endoplasmic reticulum proteins. Bioinformatics **20:**937–944.
56. **Shi, X., D. F. Lappin, and R. M. Elliott.** 2004. Mapping the Golgi targeting and retention signal of Bunyamwera virus glycoproteins. J. Virol. **78:**10793–10802.
57. **Shikano, S., and M. Li.** 2003. Membrane receptor trafficking: evidence of proximal and distal zones conferred by two independent endoplasmic reticulum localization signals. Proc. Natl. Acad. Sci. USA **100:**5783–5788.
58. **Smith, D. H., R. A. Byrn, S. A. Marsters, T. Gregory, J. E. Groopman, and D. J. Capon.** 1987. Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. Science **238:**1704–1707.
59. **Spiropoulou, C. F., S. Kunz, P. E. Rollin, K. P. Campbell, and M. B. A. Oldstone.** 2002. New World arenavirus clade C, but not clade A and B viruses, utilizes α-dystroglycan as its major receptor. J. Virol. **76:**5140–5146.
60. **Strecker, T., R. Eichler, J. ter Meulen, W. Weissenhorn, H. D. Klenk, W. Garten, and O. Lenz.** 2003. Lassa virus Z protein is a matrix protein sufficient for the release of virus-like particles. J. Virol. **77:**10700–10705.
61. **Takeda, M., G. P. Leser, C. J. Russell, and R. A. Lamb.** 2003. Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. Proc. Natl. Acad. Sci. USA **100:**14610–14617.
62. **Teasdale, R. D., and M. R. Jackson.** 1996. Signal-mediated sorting of membrane proteins between the endoplasmic reticulum and the golgi apparatus. Annu. Rev. Cell Dev. Biol. **12:**27–54.
63. **Weissenhorn, W., A. Dessen, L. J. Calder, S. C. Harrison, J. J. Skehel, and D. C. Wiley.** 1999. Structural basis for membrane fusion by enveloped viruses. Mol. Membr. Biol. **16:**3–9.
64. **York, J., S. S. Agnihothram, V. Romanowski, and J. H. Nunberg.** 2005. Genetic analysis of heptad-repeat regions in the G2 fusion subunit of the Junin arenavirus envelope glycoprotein. Virology **343:**267–279.
65. **York, J., V. Romanowski, M. Lu, and J. H. Nunberg.** 2004. The signal peptide of the Junín arenavirus envelope glycoprotein is myristoylated and forms an essential subunit of the mature G1-G2 complex. J. Virol. **78:**10783–10792.
66. **Zerangue, N., M. J. Malan, S. R. Fried, P. F. Dazin, Y. N. Jan, L. Y. Jan, and B. Schwappach.** 2001. Analysis of endoplasmic reticulum trafficking signals by combinatorial screening in mammalian cells. Proc. Natl. Acad. Sci. USA **98:**2431–2436.
67. **Zerangue, N., B. Schwappach, Y. N. Jan, and L. Y. Jan.** 1999. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. Neuron **22:**537–548.

JOURNAL OF VIROLOGY, Feb. 2007, p. 2056–2064 Vol. 81, No. 4
0022-538X/07/$08.00+0 doi:10.1128/JVI.01911-06

Synergistic Attenuation of Vesicular Stomatitis Virus by Combination of Specific G Gene Truncations and N Gene Translocations▽

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Received 1 September 2006/Accepted 23 November 2006

A variety of rational approaches to attenuate growth and virulence of vesicular stomatitis virus (VSV) have been described previously. These include gene shuffling, truncation of the cytoplasmic tail of the G protein, and generation of noncytopathic M gene mutants. When separately introduced into recombinant VSV (rVSV), these mutations gave rise to viruses distinguished from their "wild-type" progenitor by diminished reproductive capacity in cell culture and/or reduced cytopathology and decreased pathogenicity in vivo. However, histopathology data from an exploratory nonhuman primate neurovirulence study indicated that some of these attenuated viruses could still cause significant levels of neurological injury. In this study, additional attenuated rVSV variants were generated by combination of the above-named three distinct classes of mutation. The resulting combination mutants were characterized by plaque size and growth kinetics in cell culture, and virulence was assessed by determination of the intracranial (IC) 50% lethal dose (LD_{50}) in mice. Compared to virus having only one type of attenuating mutation, all of the mutation combinations examined gave rise to virus with smaller plaque phenotypes, delayed growth kinetics, and 10- to 500-fold-lower peak titers in cell culture. A similar pattern of attenuation was also observed following IC inoculation of mice, where differences in LD_{50} of many orders of magnitude between viruses containing one and two types of attenuating mutation were sometimes seen. The results show synergistic rather than cumulative increases in attenuation and demonstrate a new approach to the attenuation of VSV and possibly other viruses.

Vesicular stomatitis virus (VSV) is a member of the *Vesiculovirus* genus of the family *Rhabdoviridae*. The negative-sense virus genome is 11,162 nucleotides long and contains five genes in the order 3′ N-P-M-G-L 5′, encoding the five major viral proteins (1, 3). The bullet-shaped VSV particle (160 nm by 80 nm) contains a ribonucleoprotein core (nucleocapsid) composed of genomic RNA closely associated with N protein and a RNA polymerase composed of a complex of L and P proteins enveloped in a host cell-derived plasma membrane (4, 18, 19, 44, 53, 56). Following uptake of the virus particle by susceptible cells, nucleocapsid and viral RNA polymerase are released into the cytoplasm and viral mRNA transcription ensues. A 3′-5′ gradient of viral mRNA transcription leads to abundant N protein expression and successively decreasing levels of P, M, G, and L proteins (1, 3, 15, 19, 27, 57). This gene expression gradient provides virus proteins in a suitable ratio for subsequent viral genome replication and assembly of mature virus particles. Virus replication in cell culture is rapid, and virus progeny are detectable 5 to 6 h postinfection.

Since the initial recovery of infectious recombinant VSV (rVSV) from genomic cDNA (39, 61), effort has been directed towards the development of rVSV as a vaccine vector targeting a variety of different human pathogens, including human immunodeficiency virus type 1 (HIV-1) (25, 31–34, 48–51). The major advantages of rVSV vaccine vectors and their immunogenicity and protective efficacy in animal models have been described in detail previously (12). However, VSV is both neurotropic and neurovirulent in mice (54, 58, 60) and can cause neurological disease when injected directly into the brain of cows and horses (24). The original rVSV Indiana serotype vector ($rVSV_{IN}$) developed by J. Rose and colleagues was less pathogenic following intranasal inoculation in mice than the cell culture-adapted virus from which it was derived, but the neurovirulence (NV) potential of this vector following direct intracranial (IC) inoculation was not known. To address this question, an exploratory nonhuman primate (NHP) NV study based on the methodology used for NV testing of mumps vaccine seed lots was carried out. In that pilot study, wild-type (wt) VSV_{IN} and $rVSV_{IN}$ caused clinical signs of severe neurological disease following intrathalamic inoculation of animals; two additional rVSV vectors expressing the HIV-1 Gag protein did not cause any clinical signs of disease, but histological examination of the central nervous system (CNS) in these animals revealed evidence of necrotic and inflammatory lesions (30). These findings indicated that $rVSV_{IN}$ vectors would require further attenuation before being considered suitable for clinical evaluation.

When it became possible to recover infectious VSV from genomic cDNA (39, 61), directed approaches to study rVSV attenuation were adopted. One attenuation strategy known as gene shuffling involves rearranging the natural gene order of VSV, which alters normal levels of gene expression (2, 60). Viruses modified by gene rearrangement often grow poorly in vitro and are typically less virulent in vivo (21–23, 42, 60).

A different attenuation strategy involves truncation of the

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▽ Published ahead of print on 6 December 2006.

29-amino-acid cytoplasmic tail (CT) region of the virus G protein (50, 55). Viruses with shortened CTs have slower growth rates, reach lower peak titers in vitro, and are less pathogenic in mice than unaltered viruses (49). Because N gene shuffles and G protein CT truncations involve gene translocations and deletion of part of the G gene, respectively, mutants generated by these strategies have a stable attenuation phenotype-genotype (23, 55).

A third attenuation strategy relies on nucleotide substitutions within the M gene that ablate expression of two in-frame overlapping polypeptides initiated downstream from the M protein translation start codon (29). Viruses that do not express these polypeptides demonstrate reduced cytopathology in a variety of cell lines and are highly attenuated in mice. Consequently, mutants that do not express these polypeptides have been called noncytopathic M mutants (M_{NCP}).

In this study, we sought to explore and define strategies that would allow step-wise increases in $rVSV_{IN}$ vector attenuation to levels beyond those previously described, thereby increasing the range of attenuated vectors from which to generate an ideal $rVSV_{IN}$–HIV-1 vaccine vector for future clinical evaluation. To achieve this we combined G protein CT truncations with either N gene shuffles or M_{NCP} gene mutations. Growth characteristics of the resulting $rVSV_{IN}$ combination mutants were studied in vitro, and their neurovirulence was assessed in a mouse IC 50% lethal dose (LD_{50}) model to determine degree and relative order of vector attenuation.

MATERIALS AND METHODS

Cells and virus. Vero and baby hamster kidney (BHK) cell lines were obtained from the American Type Culture Collection and propagated under conditions of 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, sodium pyruvate (20 mM), and gentamicin (50 μg/ml). The tissue culture-adapted San Juan strain of the VSV Indiana serotype (VSV_{IN}), a recombinant form of VSV_{IN} ($rVSV_{IN}$) (39), $rVSV_{IN}$ expressing HIV-1 Gag protein ($rVSV_{IN}$ gag5), and two attenuated forms of $rVSV_{IN}$ gag5 ($rVSV_{IN}$CT1 gag5 and $rVSV_{IN}$CT9 gag5) were kindly provided by J. Rose (Yale University, New Haven, CT). A modified form of vaccinia virus Ankara (MVA) that expressed bacteriophage T7 RNA polymerase (MVA-T7) (62) was obtained from Bernard Moss (National Institutes of Health, Bethesda, Maryland) and further modified to express T7 RNA polymerase under the control of an early transcription promoter (38).

Virus propagation, purification, and titration. Virus was routinely amplified on BHK cell monolayers and titrated on Vero cell monolayers. For virus amplification BHK cells were infected at a multiplicity of infection (MOI) of 0.001 to 0.05 PFU/cell. Virus inoculum was adsorbed for 15 min at room temperature (RT) followed by 30 min at 37°C. Additional growth medium was then added, and cells were incubated at 37°C until they became rounded and detached from the flask. Infected cell supernatant was clarified by centrifugation for 10 min at 3,000 × *g*. The virus suspension was then flash frozen in an ethanol-dry ice bath and stored at −80°C prior to titration. Where necessary, virus was further purified from infected cell supernatant by centrifugation through 10% (wt/vol) sucrose in 1× phosphate-buffered saline (PBS). Briefly, ~20 ml of clarified cell supernatant was underlaid with 12 to 15 ml of 10% (wt/vol) sucrose in a Beckmann Ultraclear tube followed by centrifugation at 28,000 rpm in a Beckmann SW-28 rotor for 1.5 h at 4°C. Following centrifugation, supernatant was aspirated, the virus pellet was resuspended in PBS, and the virus suspension was flash frozen and stored at −80°C prior to plaque assay.

For virus titration by plaque assay, freshly confluent Vero cell monolayers in six-well plates were infected with 0.1-ml aliquots from serial 10-fold dilutions of rVSV in growth medium. An additional 0.4 ml of medium was added to each well to prevent cell desiccation, and virus was adsorbed for 15 min at RT followed by 30 min at 37°C. The virus inoculum was then removed, and cell monolayers were overlaid with 3 ml of 0.8% (wt/vol) agarose (SeaPlaque; Cambrex Bio Science Rockland, Inc., Rockland, ME) in growth medium. After 10 min at RT to allow the agarose to solidify, cells were incubated at 37°C in 5% CO_2 for 1 to 4 days for plaque development. The agarose overlay was then removed, monolayers were rinsed once with 2 ml of PBS, and cells were stained and fixed in 0.5 ml of 70% methanol containing 2% crystal violet for 5 min at RT. Plaques were counted after removal of excess stain under running water.

Generation of attenuated $rVSV_{IN}$ genome cDNAs. The generation of both CT1 and CT9 mutants has been previously described in detail (49, 55). The corresponding rVSV genomic cDNAs were generously provided by J. Rose (Yale University, New Haven, CT) and were used in the derivation of the combination mutants described below.

A method for gene translocation within $rVSV_{IN}$ genomic cDNA has been described in detail previously (2, 60). A different method of N gene translocation was used in this study. Briefly, the N gene was first deleted from $rVSV_{IN}$ genomic cDNA by replacing the natural BsaAI-XbaI genome fragment (Fig. 1) with a DNA fragment that was generated by in vitro ligation of two PCR products, one stretching from the BsaAI site in the plasmid vector to the exact 3′ end of the virus leader sequence (positive sense) and the other spanning the region from the transcription start signal of the P gene to the downstream XbaI site. Precise ligation of DNA containing the virus leader sequence, with DNA containing the exact 3′ end of the P gene, was achieved by addition of BsmBI sites to PCR primers. The N gene was then reinserted into the ΔN genome cDNA between the P and M genes (N2), between the M and G genes (N3), and between the G and L genes (N4) by use of a similar approach. For generation of the N2 genome cDNA, a PCR product spanning the entire N gene and 3′ CT intergenic dinucleotide was ligated to flanking PCR fragments in vitro; one DNA fragment stretched from the unique XbaI site to the 3′ end of the P gene and contained the P/M intergenic dinucleotide GT. A second DNA fragment spanned the entire M gene to the unique MluI site in the G gene. Addition of BsmBI sites to the 3′ and 5′ ends of the P and M gene fragments, respectively, and to 3′ and 5′ ends of the N gene fragment allowed all three DNA fragments to be ligated in vitro and then cloned into the XbaI and MluI sites of the ΔN genome cDNA. The N3 cDNA genome was constructed in a similar fashion. A PCR fragment spanning the region from the unique XbaI site in the P gene to the end of the M gene, including the 3′ CT intergenic dinucleotide, was ligated to a PCR fragment spanning the entire N gene, a 3′ CT intergenic dinucleotide, and the first 32 nucleotides of the G gene containing the unique MluI site. Both DNAs were ligated through BsmBI sites at the 3′ end of the P/M fragment and the 5′ end of the N gene fragment. This DNA fragment was then cloned into the unique XbaI and MluI sites of the ΔN cDNA genome. For generation of the N4 genome cDNA, a PCR product spanning the entire N gene was joined with flanking PCR products, one stretching from the unique MluI site to the end of the G gene, including the 3′ CT intergenic dinucleotide, and the other containing the G/L intergenic dinucleotide CT and the region from the 5′ end of the L gene to the unique HpaI site. All three fragments were joined by the addition of BsmBI sites to the 3′ and 5′ ends of the G and L gene fragments, respectively, and to the 3′ and 5′ ends of the N gene DNA fragment. The resulting contiguous DNA fragment was then cloned into the MluI and HpaI sites of the ΔN cDNA genome.

A plasmid cDNA containing the M_{NCP} gene in the $rVSV_{IN}$ backbone was generously provided by Michael Whitt (University of Tennessee, Nashville) (29). The M_{NCP} gag5 and M_{NCP}CT1 gag5 vectors were generated by cloning a DNA fragment that spanned the mutant M_{NCP} gene and part of the P gene into the unique XbaI-MluI sites of $rVSV_{IN}$ cDNAs (generously provided by J. Rose, Yale University, New Haven, CT) containing either the HIV-1 Gag gene inserted between the G and L genes ($rVSV_{IN}$ gag5) or the HIV-1 Gag gene inserted between a truncated (CT1) form of the G gene and the L gene ($rVSV_{IN}$CT1 gag5).

Four N gene shuffle-CT combination mutants (N2CT9, N2CT1, N3CT9, and N3CT1) were generated by swapping the G genes from the N2 and N3 cDNAs with the CT1 and CT9 truncated forms of the G gene via unique flanking MluI and HpaI sites.

Recovery of $rVSV_{IN}$ from cDNA. Infectious virus was recovered from genomic cDNA following transfection of BHK cells with a mixture of plasmids expressing VSV N, P, and L proteins and full-length positive-sense genomic RNA, all under the control of the bacteriophage T7 RNA polymerase transcription promoter (39). For transfection, 95% to 100% confluent BHK cell monolayers in six-well dishes were incubated for 4 h in 3% CO_2 at 32°C in 4.5 ml/well of fresh growth medium. Meanwhile, a plasmid DNA-$CaPO_4$ precipitate was prepared for each cell monolayer by mixing 2 to 4 μg of plasmid containing full-length genomic cDNA, 1.0 μg of N plasmid, 0.5 μg of P plasmid, 0.15 μg of L plasmid, 25 μl of $CaCl_2$ (2.5 M), and water to achieve a 250-μl final volume. The DNA-$CaPO_4$ precipitate was then formed by dropwise addition of 250 μl of 2× BBS (280 mM NaCl, 50 mM BES, 1.5 mM Na_2HPO_4, pH 6.95 to 6.98) with gentle vortexing. The mixture was incubated at RT for 20 min to allow precipitate formation and then added dropwise to cells with gentle swirling. To provide a source of T7 RNA

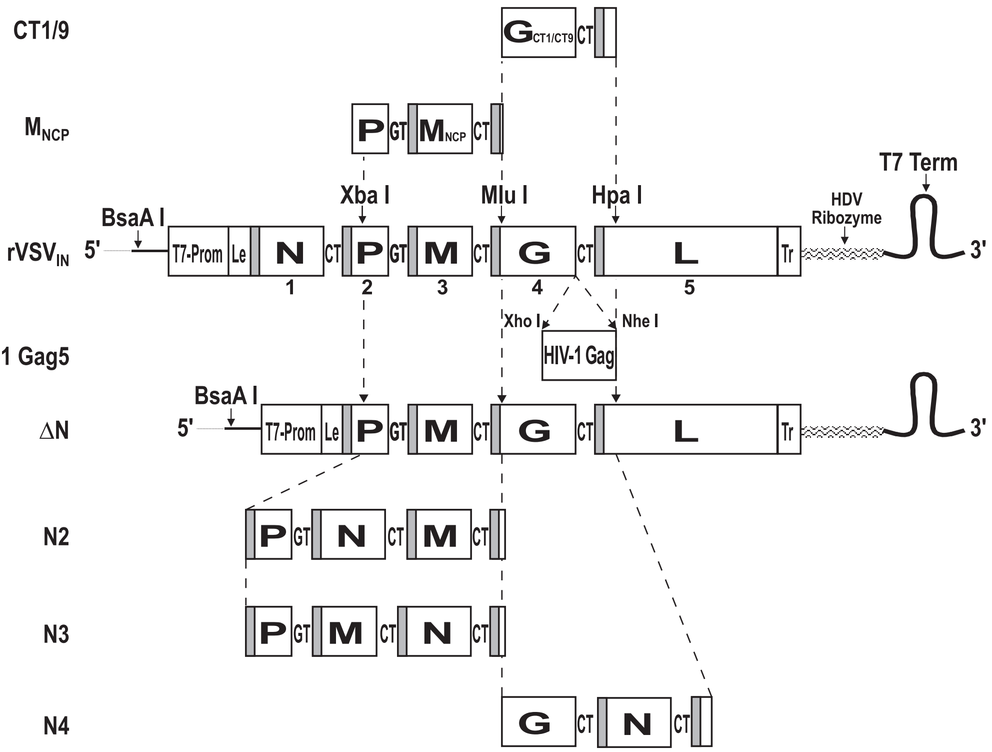

FIG. 1. Construction of $rVSV_{IN}$ mutant cDNA. The BsaAI, XbaI, MluI, and HpaI endonuclease sites used for construction of N gene shuffles and insertion of G genes containing CT truncations and M_{NCP} mutations are indicated with arrows. Virus leader (Le), trailer (Tr), GT, and CT intergenic dinucleotides and transcriptional start signals (shaded boxes) at the beginning of each gene are shown. Synthesis of positive-sense genomic RNA was under control of the T7 RNA polymerase transcription promoter (T7-Prom) and was terminated by a T7 transcription terminator (T7 Term). Hepatitis delta virus ribozyme (HDV Ribozyme) was used to generate the precise viral 3′ end on the positive-sense genomic RNA transcript.

polymerase, MVA-T7-GK16 (38) was then added to each well at an MOI of 3 to 4 PFU/cell along with 20 μg/ml cytosine arabinoside to inhibit amplification of MVA-T7. Cells were then incubated at 32°C in 3% CO_2 for 3 h followed by a 2-h heat shock at 43°C in 3% CO_2 (43). Following heat shock, cells were incubated at 32°C in 3% CO_2 for 18 to 24 h. Transfection medium was then replaced with 2 ml of fresh growth medium containing cytosine arabinoside, and cells were further incubated at 37°C in 5% CO_2 for 48 to 72 h. Transfected cells were then scraped into suspension, gently pipetted repeatedly to reduce cell clumping, and transferred to 95% to 100% confluent Vero cell monolayers in six-well dishes. The following day, cocultures were supplemented with 1 ml of fresh growth medium and incubation was continued for a further 3 to 5 days, during which time VSV cytopathic effect (CPE) became apparent. Rescued virus was then triple plaque purified and further amplified prior to in vitro and in vivo analysis.

In vitro growth studies. For comparison of $rVSV_{IN}$ mutant plaque sizes, plaque assays were performed in duplicate on replicate Vero cell monolayers as described above. For growth kinetics studies, replicate Vero cell monolayers in 25 cm^2 flasks were infected in duplicate at an MOI of 5 PFU/cell. Virus was adsorbed in 0.5 ml of growth medium for 15 min at RT followed by 30 min at 37°C with occasional rocking to prevent cell desiccation. After removal of the inoculum, monolayers were rinsed three times with 5 ml of PBS to remove unbound virus; 5 ml of growth medium was then added to each monolayer, and a 0.5-ml aliquot was immediately removed as a "time h 0" (T_0) sample and replaced with 0.5 ml of fresh medium. Incubation was continued at 37°C in 5% CO_2 for 48 to 72 h, and further samples were taken at T_3 to T_{48}. All samples were flash frozen in ethanol-dry ice and stored at −80°C for titration.

Mouse IC LD_{50} studies. Five-week-old female Swiss Webster mice (Taconic Laboratory Animals and Services, Germantown, NY) were anesthetized and injected IC with log_{10}-fold dilutions of virus in 30 μl PBS (10 mice per dilution, with dilutions adjusted to range around the anticipated LD_{50}). Weight and health status were recorded daily for 2 weeks. Mice becoming either bilaterally paralyzed or showing significant signs of distress or severe illness were sacrificed and recorded as succumbing to VSV disease. The LD_{50} and the 50% paralyzing dose (PD_{50}) were determined by the method of Reed and Muench (45) based on the number of mice that became paralyzed. All animal care and procedures conformed to Institutional Animal Care and Use Committee guidelines. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

RESULTS

Recovery of attenuated $rVSV_{IN}$ vectors from genomic cDNA. The complete spectra of $rVSV_{IN}$ vectors recovered from genomic cDNA and subsequently used for in vitro and in vivo attenuation studies are shown in Fig. 2A. Attenuated $rVSV_{IN}$ mutants were generated using three different attenuation strategies and combinations thereof. In one strategy the N gene was translocated (shuffled) to the second, third, and fourth gene positions (N2, N3, and N4, respectively) in the $rVSV_{IN}$ genome. In another strategy, the G protein CT was truncated to either nine (CT9) amino acids or one (CT1) amino acid. A third attenuation strategy abolished expression of two overlapping polypeptides encoded within the M gene open reading frame, generating the M_{NCP} gag5 mutant containing the HIV-1 gag gene at position 5 in the genome. Both the CT9 and N4 mutants contain an additional "empty" transcriptional unit (TU) at the fifth position in the genome. This TU contains an XhoI-NheI cassette flanked by transcription start and stop signals to facilitate insertion and expression of foreign genes (50, 51). Because results from previous murine NV studies (60) and an exploratory NHP NV study (30) indicated that N gene shuffles and G protein CT truncations on their own might not

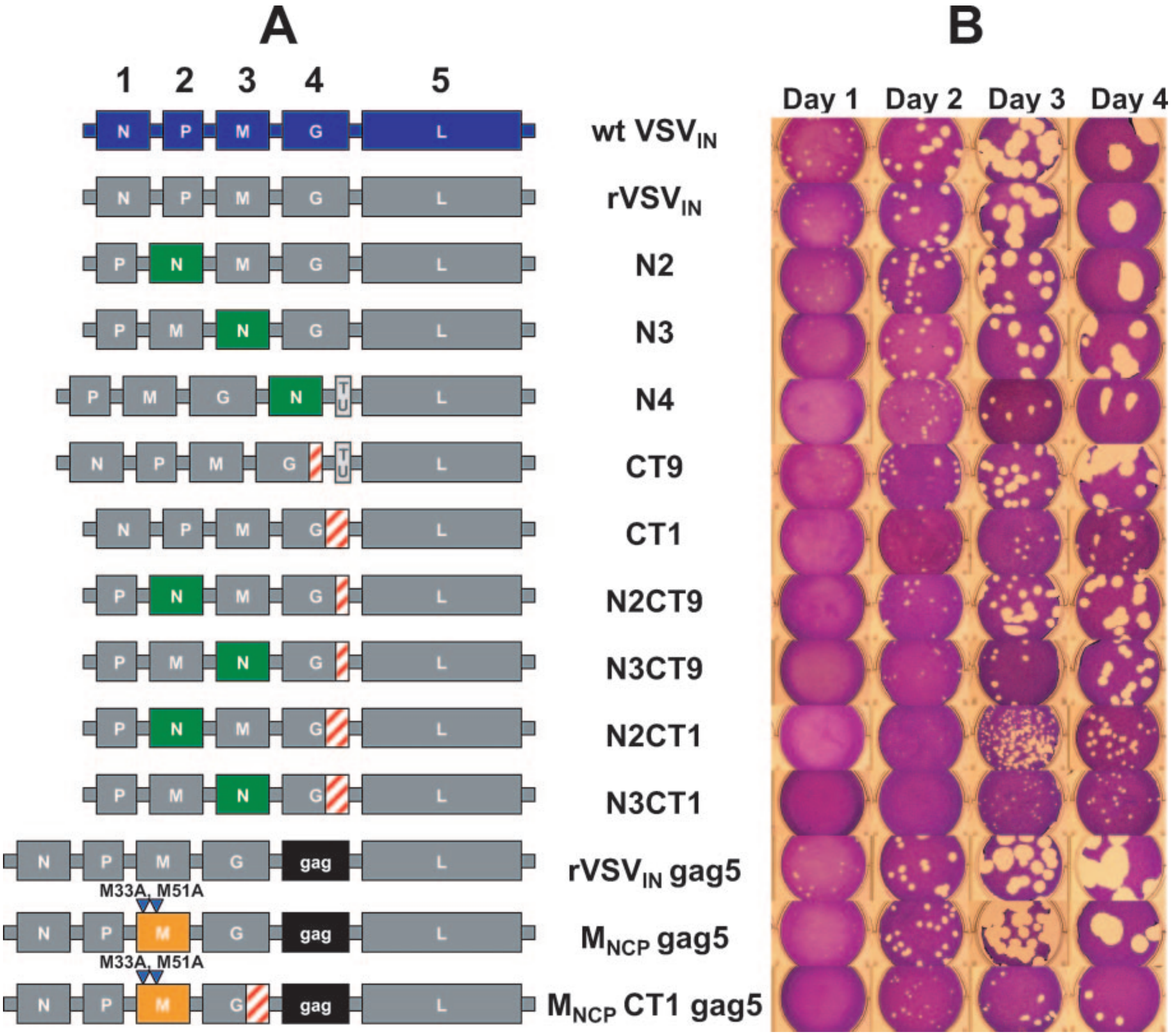

FIG. 2. Genetic organization of rVSV$_{IN}$ mutants and plaque size comparison. (A) Mutants were named to reflect genomic organization and attenuating mutations. The N gene shuffle mutants N2, N3, and N4 were named according to the position of the N gene relative to that of wt VSV$_{IN}$. The G protein CT truncation mutants CT1 and CT9 were named according to the number of amino acids retained in the cytoplasmic tail region of the G protein. Vectors containing noncytopathic M gene mutations (M33A and M51A [triangles]) were named M$_{NCP}$ mutants. Combination mutants were named N2CT1, N3CT1, N2CT9, N3CT9, and M$_{NCP}$CT1 to reflect contributing mutations. An additional empty TU containing transcription start and stop signals but no additional gene was present in N4 and CT9 mutants. The HIV-1 gag gene was present in the fifth position of virus genomes as indicated. (B) Representative plaques produced by wt VSV$_{IN}$ and rVSV$_{IN}$ variants following plaque assay on replicate Vero cell monolayers at 37°C for 1 to 4 days.

attenuate rVSV$_{IN}$ sufficiently for use as a vaccine vector in humans, these mutations were also combined in different configurations in an effort to produce more highly attenuated variants. Most double mutants were generated by combining N gene shuffles with G protein CT9 and CT1 truncations (shuffle-CT mutants), giving rise to N2CT9, N3CT9, N2CT1, and N3CT1 vectors; another double mutant containing the HIV-1 gag gene at position 5 in the genome was generated by combining the G protein CT1 truncation with the M$_{NCP}$ mutations (M$_{NCP}$ CT1 gag5). Even though it was anticipated that the mutant vectors would be more growth attenuated in vitro than the prototype rVSV$_{IN}$ vector developed by J. Rose and colleagues, all single and combination mutants were recoverable from cDNA.

Comparison of rVSV$_{IN}$ mutant plaque size. To gain a first impression on the relative attenuation levels of rVSV$_{IN}$ mutants, plaque sizes on Vero cell monolayers were compared at different times postinfection (Fig. 2B). From this analysis a number of trends emerged. Plaques produced by rVSV$_{IN}$ and wt VSV$_{IN}$ were almost the same size at all time points, indicating that rVSV$_{IN}$ growth was little more attenuated than wt VSV$_{IN}$ growth in cell culture. Virus containing only M$_{NCP}$-attenuating mutations (M$_{NCP}$ gag5) produced a delayed cell CPE, as previously observed (29), that resulted in very small plaques by day 1. At later time points, plaques were only slightly smaller than those made by rVSV$_{IN}$, indicating efficient growth and spread of this mutant in vitro. However, it should be noted that although M$_{NCP}$ plaques were similar in size to

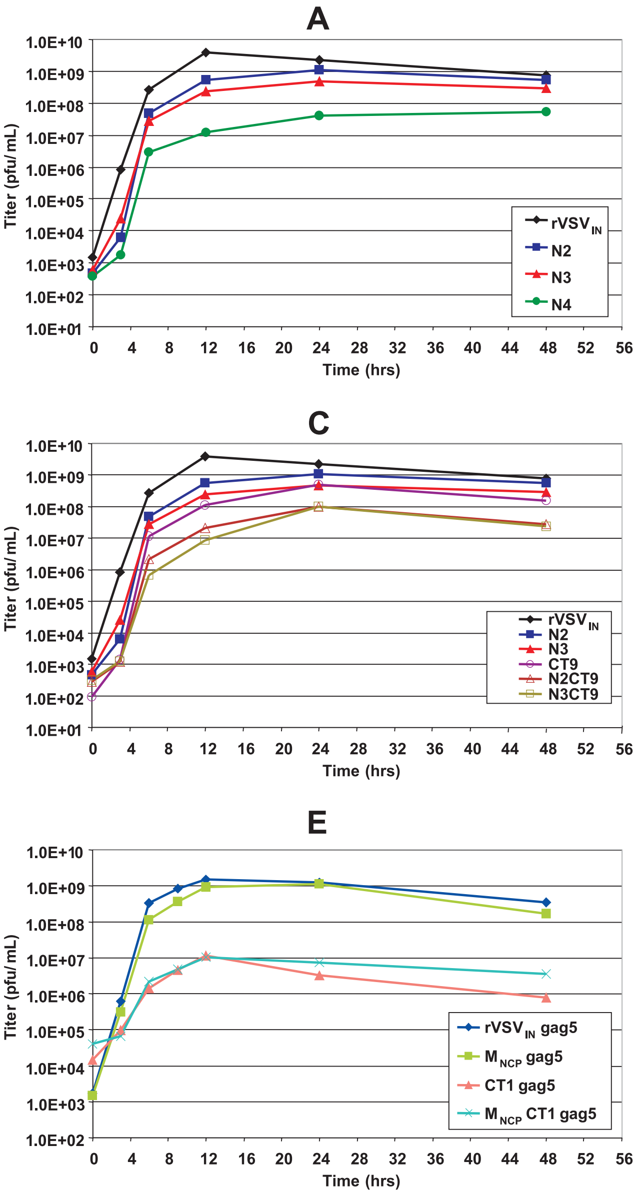

FIG. 3. Growth kinetics of rVSV$_{IN}$ mutants on Vero cell monolayers. Replicate Vero cell monolayers in 25 cm^2 flasks were infected in duplicate at an MOI of 5 PFU/cell. Infected-cell supernatants were collected at intervals postinfection and titrated on Vero cell monolayers. All datum points represent the average titers of samples taken from duplicate infections. Growth curves are shown for mutants containing N gene shuffles (A), CT truncations (B), N gene shuffle-CT truncation combinations (C and D), and M$_{NCP}$ mutations (E).

those made by rVSV$_{IN}$ and rVSV$_{IN}$ gag5 at days 2 to 4, cells within the M$_{NCP}$ gag5 plaques displayed a reduced CPE at all time points. The M$_{NCP}$ CT1 gag5 combination mutant produced plaques that were much smaller than M$_{NCP}$ gag5 plaques and similar in size to those produced by the CT1 virus, indicating that the CT1 truncation was the dominant attenuating mutation affecting virus growth and spread in vitro. Importantly, both CT and N gene shuffle mutants had plaque sizes commensurate with degrees of genetic alteration. For example, CT1 mutants produced smaller plaques than CT9 mutants, and there was a gradient of decreasing plaque size as the N gene was moved further away from the 3′ transcription promoter (N2 to N4), as previously reported (60). When N gene shuffle and CT mutations were combined, plaque size was decreased relative to the results seen with mutants having only one of the two mutations. For example, N2CT1 and N3CT1 produced plaques that were on average smaller than plaques produced by N2, N3, or CT1 mutants at all time intervals. This effect was also seen for N3CT9 but was less notable for N2CT9 except at day 1. Plaque sizes for N shuffle-CT combination mutants also varied incrementally with degree of genetic alteration, and a gradient of decreasing plaque size (N2CT9→N3CT9→N2CT1→N3CT1) was seen. Overall observations of plaque size indicated that the combination of N gene shuffles and CT truncations can attenuate virus incrementally and to a greater degree than either single form of mutation.

In vitro growth kinetics of rVSV$_{IN}$ mutants. We next performed a series of growth kinetic studies measuring the rate and extent of virus growth to further compare relative in vitro attenuation levels among rVSV$_{IN}$ mutants (Fig. 3). As shown in Fig. 3A, the rate of virus growth was reduced in relation to the position (N2 to N4) of the N gene, with a reduction in peak virus titer of 5-fold for N2, 10-fold for N3, and 100-fold for N4 compared to rVSV$_{IN}$ results. The CT9 and CT1 gag5 mutants (Fig. 3B) also had reduced growth rates and reached 10-fold and 100-fold-lower peak titer respectively than rVSV$_{IN}$, in general agreement with previous reports (49, 55). It should be noted that the addition of the HIV-1 gag gene between the G and L genes of rVSV$_{IN}$ did not significantly reduce growth of virus in cell culture and that the CT1 and CT1 gag5 mutants displayed almost identical growth kinetics in vitro (data not shown). More importantly, when N gene shuffles were combined with G protein CT truncations a series of virus mutants was generated that had reduced growth rates and a reduction in peak infectious particle production compared to virus containing either form of mutation alone (Fig. 3C and 3D). Overall, the growth kinetic studies indicated a gradient of increasing virus attenuation (N2CT9→N3CT9→N2CT1→N3CT1) identical to that observed in plaque size comparisons. In this combinatorial approach to virus attenuation, N3CT1 had 1,000-fold-lower peak virus titer than rVSV$_{IN}$ and 50- and 500-fold-lower peak titer than CT1 and N3 mutants, respectively (Fig. 3D). A separate series of growth kinetic studies were performed for the M$_{NCP}$ mutants, as they were originally generated with the HIV-1 gag gene inserted between the G and L genes of the genome(s). As previously described, virus containing the M$_{NCP}$ mutations replicated to a nearly normal peak titer in cell culture (Fig. 3E) but with a delayed onset of CPE in most cell types (29). Unlike the synergistic attenuation of virus growth seen with N gene-CT combination mutants, combining M$_{NCP}$ mutations with the CT1 truncation did not significantly alter growth in cell culture compared to the results seen with the CT1 mutant alone, indicating that the CT1 mutation was the dominant attenuating mutation in vitro.

Assessment of rVSV$_{IN}$ vector neurovirulence in mice. Young mice are much more sensitive to infection with VSV following IC inoculation than following intranasal inoculation (54, 60). Moreover, unlike wt VSV$_{IN}$, attenuated rVSV$_{IN}$ mutants containing either cytoplasmic tail truncations (CT1 and CT9) or N gene shuffles (N2, N3, and N4) do not cause death following intranasal inoculation (25, 60). Therefore, to measure differences in virulence among the attenuated rVSV$_{IN}$ mutants, mice were inoculated IC, and the cumulative animal deaths, time until death, and frequency and severity of paralysis were measured. The LD$_{50}$s and the PD$_{50}$s were calculated based on the method of Reed and Muench and are shown in Fig. 4A. The time to death in animals receiving a lethal dose is shown in Fig. 4B.

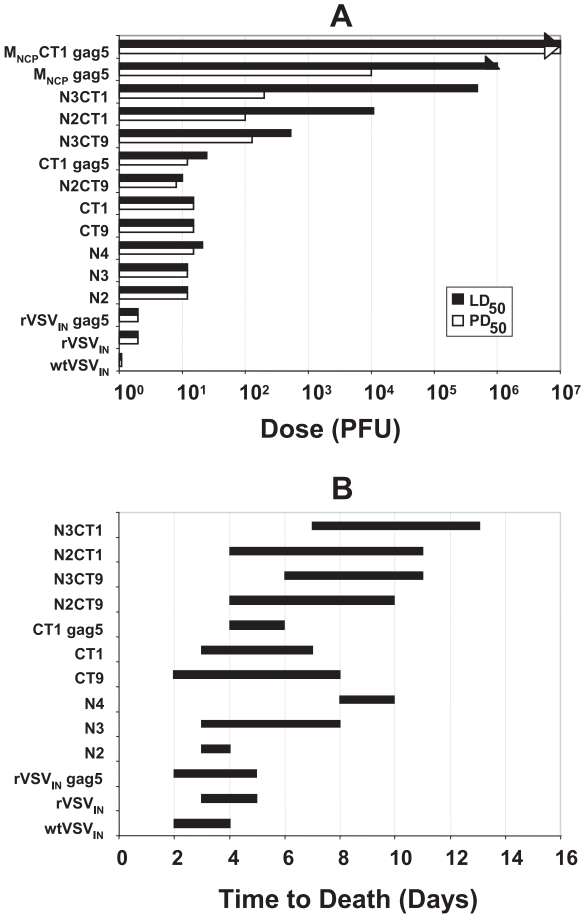

FIG. 4. Neurovirulence properties of rVSV$_{IN}$ mutants in mice following IC inoculation. In a series of experiments, 5-week-old Swiss Webster mice were inoculated IC with log$_{10}$-fold dilutions of virus. Mice were monitored for 2 weeks for mortality and morbidity (paralysis). (A) The LD$_{50}$ and PD$_{50}$ values were determined by the method of Reed and Muench. (B) Time to death was recorded for mice in the group receiving the dose immediately above the determined LD$_{50}$. Arrowheads indicate results in which LD$_{50}$ and PD$_{50}$ were not achieved.

Mice receiving wt VSV$_{IN}$ reproducibly died 2 to 4 days postinoculation, and the LD$_{50}$ was only 1 to 2 PFU. In agreement with plaque size comparisons and growth kinetics studies, rVSV$_{IN}$, with and without HIV gag inserted between the G and L genes, was only marginally more attenuated than wt VSV, with an LD$_{50}$ of approximately 2 to 5 PFU and a slightly delayed onset of death at 2 to 5 days. Viruses containing either CT truncations or N gene shuffles alone were slightly more attenuated than rVSV$_{IN}$, with LD$_{50}$ values of 12 to 21 PFU for CT9, CT1, N2, N3, and N4. Although the LD$_{50}$s of N2, N3, and N4 mutants were similar, there was a respective incremental increase in time to onset of death. However, a dramatic decrease in virulence was seen when the CT1 mutation was combined with N gene shuffles. Most notably, for N3CT1 the LD$_{50}$ increased to $>10^5$ PFU compared to 15 PFU and 12 PFU for CT1 and N3 viruses, respectively, and the LD$_{50}$ for N2CT1 was 1.1×10^4 PFU, demonstrating powerful synergistic attenuation of virulence for these combinations of mutations. Moreover, when animals died at higher doses, onset to death was delayed to 4 to 11 days postinoculation for N2CT1 and 6 to 14 days for N3CT1. To a lesser extent, and consistent with the order of attenuation observed in vitro, synergistic attenuation was also observed for N3CT9, with an LD$_{50}$ of 524 PFU and delayed onset of death. As the least attenuated among the combination mutants in vitro, the LD$_{50}$ dose for N2CT9 was very similar to the LD$_{50}$s for N2 and CT9; however, time to onset of death was delayed compared to that seen with the N2 and CT9 variants.

Many of the mice inoculated with the combination mutants, in particular, N3CT1 and N2CT1, displayed morbidity in the form of paresis and unilateral paralysis, from which they started to recover by week 3 postinfection, without mortality. Thus, the PD_{50}s for N3CT1 and N2CT1 were less than their respective LD_{50}s. Mice receiving the more virulent viruses died quickly without a measurable paralytic phase. Therefore, the PD_{50} and LD_{50} values for these viruses were recorded as being identical. Overall, the gradient of attenuation for the N gene shuffle-CT combination mutants observed in vivo was identical to that observed in vitro.

IC infection with M_{NCP} gag5 primarily caused some mild paralysis (PD_{50} of 10^4 PFU) but not death at up to the highest dose (10^6 PFU) tested, and an LD_{50} dose was not achieved. However, combining the M_{NCP} and CT1 mutations reduced the amount of paralysis compared to the results seen with M_{NCP} gag5 alone such that neither a LD_{50} nor a PD_{50} could be calculated for M_{NCP}CT1 gag5. The very high level of attenuation observed for the M_{NCP} gag and M_{NCP}CT1 gag5 mutants in vivo and the absence of a measurable LD_{50} for both mutants at input levels that were approaching a practical limit for M_{NCP}CT1 gag5 prevented any clear conclusions concerning the synergistic effect of combining the M_{NCP} and CT1 mutations. However, the M_{NCP} mutation was clearly the dominant attenuating mutation in vivo, while the CT1 mutation was clearly dominant in vitro.

DISCUSSION

An exploratory NV study of NHPs indicated that the $rVSV_{IN}$–HIV-1 vaccine vectors pioneered by J. Rose and colleagues retained significant levels of virulence and might be insufficiently attenuated for clinical evaluation (30). The present study was undertaken to investigate strategies for further attenuation of $rVSV_{IN}$ and to identify less-virulent variants that might be more suitable as vaccine vectors for HIV-1 and other pathogens.

Variants containing only a single form of attenuating mutation were more growth attenuated than the prototypic $rVSV_{IN}$ vector in vitro but, except for the M_{NCP} mutant, were still highly neurovirulent when tested in the murine IC NV model. Virus containing only the CT1 mutation also caused significant neuropathology in an exploratory NHP NV study (30). In an effort to further increase $rVSV_{IN}$ vector attenuation, CT truncations were combined with either N gene shuffles or M_{NCP} mutations. Most of the resulting combination mutants were more growth attenuated in vitro than vectors containing either single form of mutation. Growth kinetics studies showed that N2CT1 and N3CT1 reached approximately 500- to 1,000-fold-lower peak titers than $rVSV_{IN}$ and approximately 50- to 500-fold-lower peak titers than N2, N3, or CT1 mutants. Furthermore, the degree of vector attenuation could be altered incrementally depending on the pairing of specific N gene shuffle and CT mutations. For example, the N3CT1 mutant was more growth attenuated than N2CT1, which was more attenuated than N3CT9 and N2CT9 mutants. The gradient of increasing virus attenuation for these combination mutants was N2CT9→N3CT9→N2CT1→N3CT1. The same order of attenuation was also observed in vivo, but differences in attenuation between combination mutants and virus with only one type of attenuating mutation were even more dramatic. The N2, N3, CT9, and CT1 mutants still retained high levels of virulence following IC inoculation of mice (LD_{50}s of 12 to 21 PFU) consistent with previously published data for N gene shuffles (60). In contrast, N gene shuffle-CT combination mutants had incrementally increasing LD_{50}s ranging from 10 PFU for N2CT9 to $>10^5$ PFU for N3CT1. Specifically, attenuation synergy appeared to be greater when the CT1 truncation was combined with N2 and N3 gene shuffles.

The differences in relative attenuation between single and combination mutants observed in vitro probably reflect predominantly virus-specific growth attenuation factors. It is thought that the length of the CT tail of VSV G protein may affect the efficiency of virus budding from the cytoplasmic membrane of infected cells. Shorter CTs reduce the rate of particle formation and peak virus titer produced in vitro, possibly due to impaired CT interaction with viral core proteins (16, 28, 50, 55). The N gene shuffles attenuate virus by a different mechanism. During virus replication, N protein is essential for the encapsidation of nascent genomic RNA, and the resulting nucleocapsid structure is the functional template for mRNA transcription and further genome replication. When the N gene is translocated further away from the single 3′ transcription promoter, N protein expression decreases (60). Consequently, limiting N protein reduces the level of nucleocapsid available for transcription, replication, and subsequent incorporation into virus progeny. When transcription is reduced, all virus proteins are expressed less abundantly, placing additional constraints on the availability of all the components needed for assembly and morphogenesis of virus progeny. When both attenuation strategies are combined, not only are viral nucleocapsid and truncated G protein, along with other virus proteins, limiting for viral morphogenesis but impaired interactions between viral nucleocapsid core and the truncated G protein CT likely also further constrain the efficiency of mature particle formation. In vivo, innate and cellular immune responses are additionally superimposed on these growth-attenuating virus-specific factors and likely contribute to the level of attenuation observed in mice.

Innate immunity is usually rapidly induced in response to viral infection in the periphery and likely also plays an important role in controlling virus replication early (days 1 to 5) following IC inoculation of mice (7, 10, 46). Recently, a role for type I interferon has been proposed for control of attenuated but not pathogenic strains of rabies virus, a relative of VSV, following IC inoculation of mice (59), and it is possible that differences in virulence between attenuated and pathogenic strains of VSV can also be explained by differential stimulation of alpha/beta interferon in the CNS. VSV growth in the brain may also be controlled by the induction of nitric oxide, which can inhibit VSV replication in vitro and in neurons (5, 8, 11, 13, 35–37, 47). Acquired cellular immunity likely also plays an important role in killing some types of infected cells and clearance of virus in the CNS early (days 1 to 8) in the infection (11, 46). In contrast, the humoral immune response does not appear to have a significant role in the control and clearance of VSV from the CNS following direct IC inoculation of mice (7, 11).

In view of the host-specific responses to VSV infection of the CNS described above, it is possible that the more slowly replicating, highly attenuated N gene shuffle-CT truncation combination mutants less efficiently down-regulate innate immune responses, leading to a more potent antiviral state (14). For example, reduced expression of the VSV M protein and associated polypeptides can diminish the efficiency of host cell protein shutoff, allowing more efficient induction of innate immune responses (9, 20, 26, 29). Since the N gene shuffle-CT truncation combination mutants also down-regulate viral gene expression, including the M gene, the innate immune response may be better able to control these viruses. Similar reasoning likely also explains the observed differences between in vitro and in vivo attenuation of M_{NCP} mutants described here. In vitro, the M_{NCP} gag5 and M_{NCP}CT1 gag5 mutants are not subject to innate immune responses, and peak titer is close to that of rVSV gag5 and CT1 gag5, respectively, indicating that the CT1 mutation was the dominant attenuating mutation in vitro. However, both M_{NCP} gag5 and M_{NCP}CT1 gag5 had highly attenuated phenotypes in mice, indicating that the M_{NCP} mutations were dominant in vivo, presumably due to the reduced ability of these viruses to interfere with innate immune responses.

In general, the mouse IC LD_{50} NV model proved to be highly sensitive and capable of discriminating changes in virulence within the range of attenuated rVSV vectors tested in this study. Rodent models have also been used to assess the NV potential of other virus vaccine vectors and some licensed live virus vaccines and vaccine candidates, including smallpox vaccine (40), some yellow fever virus vaccine strains (6), attenuated Venezuelan equine encephalitis virus (41), the Jeryl Lynn strain of mumps virus vaccine (52) and a modified measles virus vaccine strain (17). Interestingly, some of the more highly attenuated rVSV vectors described here produced less morbidity and mortality following IC inoculation than some of the licensed live virus vaccines. However, it should be emphasized that differences in virus biology and the natural susceptibility of different mouse strains to virus infection and replication make direct comparison of attenuation levels among different virus vaccines and candidate vaccines extremely difficult.

In summary, the net effect of combining specific N gene shuffles and G protein CT truncations was a measurable synergistic attenuation of rVSV$_{IN}$ growth in vitro and a dramatic reduction of virulence in the very sensitive mouse IC LD_{50} model. These findings suggest that combining mutations that interfere with viral morphogenesis by impairing interactions between structural proteins with mutations that lead to down-regulation of viral structural protein expression may be a useful general mechanism for synergistic attenuation of rVSV$_{IN}$ and other RNA and DNA viruses. Because of the potential of rVSV$_{IN}$ as a vaccine vector for HIV-1 and other human pathogens, experiments are now under way to confirm attenuation of the combination mutants in NHP NV studies and explore the immunogenicity of these highly attenuated rVSV$_{IN}$ vectors.

ACKNOWLEDGMENTS

We thank J. Rose for providing rVSV$_{IN}$, CT1, and CT9 cDNAs and Michael Whitt for providing cDNA encoding the M_{NCP} gene. We thank Chris Parks and Sue Witko for guidance on virus rescue.

This work was sponsored in part by an HIV-1 Vaccine Design and Development Team contract from the National Institutes of Health and the National Institute of Allergy and Infectious Diseases (HVDDT NO1-A1-25458).

REFERENCES

1. **Abraham, G., and A. K. Banerjee.** 1976. Sequential transcription of the genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. USA **73:**1504–1508.
2. **Ball, L. A., C. R. Pringle, B. Flanagan, V. P. Perepelitsa, and G. W. Wertz.** 1999. Phenotypic consequences of rearranging the P, M, and G genes of vesicular stomatitis virus. J. Virol. **73:**4705–4712.
3. **Ball, L. A., and C. N. White.** 1976. Order of transcription of genes of vesicular stomatitis virus. Proc. Natl. Acad. Sci. USA **73:**442–446.
4. **Baltimore, D., A. S. Huang, and M. Stampfer.** 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. II. An RNA polymerase in the virion. Proc. Natl. Acad. Sci. USA **66:**572–576.
5. **Barna, M., T. Komatsu, and C. S. Reiss.** 1996. Activation of type III nitric oxide synthase in astrocytes following a neurotropic viral infection. Virology **223:**331–343.
6. **Barrett, A. D., and E. A. Gould.** 1986. Comparison of neurovirulence of different strains of yellow fever virus in mice. J. Gen. Virol. **67**(Pt. 4)**:**631–763.
7. **Bi, Z., M. Barna, T. Komatsu, and C. S. Reiss.** 1995. Vesicular stomatitis virus infection of the central nervous system activates both innate and acquired immunity. J. Virol. **69:**6466–6472.
8. **Bi, Z., and C. S. Reiss.** 1995. Inhibition of vesicular stomatitis virus infection by nitric oxide. J. Virol. **69:**2208–2213.
9. **Black, B. L., and D. S. Lyles.** 1992. Vesicular stomatitis virus matrix protein inhibits host cell-directed transcription of target genes in vivo. J. Virol. **66:**4058–4064.
10. **Chesler, D. A., and C. S. Reiss.** 2002. The role of IFN-gamma in immune responses to viral infections of the central nervous system. Cytokine Growth Factor Rev. **13:**441–454.
11. **Christian, A. Y., M. Barna, Z. Bi, and C. S. Reiss.** 1996. Host immune response to vesicular stomatitis virus infection of the central nervous system in C57BL/6 mice. Viral Immunol. **9:**195–205.
12. **Clarke, D. K., D. Cooper, M. A. Egan, R. M. Hendry, C. L. Parks, and S. A. Udem.** 2006. Recombinant vesicular stomatitis virus as an HIV-1 vaccine vector. Springer Semin. Immunopathol. **28:**239–253. (First published 15 September 2006; doi:10.1007/s00281-006-0042-3.)
13. **Colasanti, M., T. Persichini, G. Venturini, and P. Ascenzi.** 1999. *S*-Nitrosylation of viral proteins: molecular bases for antiviral effect of nitric oxide. IUBMB Life **48:**25–31.
14. **Conzelmann, K. K.** 2005. Transcriptional activation of alpha/beta interferon genes: interference by nonsegmented negative-strand RNA viruses. J. Virol. **79:**5241–5248.
15. **Davis, N. L., and G. W. Wertz.** 1982. Synthesis of vesicular stomatitis virus negative-strand RNA in vitro: dependence on viral protein synthesis. J. Virol. **41:**821–832.
16. **Dubovi, E. J., and R. R. Wagner.** 1977. Spatial relationships of the proteins of vesicular stomatitis virus: induction of reversible oligomers by cleavable protein cross-linkers and oxidation. J. Virol. **22:**500–509.
17. **Duprex, W. P., I. Duffy, S. McQuaid, L. Hamill, S. L. Cosby, M. A. Billeter, J. Schneider-Schaulies, V. ter Meulen, and B. K. Rima.** 1999. The H gene of rodent brain-adapted measles virus confers neurovirulence to the Edmonston vaccine strain. J. Virol. **73:**6916–6922.
18. **Emerson, S. U., and R. R. Wagner.** 1972. Dissociation and reconstitution of the transcriptase and template activities of vesicular stomatitis B and T virions. J. Virol. **10:**297–309.
19. **Emerson, S. U., and Y. Yu.** 1975. Both NS and L proteins are required for in vitro RNA synthesis by vesicular stomatitis virus. J. Virol. **15:**1348–1356.
20. **Ferran, M. C., and J. M. Lucas-Lenard.** 1997. The vesicular stomatitis virus matrix protein inhibits transcription from the human beta interferon promoter. J. Virol. **71:**371–377.
21. **Flanagan, E. B., L. A. Ball, and G. W. Wertz.** 2000. Moving the glycoprotein gene of vesicular stomatitis virus to promoter-proximal positions accelerates and enhances the protective immune response. J. Virol. **74:**7895–7902.
22. **Flanagan, E. B., T. R. Schoeb, and G. W. Wertz.** 2003. Vesicular stomatitis viruses with rearranged genomes have altered invasiveness and neuropathogenesis in mice. J. Virol. **77:**5740–5748.
23. **Flanagan, E. B., J. M. Zamparo, L. A. Ball, L. L. Rodriguez, and G. W. Wertz.** 2001. Rearrangement of the genes of vesicular stomatitis virus eliminates clinical disease in the natural host: new strategy for vaccine development. J. Virol. **75:**6107–6114.
24. **Frank, A. H., A. Appleby, and H. R. Seibold.** 1945. Experimental intracerebral infection of horses, cattle, and sheep with the virus of vesicular stomatitis. Am. J. Vet. Res. **6:**28–38.
25. **Haglund, K., J. Forman, H. G. Krausslich, and J. K. Rose.** 2000. Expression of human immunodeficiency virus type 1 Gag protein precursor and envelope proteins from a vesicular stomatitis virus recombinant: high-level production of virus-like particles containing HIV envelope. Virology **268:**112–121.
26. **Her, L. S., E. Lund, and J. E. Dahlberg.** 1997. Inhibition of Ran guanosine

triphosphatase-dependent nuclear transport by the matrix protein of vesicular stomatitis virus. Science **276:**1845–1848.

27. **Iverson, L. E., and J. K. Rose.** 1981. Localized attenuation and discontinuous synthesis during vesicular stomatitis virus transcription. Cell **23:**477–484.
28. **Jayakar, H. R., E. Jeetendra, and M. A. Whitt.** 2004. Rhabdovirus assembly and budding. Virus Res. **106:**117–132.
29. **Jayakar, H. R., and M. A. Whitt.** 2002. Identification of two additional translation products from the matrix (M) gene that contribute to vesicular stomatitis virus cytopathology. J. Virol. **76:**8011–8018.
30. **Johnson, J. E., F. Nasar, J. W. Coleman, R. E. Price, A. Javadian, K. Draper, M. Lee, P. A. Reilly, D. K. Clarke, R. M. Hendry, and S. A. Udem.** 9 November 2006, posting date. Neurovirulence properties of recombinant vesicular stomatitis virus vectors in non-human primates. Virology [Online.] doi:10.1016/J.Virol.2006.10.026.
31. **Jones, S. M., H. Feldmann, U. Stroher, J. B. Geisbert, L. Fernando, A. Grolla, H. D. Klenk, N. J. Sullivan, V. E. Volchkov, E. A. Fritz, K. M. Daddario, L. E. Hensley, P. B. Jahrling, and T. W. Geisbert.** 2005. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. Nat. Med. **11:**786–790.
32. **Kahn, J. S., A. Roberts, C. Weibel, L. Buonocore, and J. K. Rose.** 2001. Replication-competent or attenuated, nonpropagating vesicular stomatitis viruses expressing respiratory syncytial virus (RSV) antigens protect mice against RSV challenge. J. Virol. **75:**11079–11087.
33. **Kahn, J. S., M. J. Schnell, L. Buonocore, and J. K. Rose.** 1999. Recombinant vesicular stomatitis virus expressing respiratory syncytial virus (RSV) glycoproteins: RSV fusion protein can mediate infection and cell fusion. Virology **254:**81–91.
34. **Kapadia, S. U., J. K. Rose, E. Lamirande, L. Vogel, K. Subbarao, and A. Roberts.** 2005. Long-term protection from SARS coronavirus infection conferred by a single immunization with an attenuated VSV-based vaccine. Virology **340:**174–182.
35. **Komatsu, T., Z. Bi, and C. S. Reiss.** 1996. Interferon-gamma induced type I nitric oxide synthase activity inhibits viral replication in neurons. J. Neuroimmunol. **68:**101–108.
36. **Komatsu, T., D. D. Ireland, N. Chen, and C. S. Reiss.** 1999. Neuronal expression of NOS-1 is required for host recovery from viral encephalitis. Virology **258:**389–395.
37. **Komatsu, T., D. D. Ireland, N. Chung, A. Dore, M. Yoder, and C. Shoshkes Reiss.** 1999. Regulation of the BBB during viral encephalitis: roles of IL-12 and NOS. Nitric Oxide **3:**327–339.
38. **Kovacs, G. R., C. L. Parks, N. Vasilakis, and S. A. Udem.** 2003. Enhanced genetic rescue of negative-strand RNA viruses: use of an MVA-T7 RNA polymerase vector and DNA replication inhibitors. J. Virol. Methods **111:**29–36.
39. **Lawson, N. D., E. A. Stillman, M. A. Whitt, and J. K. Rose.** 1995. Recombinant vesicular stomatitis viruses from DNA. Proc. Natl. Acad. Sci. USA **92:**4477–4481.
40. **Li, Z., S. A. Rubin, R. E. Taffs, M. Merchlinsky, Z. Ye, and K. M. Carbone.** 2004. Mouse neurotoxicity test for vaccinia-based smallpox vaccines. Vaccine **22:**1486–1493.
41. **Ludwig, G. V., M. J. Turell, P. Vogel, J. P. Kondig, W. K. Kell, J. F. Smith, and W. D. Pratt.** 2001. Comparative neurovirulence of attenuated and non-attenuated strains of Venezuelan equine encephalitis virus in mice. Am. J. Trop. Med. Hyg. **64:**49–55.
42. **Martinez, I., L. L. Rodriguez, C. Jimenez, S. J. Pauszek, and G. W. Wertz.** 2003. Vesicular stomatitis virus glycoprotein is a determinant of pathogenesis in swine, a natural host. J. Virol. **77:**8039–8047.
43. **Parks, C. L., R. A. Lerch, P. Walpita, M. S. Sidhu, and S. A. Udem.** 1999. Enhanced measles virus cDNA rescue and gene expression after heat shock. J. Virol. **73:**3560–3566.
44. **Patton, J. T., N. L. Davis, and G. W. Wertz.** 1983. Cell-free synthesis and assembly of vesicular stomatitis virus nucleocapsids. J. Virol. **45:**155–164.
45. **Reed, E., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27:**493–497.
46. **Reiss, C. S., D. A. Chesler, J. Hodges, D. D. Ireland, and N. Chen.** 2002. Innate immune responses in viral encephalitis. Curr. Top. Microbiol. Immunol. **265:**63–94.
47. **Reiss, C. S., and T. Komatsu.** 1998. Does nitric oxide play a critical role in viral infections? J. Virol. **72:**4547–4551.
48. **Reuter, J. D., B. E. Vivas-Gonzalez, D. Gomez, J. H. Wilson, J. L. Brandsma, H. L. Greenstone, J. K. Rose, and A. Roberts.** 2002. Intranasal vaccination with a recombinant vesicular stomatitis virus expressing cottontail rabbit papillomavirus L1 protein provides complete protection against papillomavirus-induced disease. J. Virol. **76:**8900–8909.
49. **Roberts, A., L. Buonocore, R. Price, J. Forman, and J. K. Rose.** 1999. Attenuated vesicular stomatitis viruses as vaccine vectors. J. Virol. **73:**3723–3732.
50. **Roberts, A., E. Kretzschmar, A. S. Perkins, J. Forman, R. Price, L. Buonocore, Y. Kawaoka, and J. K. Rose.** 1998. Vaccination with a recombinant vesicular stomatitis virus expressing an influenza virus hemagglutinin provides complete protection from influenza virus challenge. J. Virol. **72:**4704–4711.
51. **Rose, N. F., P. A. Marx, A. Luckay, D. F. Nixon, W. J. Moretto, S. M. Donahoe, D. Montefiori, A. Roberts, L. Buonocore, and J. K. Rose.** 2001. An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. Cell **106:**539–549.
52. **Rubin, S. A., M. Pletnikov, and K. M. Carbone.** 1998. Comparison of the neurovirulence of a vaccine and a wild-type mumps virus strain in the developing rat brain. J. Virol. **72:**8037–8042.
53. **Rubio, C., C. Kolakofsky, V. M. Hill, and D. F. Summers.** 1980. Replication and assembly of VSV nucleocapsids: protein association with RNPs and the effects of cycloheximide on replication. Virology **105:**123–135.
54. **Sabin, A., and P. Olitsky.** 1938. Influence of host factors on neuroinvasiveness of vesicular stomatitis virus. J. Exp. Med. **67:**201–227.
55. **Schnell, M. J., L. Buonocore, E. Boritz, H. P. Ghosh, R. Chernish, and J. K. Rose.** 1998. Requirement for a non-specific glycoprotein cytoplasmic domain sequence to drive efficient budding of vesicular stomatitis virus. EMBO J. **17:**1289–1296.
56. **Thomas, D., W. W. Newcomb, J. C. Brown, J. S. Wall, J. F. Hainfeld, B. L. Trus, and A. C. Steven.** 1985. Mass and molecular composition of vesicular stomatitis virus: a scanning transmission electron microscopy analysis. J. Virol. **54:**598–607.
57. **Villarreal, L. P., M. Breindl, and J. J. Holland.** 1976. Determination of molar ratios of vesicular stomatitis virus induced RNA species in BHK21 cells. Biochemistry **15:**1663–1667.
58. **Wagner, R. R.** 1974. Pathogenicity and Immunogenicity for Mice of Temperature-Sensitive Mutants of Vesicular Stomatitis Virus. Infect. Immun. **10:**309–315.
59. **Wang, Z. W., L. Sarmento, Y. Wang, X. Q. Li, V. Dhingra, T. Tseggai, B. Jiang, and Z. F. Fu.** 2005. Attenuated rabies virus activates, while pathogenic rabies virus evades, the host innate immune responses in the central nervous system. J. Virol. **79:**12554–12565.
60. **Wertz, G. W., V. P. Perepelitsa, and L. A. Ball.** 1998. Gene rearrangement attenuates expression and lethality of a nonsegmented negative strand RNA virus. Proc. Natl. Acad. Sci. USA **95:**3501–3506.
61. **Whelan, S. P., L. A. Ball, J. N. Barr, and G. T. Wertz.** 1995. Efficient recovery of infectious vesicular stomatitis virus entirely from cDNA clones. Proc. Natl. Acad. Sci. USA **92:**8388–8392.
62. **Wyatt, L. S., B. Moss, and S. Rozenblatt.** 1995. Replication-deficient vaccinia virus encoding bacteriophage T7 RNA polymerase for transient gene expression in mammalian cells. Virology **210:**202–205.

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Cytoplasmic Tail Truncation Stabilizes S1-S2 Association and Enhances S Protein Incorporation into SARS-CoV-2 Pseudovirions

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ABSTRACT Truncations of the cytoplasmic tail (CT) of entry proteins of enveloped viruses dramatically increase the infectivity of pseudoviruses (PVs) bearing these proteins. Several mechanisms have been proposed to explain this enhanced entry, including an increase in cell surface expression. However, alternative explanations have also been forwarded, and the underlying mechanisms for the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) S protein remain undetermined. Here, we show that the partial or complete deletion of the CT (residues 19 to 35) does not modify SARS-CoV-2 S protein expression on the cell surface when the S2 subunit is measured, whereas it is significantly increased when the S1 subunit is measured. We also show that the higher level of S1 in these CT-truncated S proteins reflects the decreased dissociation of the S1 subunit from the S2 subunit. In addition, we demonstrate that CT truncation further promotes S protein incorporation into PV particles, as indicated by biochemical analyses and cryo-electron microscopy. Thus, our data show that two distinct mechanisms contribute to the markedly increased infectivity of PVs carrying CT-truncated SARS-CoV-2 S proteins and help clarify the interpretation of the results of studies employing such PVs.

IMPORTANCE Various forms of PVs have been used as tools to evaluate vaccine efficacy and study virus entry steps. When PV infectivity is inherently low, such as that of SARS-CoV-2, a CT-truncated version of the viral entry glycoprotein is widely used to enhance PV infectivity, but the mechanism underlying this enhanced PV infectivity has been unclear. Here, our study identified two mechanisms by which the CT truncation of the SARS-CoV-2 S protein dramatically increases PV infectivity: a reduction of S1 shedding and an increase in S protein incorporation into PV particles. An understanding of these mechanisms can clarify the mechanistic bases for the differences observed among various assays employing such PVs.

KEYWORDS cytoplasmic tail, entry, infectivity, pseudovirus, S protein, S1 shedding, SARS-CoV-2, spike density

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) encodes 15 nonstructural proteins as well as 4 structural proteins. Of these, only the structural proteins, spike (S), membrane (M), envelope (E), and nucleocapsid (N), are incorporated into the virion. The N protein is essential for the encapsidation of the 30 kb positive-sense RNA genome, the M and E proteins contribute to virus assembly and budding via interactions with other viral proteins (1, 2), and the S protein mediates entry into the target cell.

For successful entry, the S protein needs to be activated by two sequential proteolytic cleavages. The first cleavage divides the S protein into the S1 and S2 subunits, of

Editor Tom Gallagher, Loyola University Chicago

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The authors declare no conflict of interest.

Received 20 October 2022
Accepted 23 December 2022
Published 15 February 2023

which S1 binds the receptor angiotensin-converting enzyme 2 (ACE2) and S2 mediates membrane fusion (3). In the case of the first SARS-CoV that emerged 2 decades ago, cleavage at the S1-S2 junction is accomplished in target cells by a cell surface protease, TMPRSS2, or lysosomal proteases, cathepsins (4–8). In contrast, for SARS-CoV-2, cleavage is carried out by furin, a Golgi-resident protease, in infected cells during virus maturation. The second cleavage occurs at a site internal to the S2 subunit, termed the S2′ site, and is carried out by TMPRSS2 or cathepsins for both SARS-CoV and SARS-CoV-2 when they reach the target cells. This second cleavage releases the fusion peptide that is required for subsequent fusion between the cellular and viral membranes (3).

Like SARS-CoV-2, the entry glycoproteins of many viruses are cleaved into the surface and transmembrane subunits prior to virus release from infected cells. For most of these viruses, the two subunits remain associated until the receptor-binding domain (RBD) located in the surface subunit binds the receptor. Receptor binding induces conformational changes in the entry glycoprotein and leads to the dissociation of the surface subunit and subsequent membrane fusion mediated by the transmembrane subunit. In the case of the original Wuhan-Hu-1 strain of SARS-CoV-2, the S1-S2 association is weak, and thus, S1 was easily shed from the spikes (9). To overcome this problem, the virus acquired the D614G mutation early in the pandemic, which stabilized the S1-S2 association and increased virus infectivity (9–12).

Another modification of the SARS-CoV-2 S protein that increases virus infectivity is cytoplasmic tail (CT) truncation. The understanding that CT truncation of viral entry glycoproteins enhances pseudovirus (PV) infectivity originated decades ago from intriguing observations that lentiviruses grown in human T cell lines acquired a premature stop codon in their CTs (13–16). Multiple subsequent studies reported that CT truncations of the entry glycoproteins of various viruses enhanced PV infectivity (17–21). We also showed that a 19-amino-acid truncation of the SARS-CoV S protein enhanced PV infectivity (21). Mechanistic studies showed that CT truncation upregulated the cell surface expression of the entry glycoproteins (20, 22). These observations have been interpreted to suggest that the removal of the endoplasmic reticulum (ER) retention signal present at the carboxy-terminal end of the CT was responsible for the elevated expression on the cell surface. However, other studies did not observe increased expression of the CT-truncated glycoproteins on the cell surface (23, 24). In addition, even if an increase was observed, the degree of the increase was modest, and thus, it is difficult to explain the dramatic changes in PV infectivity. An alternative mechanism, a conformational change in the ectodomain, was also proposed for various enveloped viruses to explain the increased fusogenicity upon CT truncation of their entry glycoproteins (20, 23, 25–27).

Here, we show that CT truncation of the SARS-CoV-2 S protein modestly increases cell surface expression when both S1 and S2 are measured, but no increase is observed when only S2 is detected. When only S1 is detected, however, CT-truncated S protein expression on the cell surface is substantially elevated. Further investigation shows that these differences are contributed by reduced S1 shedding in the CT-truncated S protein. We also demonstrate through biochemical and cryo-electron microscopy (cryo-EM) studies that PVs bearing the CT-truncated S protein exhibit much higher spike densities. Together, our studies show that CT truncation of the S protein enhances PV infectivity by decreasing S1 dissociation and increasing S protein incorporation into PV particles.

RESULTS

Cytoplasmic tail truncation of the S protein does not change SARS-CoV-2 PV production but dramatically enhances PV infectivity. The CT of the SARS-CoV-2 S protein consists of 37 amino acids that contain four motifs, as shown in Fig. 1A: a putative ER retention signal (KXHXX) (28, 29), a charged cluster (KFDEDDSE) (30), and two cysteine-rich motifs (CRMs), CCSCGSCC and SCCSC (30–32). To better understand the role of the CT in SARS-CoV-2 infectivity, we made truncation variants of the S protein (S-dCTs) in which these motifs were sequentially deleted, and we compared them to

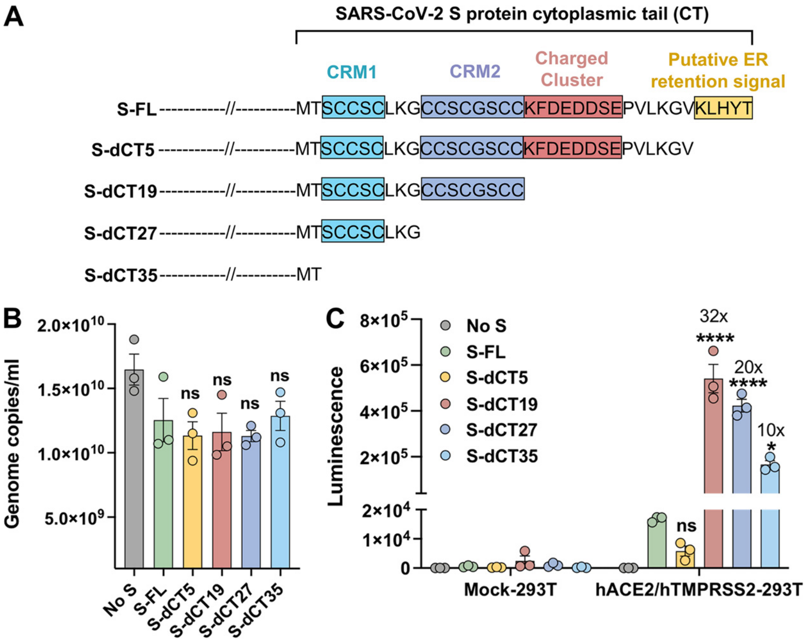

FIG 1 Cytoplasmic tail truncation of the S protein does not change SARS-CoV-2 PV production but dramatically enhances PV infectivity. (A) Diagram representing the CT truncation variants of the S protein used in this study. (B) MLV PVs bearing the full-length S protein (S-FL) or its CT truncation variants (S-dCTs) and expressing firefly luciferase were produced from HEK293T cell transfection, and their titers were quantified by RT-qPCR. (C) Infectivity of the same PVs in parental HEK293T cells or the same cells expressing human ACE2 and TMPRSS2. Cells were infected for 1 h with 5 × 10^8 genome copies per well in a 48-well plate, and infection levels were assessed by measuring luminescence at 24 h postinfection. Panels B and C show mean values ± SEM from three independent experiments conducted with three independently prepared PVs, and statistical significance was calculated by two-way ANOVA using Sidak's multiple-comparison test (*, $P < 0.05$; ****, $P < 0.0001$; ns, not significant). CRM, cysteine-rich motif.

the full-length S protein (S-FL). PVs expressing firefly luciferase (FLuc) and bearing these S-dCTs (PV-dCTs) were produced from HEK293T cell transfection. PVs bearing S-FL (PV-FL) or no S protein were used as controls. To assess the effect of CT truncation on the PV yield, PV titers were quantified by reverse transcription-quantitative PCR (RT-qPCR). Figure 1B shows that these PVs were produced at comparable levels, indicating that CT truncation did not affect PV production.

Next, we assessed their entry efficiency by infecting HEK293T cells stably expressing human ACE2 and TMPRSS2 (hACE2/hTMPRSS2-293T cells) with the same genome copy numbers. HEK293T cells transduced with empty vectors but selected with drugs in the same way as for hACE2/hTMPRSS2-293T cells were used as negative controls for infection (Mock-293T cells). As shown in Fig. 1C, S-dCT5 unexpectedly decreased PV entry compared to S-FL, indicating that the KLHYT motif, a putative ER retention signal, may play its role only in the context of other motifs. In contrast, S-dCT19 increased PV entry by more than 30-fold, which is consistent with previous reports that a deletion of the last 13 to 21 amino acids from the carboxy terminus of the CT enhances PV infectivity (23, 24, 33, 34). S-dCT27 and S-dCT35, which lack one or both CRMs, respectively, also increased PV entry compared to S-FL but to a lesser degree than did S-dCT19, which contains both CRMs (Fig. 1C). Together, these data confirm that CT truncations, except dCT5, substantially enhance the PV entry efficiency and suggest that although the CRMs may play a role in PV infectivity, they are not essential for either PV production or entry.

Cytoplasmic tail truncation does not increase S2 levels but significantly increases S1 levels on the cell surface. Whereas most viruses that are commonly used as a backbone for pseudotyping systems, including human immunodeficiency virus type 1 (HIV-1), murine leukemia virus (MLV), and vesicular stomatitis virus (VSV), bud from the plasma membrane, SARS-CoV-2 buds from the membranes of the ER or the ER-Golgi intermediate compartment (ERGIC) (35). The S protein is therefore designed to be expressed in the intracellular compartments, but when its expression level is high, it is also trafficked to the plasma membrane. Because PV production could benefit from increased S protein expressed on the plasma membrane, we investigated whether CT truncation increased S protein expression on the cell surface. We expressed S-FL and S-dCTs on HEK293T cells via transfection and detected them using convalescent-phase plasma samples derived from coronavirus disease 2019 (COVID-19) patients. Note that whereas all COVID-19 convalescent-phase plasma samples efficiently recognize the S2 subunit, they rarely recognize the S1 subunit (Fig. 2A), likely because the original SARS-CoV-2 did not have much S1 remaining on the virion, owing to its shedding (9). When plasma sample 2, which recognizes both S1 and S2, was used for detection, all S-dCTs, except S-dCT5, were expressed at modestly higher levels than S-FL; albeit modest, the difference was statistically significant (Fig. 2B). However, when plasma sample 9, which recognizes only S2, was used, S-FL and all S-dCTs, except S-dCT5, were expressed at comparable levels (Fig. 2C). Because these data suggest that different S1 levels of S-FL and S-dCTs are responsible for the disparate detection profiles of plasma samples 2 and 9, we also detected S1 alone. To detect only S1, we used hACE2-NN-Ig, the human ACE2 ectodomain that contains enzyme activity-null mutations (H374N and H378N) and is fused to the Fc region of human IgG1 (hIgG1) (21). Figure 2D shows that the S1 levels of S-dCTs, except S-dCT5, detected by hACE2-NN-Ig were approximately 4-fold higher than that of S-FL. These data show that the differences between S-FL and S-dCTs in cell surface expression levels result from their differences in S1, but not S2, contents and that S-dCTs have much higher S1 contents in the spike trimers than S-FL. These data also provide an explanation for the conflicting observations made in previous reports on the cell surface expression of CT-truncated glycoproteins (20, 22–24). Our results demonstrate that depending on which component of the glycoprotein is measured, the expression levels of the CT-truncated glycoproteins could appear to be increased or not increased.

The higher S1 levels observed with S-dCTs than with S-FL prompted us to hypothesize that CT truncation may strengthen the S1-S2 association and, consequently, decrease S1 dissociation from S2. However, because higher hACE2-NN-Ig binding to S-dCTs can result from either a decrease in S1 shedding or an increase in the RBD-up conformation, the conformation that binds the receptor (36–38), we attempted to distinguish these two possibilities by comparing the binding of hACE2-NN-Ig to that of an antibody recognizing the N-terminal domain (NTD) of S1. As this antibody binds the NTD, its binding is unlikely to be affected by the change in the RBD conformation. HEK293T cells were transfected with increasing amounts of an S-dCT19 or S-FL plasmid, their cell surface levels of S1 were measured using hACE2-NN-Ig or the NTD antibody, and their S2 levels were measured using plasma sample 9. Both the hACE2-NN-Ig and NTD antibodies detected severalfold-higher levels of S1 in S-dCT19 than in S-FL at all expression levels (Fig. 2E and F), while the S2 levels of S-dCT19 and S-FL were similar when measured using plasma sample 9 (Fig. 2G). The very similar detection profiles with hACE2-NN-Ig and the NTD antibody of S-FL and S-dCT19 suggest that the RBD-up or -down conformation does not significantly contribute to the differences in the S1 levels but that reduced S1 shedding is likely the major source of the observed S1 differences between S-FL and S-dCTs induced by CT truncation.

In contrast to the other S-dCTs, a low level of S-dCT5 was detected on the cell surface when measured using convalescent-phase plasma sample 2 (Fig. 2B). We thus assessed S-dCT5 expression inside the cell. As the relative level of S-dCT5 compared to other S proteins in permeabilized cells was similar to that on the cell surface (Fig. 2H), we further assessed the S-dCT5 level in cell lysates by Western blotting (WB). Because plasma sample 2 does not efficiently detect S protein in cell lysates, while it does in PVs, a polyclonal anti-S antibody was used for blotting. As shown in Fig. 2I, while comparable levels of the uncleaved S band were observed for all S proteins, indicating that S-dCT5 is expressed comparably to the others, much lower levels of the S1 and S2 bands were observed for S-dCT5, suggesting that they may be rapidly degraded once cleaved. Because this antibody detects S1 only very weakly, we used the NTD antibody to better visualize the S1 bands. Figure 2J shows that the S1 bands in all S-dCTs, except

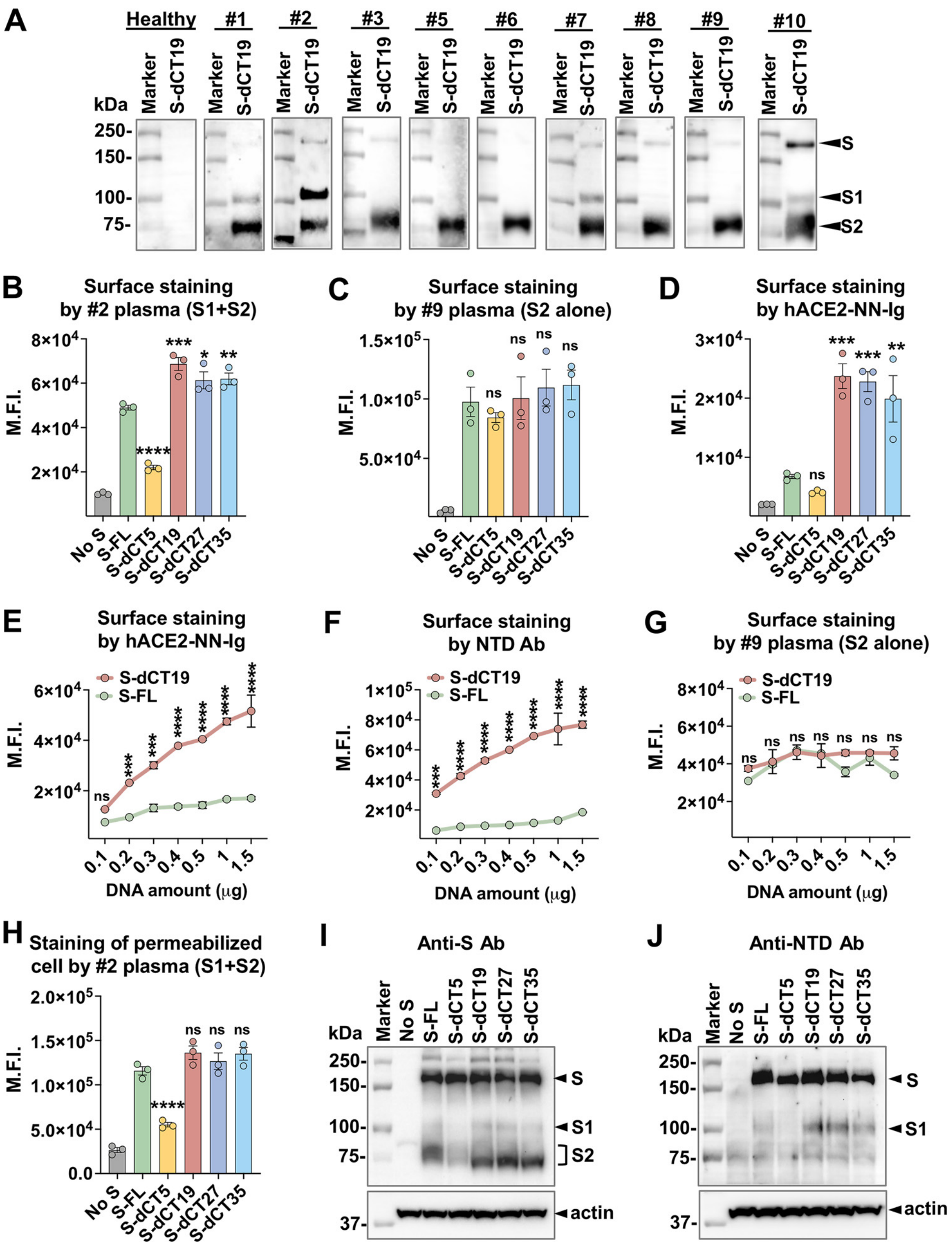

FIG 2 Cytoplasmic tail truncation does not increase S2 levels but significantly increases S1 levels on the cell surface. (A) Ten convalescent-phase plasma samples derived from individuals infected with SARS-CoV-2 early in the pandemic were screened by Western blot analyses for their ability to recognize the S1 and S2 bands of sucrose-pelleted PV-dCT19. (B to D) HEK293T cells grown on 6-well plates were transfected with 0.3 μg of a plasmid encoding the indicated S-FL or S-dCT protein, and their cell surface expression levels were assessed at 42 h posttransfection using plasma sample 2 (at a 1:200 dilution), which recognizes both S1 and S2 (B); plasma sample 9 (at a 1:200 dilution), which recognizes only S2 (C); or 3 μg/mL of hACE2-NN-Ig, which binds only S1 (D). Shown are the mean fluorescence intensity (M.F.I.) values ± SEM from three independent experiments. (E to G) HEK293T cells on 6-well plates were transfected with the indicated amounts of the plasmid encoding S-FL or S-dCT19, and cell surface staining was conducted with 3 μg/mL hACE2-NN-Ig (E), 3 μg/mL NTD antibody (Ab) (F), or plasma sample 9 (at a 1:200 dilution) recognizing only S2 (G). (H) Experiment similar to the one for panel B except that staining was conducted in cells permeabilized with 0.1% saponin. (I and J) S protein expression levels were assessed in cells lysed with dodecyl maltopyranoside and blotted with rabbit anti-S antibody (I) or the same NTD antibody used for panel F (J). The average mean fluorescence intensity values ± SEM from three independent experiments are shown. Statistical significance was analyzed by one-way ANOVA using Dunnett's multiple-comparison test (B to D) or two-way ANOVA using Sidak's multiple-comparison test (E to G) (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$; ****, $P < 0.0001$; ns, not significant).

S-dCT5, are stronger than that in S-FL. These data are consistent with our conclusion drawn from cell surface expression and confirm that S-dCTs, except S-dCT5, retain higher levels of S1 than S-FL owing to reduced S1 shedding.

Cytoplasmic tail truncation dramatically enhances functional S protein incorporation into PV particles. As the S protein mediates receptor attachment, the quantity and quality of spike trimers on the virion determine virus and PV infectivity. Thus, we measured the S protein density on PV-FL and PV-dCTs, which were pelleted through a sucrose layer ("sucrose-pelleted PV"), by WB analyses, using plasma sample 2, which recognizes both S1 and S2 (Fig. 2A). When the same numbers of PV particles were analyzed, as supported by the comparable amounts of p30, the MLV Gag protein, the intensities of the S1 and S2 bands of all PV-dCTs, except PV-dCT5, were dramatically increased compared to those of PV-FL (Fig. 3A, left), which were barely detectable only with a much longer exposure of the same blot (Fig. 3A, right). These data demonstrate that CT truncation leads to the much more efficient incorporation of the S protein into PV particles.

To confirm that the increased intensities of the S1 and S2 bands of PV-dCTs assessed by WB analyses (Fig. 3A and B) actually reflect an increased spike density on the virion, we visualized PV-FL and PV-dCT19 by cryo-electron microscopy (cryo-EM). We focused on PV-dCT19 because it exhibits the highest infectivity and virion spike density. Examined by cryo-EM, both PV-FL and PV-dCT19 were quite heterogeneous with respect to their spike densities. Although PV-dCT19 particles generally exhibited higher spike densities than PV-FL particles, to analyze them in a semiquantitative way, we sorted PV-FL and PV-dCT19 cryo-EM particles into high-, medium-, and low (or bald)-spike-coverage groups (Fig. 3C). Three cryo-EM images, each representing the three categories, are shown in Fig. 3C, left. Using these criteria, cryo-EM images of 61 PV-FL and 88 PV-dCT19 particles (see Fig. S1 in the supplemental material) were sorted with four independent counts. Note that we found only one high-density and two medium-density PV-FL particles. Most PV-FL particles belonged to the low-surface-density group. Specifically, 1.3% and 7.1% of the PV-FL particles belonged to the high- and medium-density groups, respectively, while the majority, 91.6%, had low or undetectable levels of spike (Fig. 3D). In contrast, PV-dCT19 virions were more evenly distributed among the high-, medium-, and low-surface-density groups (Fig. 3D): high at 25.4%, medium at 40.6%, and low/bald at 34.0%. These cryo-EM data are consistent with the WB results and clearly demonstrate that PV-FL overall has a much lower virion spike density than does PV-dCT19.

Cytoplasmic tail truncation significantly enhances functional S protein on PV particles. Much higher S1 levels were detected on PV-dCTs (Fig. 3A and B) as well as S-dCTs on the cell surface. To confirm that the higher level of S1 indeed reflects the more efficient retention of S1 on PV-dCTs, we analyzed the S1/S2 ratio of PV-dCTs and PV-FL. Because of the much lower S density of PV-FL, a large amount of PV-FL was compared to a much smaller amount of PV-dCTs in order to detect both the S1 and S2 bands in both PVs. As Fig. 4A and its quantification in Fig. 4B show, the S1/S2 ratio of PV-dCT19 is approximately 2.5-fold higher than that of PV-FL. This result is consistent with the differences that we observed with cell surface-expressed S-FL and S-dCTs (Fig. 2D, E, and F) and again indicates that CT truncation contributes to the greater retention of S1.

To understand the three-dimensional organization of the S protein on the surface of PVs, we performed cryo-EM tomography with PV-FL and PV-dCT19 (Fig. 4 and Fig. S2). On some of the PV surfaces, we were able to distinguish the prefusion trimers, which look like half-blossomed flowers, from the postfusion trimers, which look like thin sticks, as a result of S1 shedding and the conversion of S2 to a helical-bundle conformation (Fig. 4C) (37–39). Sequentially slabbing through the three-dimensional tomogram slices, we observed that most PV-FL particles have extremely low coverage of surface proteins and that the majority of the spike trimers are in the postfusion conformation, with a subset being in the prefusion conformation (Fig. 4D). In contrast, on PV-dCT19, prefusion spikes can be abundantly identified amid closely spaced clusters

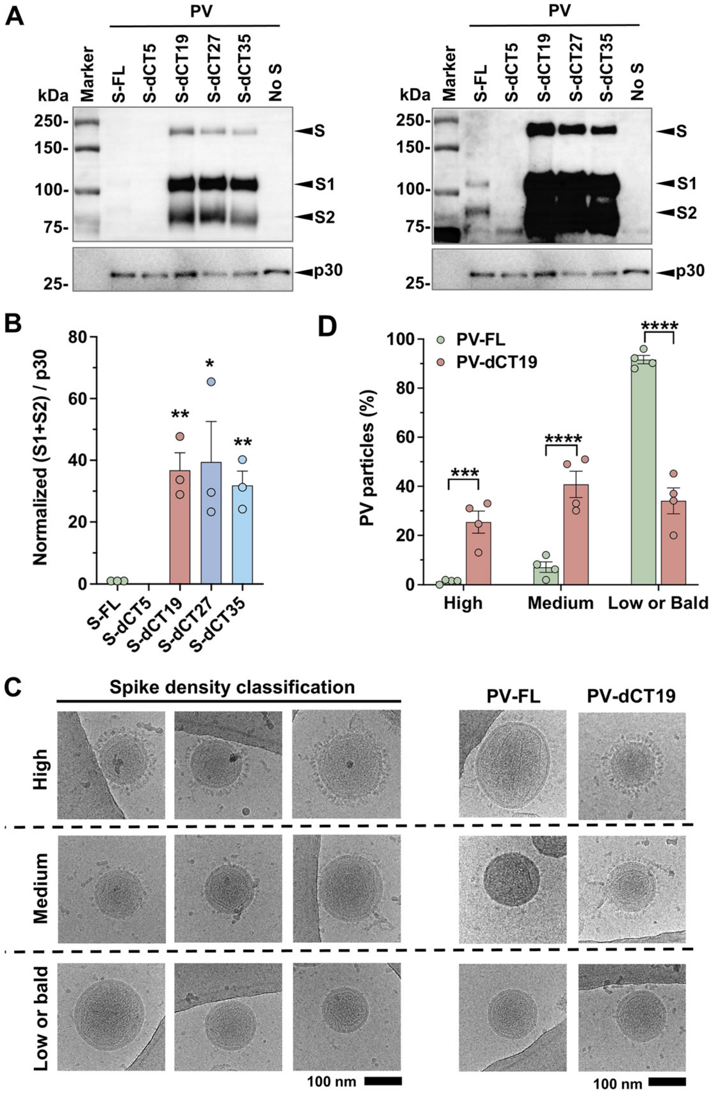

FIG 3 Cytoplasmic tail truncation dramatically enhances S protein incorporation into PV particles. (A) PVs bearing the indicated S variants and produced from HEK293T cell transfection were pelleted through a layer of 30% of sucrose and analyzed by Western blotting. The S, S1, and S2 bands were detected using convalescent-phase plasma sample 2 at a 1:200 dilution. (Left) Blot representative of results from three independent experiments. The S1 and S2 bands of S-FL and S-dCT5 are too weak to be detected. (Right) A much longer exposure of the same blot shown on the left. The S1 and S2 bands of S-FL, but not those of S-dCT5, are visible. (B) Sum of the S1 and S2 band intensities of the Western blot shown in panel A normalized to the intensity of the p30 band in the same blot. Shown are the averages from three independent experiments. (C, left) As both PV-FL and PV-dCT19 exhibit heterogeneous spike densities, to provide an objective guideline for semiquantitative evaluation, three representative cryo-EM images were selected for each of the high-, medium-, and low/bald-spike-density groups. (Right) Images selected separately from PV-FL and PV-dCT19 for each category. Note that there are only one high-density and a few medium-density particles among 61 PV-FL particles. (D) A total of 149 PV particles (61 PV-FL and 88 PV-dCT19 [shown in Fig. S1 in the supplemental material]) were evaluated by four individuals and categorized into the high-, medium-, and low/bald-density groups using the criteria described above for panel C. Mean values ± SEM are shown. Statistical significance was analyzed by an unpaired *t* test (B) and two-way ANOVA using Sidak's multiple-comparison test (D) (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.005$; ****, $P < 0.0001$).

of proteins on the PV surface resolved in three dimensions (Fig. 4E). While the high density of protein on the PV-dCT19 particles could mask the presence of some postfusion spikes, and only a portion of the spike conformations are clearly identifiable, many spike trimers appear to be in the prefusion conformation. The higher abundance of prefusion spike trimers on PV-dCT19 is consistent with the presence of intact, functional S-dCTs on the cell surface (Fig. 2) and the high S1/S2 ratios observed in WB

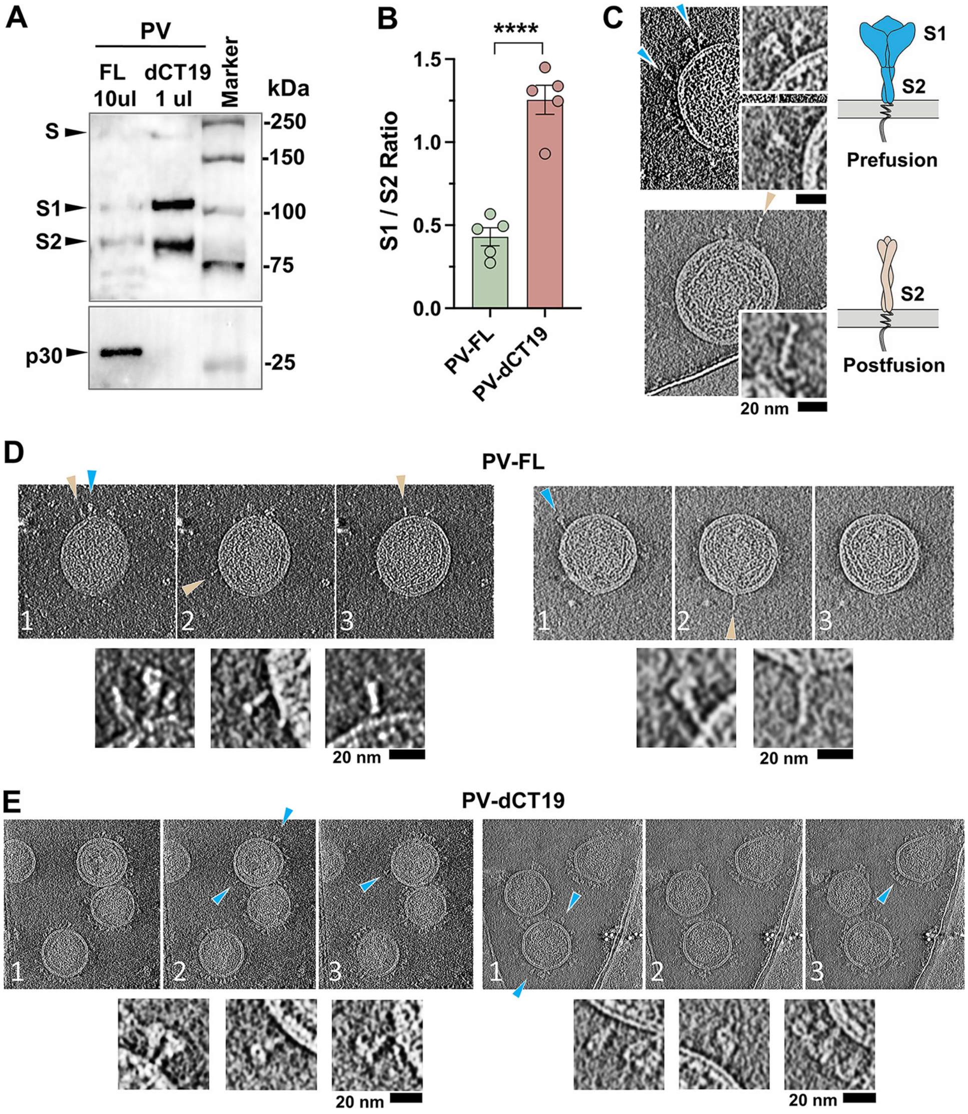

FIG 4 Cytoplasmic tail truncation significantly enhances functional S protein on PV particles. (A) The same PVs shown in Fig. 3A but with PV-FL compared to 10-times-less PV-dCT19 to more accurately measure their S1/S2 ratios. (B) Mean S1/S2 ratios ± SEM of PV-FL and PV-dCT19 analyzed from five Western blots performed with three sets of independently prepared PV batches. Statistical significance was analyzed by an unpaired *t* test (****, $P < 0.0001$). (C) Prefusion (top) (blue arrows) and postfusion (bottom) (light-brown arrow) conformations of spike trimers on sucrose-pelleted PVs examined by cryo-EM tomography. Schematic representations of these two spike conformations are presented at the right. (D) Sequential slices, indicated by the numbers at the bottom left, of PV-FL cryo-electron tomograms, with a mix of prefusion (blue arrows) and postfusion (light-brown arrows) S conformations, enlarged at the bottom. (E) Sequential slices, indicated by the numbers at the bottom left, of PV-dCT19 cryo-electron tomograms, with mostly the prefusion (blue arrows) S conformation, enlarged at the bottom. See also Fig. S2 in the supplemental material.

analyses of PV-dCTs relative to PV-FL (Fig. 4A). Taken together, these data demonstrate that CT truncation enhances S protein incorporation into PVs and S1 retention in the spike, leading to much higher numbers of prefusion spikes on the pseudovirion, thus providing an explanation for the dramatic increase in PV-dCT infectivity.

DISCUSSION

CT truncation of virus entry glycoproteins has been widely used to enhance the infectivity of various PVs. Although several potential explanations were offered, the underlying mechanism for the greatly enhanced PV infectivity is still unclear. Increased cell surface expression of viral glycoproteins upon CT truncation was proposed as an

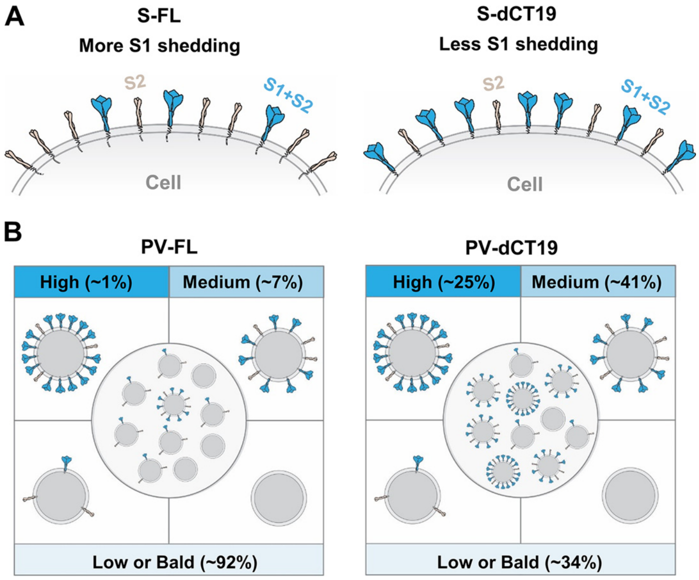

FIG 5 Schematic representation of increased functional spikes on the cell surface and in pseudoviruses upon CT truncation of the S protein. (A) Higher levels of functional S-dCTs are observed on the cell surface, while S2 levels are similar between S-dCTs and S-FL. Functional spikes (in blue), assessed using ACE2-NN-Ig or NTD antibody (Fig. 2D to F), contain both S1 and S2 in the prefusion conformation. Nonfunctional spikes (in light brown), measured using plasma sample 9, which recognizes only S2 (Fig. 2C and G), consist of only S2 in the postfusion conformation. (B) PV-dCTs exhibit much higher levels of functional spikes than PV-FL. The diagrams of four PVs represent those with a high, medium, or low spike density or bald virions. The group of 10 PV particles in the center for PV-FL and PV-dCTs reflects the approximate composition of each PV population with respect to the virion spike density. The proportion of each spike density group in the PV-FL and PV-dCT19 populations was assessed using cryo-EM micrographs (Fig. 3C and D; see also Fig. S1 in the supplemental material).

explanation for the enhanced PV infectivity, but conflicting results were also reported (20, 22–24). Furthermore, even if cell surface expression is increased, the degree of the increase is modest and thus would not be sufficient to explain the dramatic change in PV infectivity. Increased fusogenicity of the glycoproteins induced by CT truncation was also proposed for measles, murine leukemia, vesicular stomatitis, and Nipah viruses (18, 20, 23, 25–27, 33), and a conformational change in the ectodomain induced by inside-out signaling was proposed to explain such increased fusogenicity (20, 25, 27).

Our study here shows that the cell surface expression of CT-truncated SARS-CoV-2 S protein could lead to different outcomes depending on which subunit, S1, S2, or both, is measured (Fig. 5A). If S2 is measured, no significant difference is detected, while a modest increase is observed if both S1 and S2 are measured. However, if only S1 is measured, a much higher S1 level is observed with S-dCT19, -27, and -35 than with S-FL. These data provide an explanation for the conflicting reports on the cell surface expression of the CT-truncated viral entry glycoproteins (20, 22–24).

Our study further identifies two different mechanisms underlying the dramatically increased infectivity of SARS-CoV-2 PVs carrying a CT truncation. Through biochemical and cryo-electron microscopy, we show that CT truncation promotes S protein incorporation into PVs and enhances S1 retention on the S protein. These two events together result in much higher numbers of functional spikes in PV-dCT19, -27, and -35 (Fig. 5B), leading to a dramatic increase in their infectivity. Our results on the increased S1 retention on S-dCTs are consistent with previous reports in which increased fusogenicity upon CT truncation was observed for the entry glycoproteins of various viruses (18, 25–27). Those studies were conducted using syncytium formation assays, and thus, a higher level of the surface subunit of a glycoprotein expressed on the cell surface would have resulted in more efficient syncytium formation. Although further studies are necessary, the increased S1 retention on S-dCTs may arise from the enhanced flexibility of the S2 subunit, which accommodates a more stable S1-S2 interaction.

Our data also show that the differences in spike density induced by CT truncation are much greater in PVs than on the cell surface; while the S2 levels are similar between S-FL and S-dCTs on the cell surface (Fig. 2C), they are much higher in PV-dCTs than in PV-FL (Fig. 3A). A higher spike density in PV-dCTs would be possible if S-dCTs are more enriched in lipid rafts than S-FL because many viruses bud from this microdomain. However, S-dCT27 and S-dCT35 lack some or all of the motifs for palmitoylation, a modification that promotes protein trafficking into lipid rafts (40), and thus, it is unlikely that S-dCTs are enriched in lipid rafts and more efficiently incorporated into the virion. A more likely explanation is that S-dCTs can be more freely incorporated into PVs because they lack the binding motif for Ezrin-Moesin-Radixin proteins that anchor membrane proteins to the cellular cytoskeleton (41). An alternative explanation is that the steric hindrance formed between the structural proteins of PVs (Gag or matrix protein) and the large CT of S-FL makes it difficult to be incorporated into PVs, whereas the smaller CT of S-dCTs allows more efficient incorporation into PVs, as we previously proposed (21).

MATERIALS AND METHODS

Plasmids. The SARS-CoV-2 S protein gene was codon optimized and synthesized by Integrated DNA Technologies based on the protein sequence (Wuhan-Hu-1 strain [GenBank accession number YP_009724390]) and cloned into the pCAGGS vector (42). The genes for cytoplasmic-tail-truncated variants of the SARS-CoV-2 S protein were also synthesized and cloned into the pCAGGS vector. None of these genes contain a tag. The retroviral vector pQCXIX (Clontech), encoding enhanced green fluorescent protein (eGFP) or firefly luciferase (FLuc), and the plasmid expressing the MLV Gag and Pol proteins or the G protein of VSV (VSV-G) were previously described (43). An hTMPRSS2 expressor plasmid was constructed by cloning its residues 1-492 (GenBank accession number NP_005647) into the retroviral vector pQCXIB (44). The hACE2 expressor plasmid was constructed by cloning its residues 20 to 805 (GenBank accession number NM_021804) downstream of the mouse ACE2 signal sequence (MSSSSWLLLSLVAVTTAQS) and the Myc tag sequence into the retroviral vector pQCXIP. The expression plasmid for hACE2-NN-Ig was previously described (45): the hACE2 ectodomain fragment (residues 20 to 615 [GenBank accession number NM_021804]) containing the H374N and H378N mutations was cloned into pcDNA3.1 containing the CD5 signal sequence and the Fc region of human IgG1.

Cells. Human embryonic kidney HEK293T cells were obtained from the ATCC and maintained in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO_2. HEK293T cells transduced to stably express hACE2 and hTMPRSS2 (hACE2/hTMPRSS2-293T cells) or mock transduced (Mock-293T cells) were selected and maintained in medium supplemented with 1 μg/mL puromycin and 10 μg/mL blasticidin (InvivoGen). The transduction vectors for hACE2 and hTMPRSS2 were produced by transfecting pQCXIP-hACE2 or pQCXIB-hTMPRSS2 into HEK293T cells together with the plasmid encoding murine leukemia virus Gag-Pol and the plasmid encoding the G protein of vesicular stomatitis virus. The vector for mock transduction was produced similarly using the empty pQCXIP or pQCXIB plasmid.

MLV PV production and sucrose pelleting. HEK293T cells at ~60% confluence in T75 flasks were transfected using a calcium-phosphate method with 24 μg of total DNA at a ratio of 5:5:1 (by mass) of the retroviral vector pQCXIX encoding eGFP or FLuc, the plasmid expressing MLV Gag and Pol proteins, and the plasmid expressing either the full-length S protein or the truncated version of the S protein of SARS-CoV-2 (Wuhan-Hu-1). Transfected cells were washed at 6 h posttransfection and replenished with 10 mL DMEM supplemented with 10% FBS. The PV-containing culture supernatants were collected at 43 h posttransfection, cleared through 0.45-μm filters, and either immediately aliquoted and stored at −80°C or used for entry experiments.

For sucrose-pelleted PV preparation, 10 mL of the cleared culture supernatants containing PVs precleared by centrifugation in a TX-400 swinging-bucket rotor at 3,000 rpm for 10 min followed by filtration through 0.22-μm filters was loaded onto 2 mL of 30% sucrose (catalog number S7903; Sigma-Aldrich) in NTE buffer (120 mM NaCl, 20 mM Tris, 2 mM EDTA [pH 8.0]) and centrifuged at 50,000 $\times$ g in an SW41 rotor for 2 h at 10°C. The PV pellets were resuspended in 20 μL NTE buffer with gentle shaking on ice and either used immediately or aliquoted and frozen at −80°C.

PV quantification. PVs were quantified by RT-qPCR using primers and a probe that target the cytomegalovirus (CMV) promoter. Culture supernatants containing PVs were treated with 100 μg/mL RNase A for 1 h at 37°C to degrade RNAs that were not packaged inside the virion, and RNA was extracted with TRIzol and GlycoBlue coprecipitant and digested for 30 min at 37°C with DNase I at 1 IU per 1 μg extracted RNA. DNase I was inactivated by incubation for 10 min at 65°C with EDTA added to a final concentration of 5 mM. DNase-treated RNA was reverse transcribed using a high-capacity cDNA reverse transcription kit (catalog number 4374966; Applied Biosystems). qPCR was performed, using Luna universal probe qPCR master mix (catalog number M3004E; New England BioLabs) with a known quantity of the pQCXIX vector (Clontech), to generate standard curves, and data were collected with CFX Manager 3.1 (Bio-Rad). The primers and probe were synthesized by Integrated DNA Technologies (sense primer 5′-TCACGGGGATTTCCAAGTCTC-3′, antisense primer 5′-AATGGGGCGGAGTTGTTACGAC-3′, and probe 5′-FAM [6-carboxyfluorescein]-AAACAAACT-[ZEN]-CCCATTGACGTCA-IBFQ-3′).

PV entry assay in hACE2-hTMPRSS2-293T cells. A PV entry (transduction) assay was performed by incubating Mock-293T or hACE2/hTMPRSS2-293T cells on 48-well plates with PVs (5 × 10^8 genome copies in 200 μL per well) for 1 h at 37°C in a CO_2 incubator. Medium was replaced with DMEM containing 10% FBS. The entry levels of PVs expressing firefly luciferase were assessed by measuring luciferase activity using the Luc-Pair firefly luciferase HS assay kit (catalog number LF009; GeneCopoeia) and reading the plates on the SpectraMax paradigm multimode detection platform using SoftMax Pro 6.3 (Molecular Devices).

Screening of human plasma. Deidentified plasma samples were obtained by the Allergy, Asthma, and Immunology Specialists of South Florida, LLC, in mid-2020 for COVID-19 serotyping, and these were exempt (IRB-20-7580) from human subject research under CFR 45.101(b). These plasma samples were screened by Western blotting at a 1:200 dilution for their ability to recognize the S1 and S2 subunits of the SARS-CoV-2 S protein on sucrose-pelleted PV-dCT19. The blot was visualized using 1:10,000-diluted goat anti-human IgG conjugated with horseradish peroxidase (HRP) (catalog number 109-035-098; Jackson ImmunoResearch).

Cell surface and total expression of the spike protein. HEK293T cells at approximately 70% confluence in 6-well plates were transfected with 0.3 μg, unless indicated otherwise, of the plasmid expressing the indicated S protein variant. Cells were detached at 42 h posttransfection in phosphate-buffered saline (PBS) containing 2 mM EDTA. Approximately 1 × 10^6 cells were fixed with a 2% formaldehyde solution in PBS for 30 min on ice and blocked with PBS containing 2% bovine serum albumin (BSA) (Sigma-Aldrich) and 5% goat serum (Gibco) for 30 min on ice. These cells were then incubated on ice for 90 min in 100 μL of PBS containing either SARS-CoV-2 convalescent-phase plasma at a 1:200 dilution, 3 μg/mL of purified hACE2-NN-Ig (9), or 3 μg/mL of anti-NTD monoclonal antibodies (catalog number SPD-M121; Acro Biosystems), followed by 1:600-diluted anti-hIgG–Alexa Fluor 647 (AF647) (catalog number 109-605-003; Jackson ImmunoResearch). Stained cells were analyzed using an Accuri flow cytometer (BD Biosciences) equipped with the HyperCyt autosampler (IntelliCyt) and ForeCyt 6.2R3 software (IntelliCyt). To measure the total expression levels of the S protein, aliquots of the same cells were permeabilized with 0.1% saponin (Alfa Aesar) in PBS for 10 min at room temperature and subjected to staining on ice as described above.

Western blot analysis of S protein in cell lysates and on pseudovirions. For the detection of S protein bands in the cell lysates, HEK293T cells in 6-well plates were transfected to express S-FL or S-dCT5 and lysed with 0.2 mL of PBS containing 0.5% dodecyl maltopyranoside (Anatrace) and a protease inhibitor cocktail (catalog number A32955; Thermo Scientific). Fifteen microliters of the lysates was analyzed by WB using rabbit anti-S protein antibody (catalog number NR-52947; BEI Resources) at a 1:5,000 dilution or 1 μg/mL of NTD antibody (catalog number SPD-M121; Acro Biosystems) For the analyses of the S protein density on the virion, sucrose-pelleted PVs were analyzed by SDS-PAGE and WB. Unless indicated otherwise, 5 μL PVs was analyzed in each WB analysis. PV proteins were separated on a 4 to 12% Bis-Tris gel (catalog number NW041122; Life Technologies) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% milk in 1× Tris-buffered saline containing 0.1% Tween 20 for 1 h and blotted with human SARS-CoV-2 convalescent-phase plasma at a 1:200 dilution to detect the S protein bands or 1 μg/mL anti-p30 MLV Gag antibody (catalog number ab130757; Abcam) to detect p30 bands as a PV quantity control. S protein bands were detected using 10 ng/mL mouse anti-human IgG antibody conjugated with polymerized HRP (catalog number 61R-I166AHRP40; Fitzgerald), and p30 bands were detected using 1:10,000-diluted goat anti-mouse IgG–HRP polyclonal antibody (catalog number 115-036-062; Jackson Immuno Laboratory). Bands were visualized using the SuperSignal West Atto ultimate-sensitivity substrate (catalog number A38555; Thermo Scientific), and the band intensities were measured using Image Lab software (Bio-Rad).

Cryo-EM of full-length and dCT19 spike proteins. For cryo-EM samples, 300-mesh R2/2 copper Quantifoil grids (Electron Microscopy Sciences [EMS]) were negatively glow discharged for 30 s. The PV sample (3.5 μL) was applied onto the grid and incubated for 30 s, followed by plunge-freezing into liquid ethane with a Vitrobot Mark IV instrument (100% humidity, 4°C, and 4.5 s per blot). The grids were imaged on an FEI Tecnai T12 120-kV electron microscope.

For cryo-electron tomography, grids were prepared as described above, with the addition of 10-nm gold beads at a ratio of 14:1 (vol/vol) before plunge-freezing. Dose-symmetric tilt series (to ±48° with 3° increments) were collected on a Titan Krios instrument with a K3 detector (total dose of 100 $e^-/Å^2$; 7 frames per angle) and a 20-eV energy filter. The data set was motion corrected with MotionCor2 (46), reconstructed with IMOD (47), denoised with Topaz (48), and processed using ImageJ software.

Statistical analysis. All of the statistical details of specific experiments, which included the statistical tests used, numbers of samples, mean values, standard errors of the means (SEM), and *P* values derived from the indicated tests, are described in the figure legends and shown in the figures. Statistical analyses were conducted utilizing GraphPad Prism version 8.0 (GraphPad Software Inc.). Triplicate and other replicative data are presented as means ± SEM. A *P* value of <0.05 was considered to be statistically significant. For comparisons between two treatments, Student's *t* test (unpaired) was used. For comparisons of each group with the mean of every other group within a data set containing more than two groups, either one-way analysis of variance (ANOVA) with Dunnett's multiple-comparison test or two-way ANOVA with Sidak's multiple-comparison test was used.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

ACKNOWLEDGMENTS

We thank the University of Washington Arnold and Mabel Beckman Cryo-EM Center for data collection time and support.

This work was supported by NIH grant R01 AI129868 for coronavirus research supplement to H.C. and NIH grant S10 OD032290 and NIH NIGMS grant R01 GM099989 to K.K.L.

Conceptualization, L.Z., K.K.L., and H.C.; Methodology, L.Z., N.H., A.O., K.K.L., and H.C.; Investigation, L.Z., N.H., A.O., and K.N.L.; Data curation, L.Z., N.H., and A.O.; Resources, H.M., K.K.L., and H.C.; Writing – original draft, L.Z., A.O., K.K.L., and H.C.; Writing – review and editing, all authors; Supervision, K.K.L. and H.C.; Funding acquisition, K.K.L. and H.C.

We declare no competing interests.

REFERENCES

1. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, Droese B, Klaus JP, Makino S, Sawicki SG, Siddell SG, Stamou DG, Wilson IA, Kuhn P, Buchmeier MJ. 2011. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol 174:11–22. https://doi.org/10.1016/j.jsb.2010.11.021.
2. Schoeman D, Fielding BC. 2019. Coronavirus envelope protein: current knowledge. Virol J 16:69. https://doi.org/10.1186/s12985-019-1182-0.
3. Jackson CB, Farzan M, Chen B, Choe H. 2022. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol 23:3–20. https://doi.org/10.1038/s41580-021-00418-x.
4. Bosch BJ, Bartelink W, Rottier PJ. 2008. Cathepsin L functionally cleaves the severe acute respiratory syndrome coronavirus class I fusion protein upstream of rather than adjacent to the fusion peptide. J Virol 82:8887–8890. https://doi.org/10.1128/JVI.00415-08.
5. Huang IC, Bosch BJ, Li F, Li W, Lee KH, Ghiran S, Vasilieva N, Dermody TS, Harrison SC, Dormitzer PR, Farzan M, Rottier PJ, Choe H. 2006. SARS coronavirus, but not human coronavirus NL63, utilizes cathepsin L to infect ACE2-expressing cells. J Biol Chem 281:3198–3203. https://doi.org/10.1074/jbc.M508381200.
6. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F. 2010. Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. J Virol 84:12658–12664. https://doi.org/10.1128/JVI.01542-10.
7. Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P. 2005. Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry. Proc Natl Acad Sci U S A 102:11876–11881. https://doi.org/10.1073/pnas.0505577102.
8. Bertram S, Glowacka I, Blazejewska P, Soilleux E, Allen P, Danisch S, Steffen I, Choi SY, Park Y, Schneider H, Schughart K, Pohlmann S. 2010. TMPRSS2 and TMPRSS4 facilitate trypsin-independent spread of influenza virus in Caco-2 cells. J Virol 84:10016–10025. https://doi.org/10.1128/JVI.00239-10.
9. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, Rangarajan ES, Pan A, Vanderheiden A, Suthar MS, Li W, Izard T, Rader C, Farzan M, Choe H. 2020. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun 11:6013. https://doi.org/10.1038/s41467-020-19808-4.
10. Hou YJ, Chiba S, Halfmann P, Ehre C, Kuroda M, Dinnon KH, III, Leist SR, Schafer A, Nakajima N, Takahashi K, Lee RE, Mascenik TM, Graham R, Edwards CE, Tse LV, Okuda K, Markmann AJ, Bartelt L, de Silva A, Margolis DM, Boucher RC, Randell SH, Suzuki T, Gralinski LE, Kawaoka Y, Baric RS. 2020. SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. Science 370:1464–1468. https://doi.org/10.1126/science.abe8499.
11. Michaud WA, Boland GM, Rabi SA. 2020. The SARS-CoV-2 spike mutation D614G increases entry fitness across a range of ACE2 levels, directly outcompetes the wild type, and is preferentially incorporated into trimers. bioRxiv. https://doi.org/10.1101/2020.08.25.267500.
12. Fernández A. 2020. Structural impact of mutation D614G in SARS-CoV-2 spike protein: enhanced infectivity and therapeutic opportunity. ACS Med Chem Lett 11:1667–1670. https://doi.org/10.1021/acsmedchemlett.0c00410.
13. Fultz PN, McClure HM, Anderson DC, Swenson RB, Anand R, Srinivasan A. 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (Cercocebus atys). Proc Natl Acad Sci U S A 83:5286–5290. https://doi.org/10.1073/pnas.83.14.5286.
14. Kanki PJ, M'Boup S, Ricard D, Barin F, Denis F, Boye C, Sangare L, Travers K, Albaum M, Marlink R, Romet-lemonne JL, Essex M. 1987. Human T-lymphotropic virus type 4 and the human immunodeficiency virus in West Africa. Science 236:827–831. https://doi.org/10.1126/science.3033826.
15. Chakrabarti L, Guyader M, Alizon M, Daniel MD, Desrosiers RC, Tiollais P, Sonigo P. 1987. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. Nature 328:543–547. https://doi.org/10.1038/328543a0.
16. Hahn BH, Kong LI, Lee SW, Kumar P, Taylor ME, Arya SK, Shaw GM. 1987. Relation of HTLV-4 to simian and human immunodeficiency-associated viruses. Nature 330:184–186. https://doi.org/10.1038/330184a0.
17. Mammano F, Salvatori F, Indraccolo S, De Rossi A, Chieco-Bianchi L, Göttlinger HG. 1997. Truncation of the human immunodeficiency virus type 1 envelope glycoprotein allows efficient pseudotyping of Moloney murine leukemia virus particles and gene transfer into CD4$^+$ cells. J Virol 71:3341–3345. https://doi.org/10.1128/JVI.71.4.3341-3345.1997.
18. Zingler K, Littman DR. 1993. Truncation of the cytoplasmic domain of the simian immunodeficiency virus envelope glycoprotein increases Env incorporation into particles and fusogenicity and infectivity. J Virol 67:2824–2831. https://doi.org/10.1128/JVI.67.5.2824-2831.1993.
19. Christodoulopoulos I, Cannon PM. 2001. Sequences in the cytoplasmic tail of the gibbon ape leukemia virus envelope protein that prevent its incorporation into lentivirus vectors. J Virol 75:4129–4138. https://doi.org/10.1128/JVI.75.9.4129-4138.2001.
20. Aguilar HC, Anderson WF, Cannon PM. 2003. Cytoplasmic tail of Moloney murine leukemia virus envelope protein influences the conformation of the extracellular domain: implications for mechanism of action of the R peptide. J Virol 77:1281–1291. https://doi.org/10.1128/jvi.77.2.1281-1291.2003.
21. Moore MJ, Dorfman T, Li W, Wong SK, Li Y, Kuhn JH, Coderre J, Vasilieva N, Han Z, Greenough TC, Farzan M, Choe H. 2004. Retroviruses pseudotyped with the severe acute respiratory syndrome coronavirus spike protein efficiently infect cells expressing angiotensin-converting enzyme 2. J Virol 78:10628–10635. https://doi.org/10.1128/JVI.78.19.10628-10635.2004.
22. Yao QH, Compans RW. 1995. Differences in the role of the cytoplasmic domain of human parainfluenza virus fusion proteins. J Virol 69:7045–7053. https://doi.org/10.1128/JVI.69.11.7045-7053.1995.
23. Havranek KE, Jimenez AR, Acciani MD, Lay Mendoza MF, Reyes Ballista JM, Diaz DA, Brindley MA. 2020. SARS-CoV-2 spike alterations enhance pseudoparticle titers and replication-competent VSV-SARS-CoV-2 virus. Viruses 12:1465. https://doi.org/10.3390/v12121465.
24. Yu J, Li Z, He X, Gebre MS, Bondzie EA, Wan H, Jacob-Dolan C, Martinez DR, Nkolola JP, Baric RS, Barouch DH. 2021. Deletion of the SARS-CoV-2 spike cytoplasmic tail increases infectivity in pseudovirus neutralization assays. J Virol 95:e00044-21. https://doi.org/10.1128/JVI.00044-21.
25. Aguilar HC, Matreyek KA, Choi DY, Filone CM, Young S, Lee B. 2007. Polybasic KKR motif in the cytoplasmic tail of Nipah virus fusion protein modulates membrane fusion by inside-out signaling. J Virol 81:4520–4532. https://doi.org/10.1128/JVI.02205-06.
26. Cathomen T, Naim HY, Cattaneo R. 1998. Measles viruses with altered envelope protein cytoplasmic tails gain cell fusion competence. J Virol 72:1224–1234. https://doi.org/10.1128/JVI.72.2.1224-1234.1998.

27. Zhao Y, Zhu LJ, Benedict CA, Chen DG, Anderson WF, Cannon PM. 1998. Functional domains in the retroviral transmembrane protein. J Virol 72:5392–5398. https://doi.org/10.1128/JVI.72.7.5392-5398.1998.
28. Lontok E, Corse E, Machamer CE. 2004. Intracellular targeting signals contribute to localization of coronavirus spike proteins near the virus assembly site. J Virol 78:5913–5922. https://doi.org/10.1128/JVI.78.11.5913-5922.2004.
29. McBride CE, Li J, Machamer CE. 2007. The cytoplasmic tail of the severe acute respiratory syndrome coronavirus spike protein contains a novel endoplasmic reticulum retrieval signal that binds COPI and promotes interaction with membrane protein. J Virol 81:2418–2428. https://doi.org/10.1128/JVI.02146-06.
30. Petit CM, Melancon JM, Chouljenko VN, Colgrove R, Farzan M, Knipe DM, Kousoulas KG. 2005. Genetic analysis of the SARS-coronavirus spike glycoprotein functional domains involved in cell-surface expression and cell-to-cell fusion. Virology 341:215–230. https://doi.org/10.1016/j.virol.2005.06.046.
31. Corver J, Broer R, van Kasteren P, Spaan W. 2009. Mutagenesis of the transmembrane domain of the SARS coronavirus spike glycoprotein: refinement of the requirements for SARS coronavirus cell entry. Virol J 6:230. https://doi.org/10.1186/1743-422X-6-230.
32. Petit CM, Chouljenko VN, Iyer A, Colgrove R, Farzan M, Knipe DM, Kousoulas KG. 2007. Palmitoylation of the cysteine-rich endodomain of the SARS-coronavirus spike glycoprotein is important for spike-mediated cell fusion. Virology 360:264–274. https://doi.org/10.1016/j.virol.2006.10.034.
33. Chen HY, Huang C, Tian L, Huang X, Zhang C, Llewellyn GN, Rogers GL, Andresen K, O'Gorman MRG, Chen YW, Cannon PM. 2021. Cytoplasmic tail truncation of SARS-CoV-2 spike protein enhances titer of pseudotyped vectors but masks the effect of the D614G mutation. J Virol 95:e00966-21. https://doi.org/10.1128/JVI.00966-21.
34. Xiong H-L, Wu Y-T, Cao J-L, Yang R, Liu Y-X, Ma J, Qiao X-Y, Yao X-Y, Zhang B-H, Zhang Y-L, Hou W-H, Shi Y, Xu J-J, Zhang L, Wang S-J, Fu B-R, Yang T, Ge S-X, Zhang J, Yuan Q, Huang B-Y, Li Z-Y, Zhang T-Y, Xia N-S. 2020. Robust neutralization assay based on SARS-CoV-2 S-protein-bearing vesicular stomatitis virus (VSV) pseudovirus and ACE2-overexpressing BHK21 cells. Emerg Microbes Infect 9:2105–2113. https://doi.org/10.1080/22221751.2020.1815589.
35. Wolff G, Limpens R, Zevenhoven-Dobbe JC, Laugks U, Zheng S, de Jong AWM, Koning RI, Agard DA, Grunewald K, Koster AJ, Snijder EJ, Barcena M. 2020. A molecular pore spans the double membrane of the coronavirus replication organelle. Science 369:1395–1398. https://doi.org/10.1126/science.abd3629.
36. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181:281–292.e6. https://doi.org/10.1016/j.cell.2020.02.058.
37. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 367:1260–1263. https://doi.org/10.1126/science.abb2507.
38. Cai Y, Zhang J, Xiao T, Peng H, Sterling SM, Walsh RM, Jr, Rawson S, Rits-Volloch S, Chen B. 2020. Distinct conformational states of SARS-CoV-2 spike protein. Science 369:1586–1592. https://doi.org/10.1126/science.abd4251.
39. Liu C, Mendonca L, Yang Y, Gao Y, Shen C, Liu J, Ni T, Ju B, Liu C, Tang X, Wei J, Ma X, Zhu Y, Liu W, Xu S, Liu Y, Yuan J, Wu J, Liu Z, Zhang Z, Liu L, Wang P, Zhang P. 2020. The architecture of inactivated SARS-CoV-2 with postfusion spikes revealed by cryo-EM and cryo-ET. Structure 28:1218–1224.e4. https://doi.org/10.1016/j.str.2020.10.001.
40. Resh MD. 1999. Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. Biochim Biophys Acta 1451:1–16. https://doi.org/10.1016/S0167-4889(99)00075-0.
41. Tsukita S, Yonemura S, Tsukita S. 1997. ERM proteins: head-to-tail regulation of actin-plasma membrane interaction. Trends Biochem Sci 22:53–58. https://doi.org/10.1016/s0968-0004(96)10071-2.
42. Niwa H, Yamamura K, Miyazaki J. 1991. Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 108:193–199. https://doi.org/10.1016/0378-1119(91)90434-d.
43. Wong SK, Li W, Moore MJ, Choe H, Farzan M. 2004. A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 279:3197–3201. https://doi.org/10.1074/jbc.C300520200.
44. Campeau E, Ruhl VE, Rodier F, Smith CL, Rahmberg BL, Fuss JO, Campisi J, Yaswen P, Cooper PK, Kaufman PD. 2009. A versatile viral system for expression and depletion of proteins in mammalian cells. PLoS One 4:e6529. https://doi.org/10.1371/journal.pone.0006529.
45. Mou H, Quinlan BD, Peng H, Liu G, Guo Y, Peng S, Zhang L, Davis-Gardner ME, Gardner MR, Crynen G, DeVaux LB, Voo ZX, Bailey CC, Alpert MD, Rader C, Gack MU, Choe H, Farzan M. 2021. Mutations derived from horseshoe bat ACE2 orthologs enhance ACE2-Fc neutralization of SARS-CoV-2. PLoS Pathog 17:e1009501. https://doi.org/10.1371/journal.ppat.1009501.
46. Zheng SQ, Palovcak E, Armache JP, Verba KA, Cheng Y, Agard DA. 2017. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat Methods 14:331–332. https://doi.org/10.1038/nmeth.4193.
47. Kremer JR, Mastronarde DN, McIntosh JR. 1996. Computer visualization of three-dimensional image data using IMOD. J Struct Biol 116:71–76. https://doi.org/10.1006/jsbi.1996.0013.
48. Bepler T, Kelley K, Noble AJ, Berger B. 2020. Topaz-Denoise: general deep denoising models for cryoEM and cryoET. Nat Commun 11:5208. https://doi.org/10.1038/s41467-020-18952-1.

JOURNAL OF VIROLOGY, May 2007, p. 4520–4532
0022-538X/07/$08.00+0 doi:10.1128/JVI.02205-06

Vol. 81, No. 9

Polybasic KKR Motif in the Cytoplasmic Tail of Nipah Virus Fusion Protein Modulates Membrane Fusion by Inside-Out Signaling[▽]

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Received 6 October 2006/Accepted 31 January 2007

The cytoplasmic tails of the envelope proteins from multiple viruses are known to contain determinants that affect their fusogenic capacities. Here we report that specific residues in the cytoplasmic tail of the Nipah virus fusion protein (NiV-F) modulate its fusogenic activity. Truncation of the cytoplasmic tail of NiV-F greatly inhibited cell-cell fusion. Deletion and alanine scan analysis identified a tribasic KKR motif in the membrane-adjacent region as important for modulating cell-cell fusion. The K1A mutation increased fusion 5.5-fold, while the K2A and R3A mutations decreased fusion 3- to 5-fold. These results were corroborated in a reverse-pseudotyped viral entry assay, where receptor-pseudotyped reporter virus was used to infect cells expressing wild-type or mutant NiV envelope glycoproteins. Differential monoclonal antibody binding data indicated that hyper- or hypofusogenic mutations in the KKR motif affected the ectodomain conformation of NiV-F, which in turn resulted in faster or slower six-helix bundle formation, respectively. However, we also present evidence that the hypofusogenic phenotypes of the K2A and R3A mutants were effected via distinct mechanisms. Interestingly, the K2A mutant was also markedly excluded from lipid rafts, where ~20% of wild-type F and the other mutants can be found. Finally, we found a strong negative correlation between the relative fusogenic capacities of these cytoplasmic-tail mutants and the avidities of NiV-F and NiV-G interactions ($P = 0.007$, $r^2 = 0.82$). In toto, our data suggest that inside-out signaling by specific residues in the cytoplasmic tail of NiV-F can modulate its fusogenicity by multiple distinct mechanisms.

Nipah virus (NiV) and *Hendra virus* (HeV) are deadly emerging zoonotic viruses belonging to the new *Henipavirus* genus within the family *Paramyxoviridae* (66). NiV infections result in respiratory and neurological symptoms, often leading to fatal encephalitis, the primary reason for death in humans (32, 64). Microvascular endothelial cell syncytium formation is a hallmark of NiV infection, associated with endothelial cell death, vascular inflammation, and necrosis (70). The mortality rate of NiV-infected humans ranges from ~40% in the original outbreaks in Malaysia and Singapore in 1999 to 2000 to ~70% in Bangladesh in 2005 (5, 6). The natural reservoir for NiV has been determined to be fruit bats of the genus *Pteropus* (46), and pigs served as the intermediate amplifying host in the original Malaysian-Singaporean outbreaks. Ominously, even though human-to-human transmission was not documented in the original outbreaks, direct bat-to-human and human-to-human transmissions have been reported in the later outbreaks in Bangladesh (5, 6). NiV is classified as a BSL4 pathogen and has also been designated as a select agent because of its bio- or agroterrorism potential. These characteristics of NiV underscore the need for research and treatment development against this perilous pathogen and the need for understanding of the necessary components and mechanisms of virus-cell and cell-cell fusions in order to inhibit viral infection and spread.

For paramyxoviruses, two separate membrane proteins are involved in the fusion process, the attachment protein (H, HN, or G), which binds to the receptor molecule in the target cell membrane, and the fusion protein (F) that actually carries out membrane fusion. For most paramyxoviruses, both F and its homotypic attachment protein are necessary for membrane fusion, except for rare cases like the hyperfusogenic simian virus 5 (SV5) W3A isolate (27, 48). Activation of F is believed to occur through the following three steps: (i) binding of the attachment protein to the receptor, (ii) interaction of the attachment and F proteins (or changes thereof), and (iii) conformational changes in F that mediate membrane fusion. The fusion (F) and attachment (G) envelope glycoproteins in NiV or HeV are both necessary for cell-cell fusion, syncytium formation, and viral entry. G is responsible for binding to its cognate receptor, ephrinB2 (9, 44), and at least for NiV, ephrinB3 can also be used as an alternative receptor (45). The high expression of ephrinB2 on neurons and endothelial cells and the patterns of expression of ephrinB3 in the central nervous system largely account for the cell tropism of NiV and HeV (9, 44, 45). However, much less is known about the components necessary for the subsequent steps in the activation of NiV fusion (NiV-F) or HeV-F protein.

Paramyxovirus F proteins belong to the class I fusion proteins that share several structural and functional characteristics. The structures of the retroviral Moloney murine leukemia virus (MoMuLV) p15E, lentiviral human immunodeficiency virus type 1 (HIV-1) gp41, Ebola virus GP2, paramyxovirus SV5 F, and influenza virus hemagglutinin (HA) fusion proteins have all been shown to have similar trimeric coiled-coil core

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[▽] Published ahead of print on 14 February 2007.

structures, suggesting similar membrane fusion mechanisms (8, 14, 20, 72). Class I fusion proteins of enveloped viruses are synthesized as precursors that must be cleaved and hence activated into a metastable conformation that is ready for enabling virus-cell membrane fusion. Typically, cleavage generates a new N terminus that contains a hydrophobic fusion peptide motif. Upon activation of the fusion protein through receptor binding and/or endosomal low pH, the fusion peptide gets inserted into the host cell target membrane. Class I fusion proteins also contain two heptad repeat regions (HR1 and HR2); the C-terminal HR2 region is generally thought to be preformed, but the N-terminal HR1 region is formed only upon fusion peptide insertion (14, 35, 72, 73). Class I fusion proteins function as trimers, and the HR1 and HR2 regions have a strong propensity to fold into coiled-coil domains during six-helix bundle (6HB) formation. The free energy released from fusion protein refolding from the metastable prefusion state to the stable postfusion 6HB state likely drives the virus-host cell membranes together, overcoming the electrostatic repulsion intrinsic to the negatively charged phospholipids' head groups of the two membranes (38, 55).

For NiV and HeV, the fusion protein is cleaved within the endosomal compartment from the precursor F_0 to the F_1 and F_2 subunits (18, 39). Such cleavage is likely required for activation of the F protein into the metastable state. For NiV, after activation into the metastable state, not much is known about the subsequent steps in the triggering of the fusion protein that leads to eventual membrane fusion. We and others have recently reported that N-glycans in both the NiV-F and HeV-F proteins have some effects on protein expression and membrane fusion (3, 15, 40). In addition, we identified N-glycans in NiV-F that both reduce fusion and viral entry and protect the virus against neutralizing antibodies (Abs) (3). These results show some uniqueness of the *Henipavirus* genus fusion proteins. However, little is known about other domains in NiV-F or HeV-F that may have an important role in membrane fusion. Triggering of fusion is usually envisioned to involve primarily the ectodomain of the fusion protein. However, accumulating evidence from retroviral (13, 25, 50, 54), lentiviral (41, 42), and other paramyxoviral (65, 67) envelope (Env) proteins suggests that the Env cytoplasmic tail (CT) is involved in regulating the fusion process.

Multiple reports indicate that fusion mediated by the ectodomain of the retrovirus MoMuLV (2), the lentiviruses simian immunodeficiency virus (SIV) (61) and HIV-1 (71), and the paramyxovirus SV5 (67) fusion proteins can be modulated by inside-out signaling from the CT. Truncation of the long CT of lentiviral Env proteins occurs under certain culture conditions, and increased fusogenicity has been reported for truncated versions of SIV, HIV-1, and HIV-2 Env (16, 31, 41, 60, 61, 76). For the paramyxovirus SV5 F protein, isolates with a short (20-residue) CT (W3A and WR) cause extensive cell-cell fusion, whereas isolates with an extended CT (T1 and SER) cause little or no cell-cell fusion, and truncation of the CT restores fusion to levels seen in W3A and WR isolates (28, 65). For MoMuLV, SIV, and SV5, the hyperfusogenicity caused by truncation of the CT is linked to overall conformational changes in the ectodomain of the protein (2, 61, 67). In MoMuLV and the Mason-Pfizer monkey virus, the CT is even protease cleaved during viral maturation to "prime" the fusion protein for fusogenicity (2, 13). Here we investigated the potential role(s) of the CT of the NiV fusion protein in cell surface expression (CSE), processing, membrane fusion, and viral entry and defined specific residues in a polybasic motif in the CT that can affect the conformation of the ectodomain, fusogenicity, and interaction of the fusion protein with the attachment glycoprotein.

MATERIALS AND METHODS

Expression plasmids and codon optimization. The codon-optimized NiV-G and NiV-F genes were tagged at their C termini with HA and AU1 tags, respectively, as previously described (33). The NiV-HR2-Fc construct was made by fusing the heptad repeat region 2 sequence of NiV-F (amino acids 447 to 488) with the human immunoglobulin G1 Fc constant region as previously described (3, 44). The deletion mutants −T, −T1, −T2, −T3, −T4, −T12, and −T234 and point mutants K1A, K2A, R3A, N4A, and T5A were made by deleting or mutating the codon-optimized wild-type (WT) NiV-F plasmid with appropriately designed primers and the QuikChange site-directed mutagenesis kit (Stratagene, Cedar Creek, TX). All mutations and deletions were confirmed by sequencing the entire open reading frame.

Cell culture. Vero cells were cultured in minimal essential medium alpha with 10% fetal bovine serum (FBS). PK13 and 293T cells were cultured in Dulbecco's modified Eagle's medium with 10% FBS. We obtained 293T and Vero cells from the American Type Culture Collection, and PK13 (porcine fibroblasts) cells were a kind gift from Irvin Chen at the University of California Los Angeles.

Quantitation of cell-cell fusion. Codon-optimized NiV-G and codon-optimized WT or mutant NiV-F expression plasmids (1:1 ratio, 1 μg total) (3, 33) were transfected with 1.5 μg pcDNA3.1 plasmid as filler DNA into 293T or Vero cells growing in 12-well plates at 80% confluence, as indicated. At 12 to 18 h posttransfection, cells were stained with 4′,6′-diamidino-2-phenylindole (DAPI) and syncytium formation was quantified by counting the nuclei in syncytia per ×100 field (at least 10 fields were counted per condition). Syncytia were defined as four or more nuclei visualized within a common cell membrane, as indicated previously (3).

Quantification of NiV-F and NiV-G CSE levels by flow cytometry. Production of antisera from genetically immunized rabbits (with NiV-M and -F or -G expression plasmids) was previously described (44). Sera containing anti-F or anti-G specific activities were used for flow cytometry on NiV-F- or -G-transfected cells at a 1:1,000 dilution. Bound Ab was detected with phycoerythrin-conjugated goat anti-rabbit Abs (Caltag, Burlingame, CA). Antisera were also raised in rabbits immunized with peptides corresponding to amino acids 39 to 57 and 331 to 348 of NiV-F_2 and NiV-G, respectively, as previously described (3, 33). These regions were previously shown to be immunogenic (10). For quantitation of binding of the monoclonal Abs (MAbs), flow cytometry was performed with MAb concentrations of 0.03 to 3 μg/ml. For calculating the binding ratios of any given pair of Abs, data obtained from equal concentrations of the respective Abs were used.

Reverse pseudotype viral entry assay. The ephrinB2 NiV receptor protein was pseudotyped onto a reporter virus, vesicular stomatitis virus (VSV), by transfecting an ephrinB2 expression plasmid into 293T cells and subsequently infecting these cells with recombinant VSV expressing the *Renilla* Luc reporter gene (VSV-ΔG-rLuc), similarly to the procedure described previously for preparation of NiV-F- and -G-pseudotyped VSVrLuc virions (3, 44, 45). ephrinB2 reverse-pseudotyped virions were purified over a 20% sucrose cushion as for NiV-F- and -G-pseudotyped viruses. 293T cells plated in 96-well plates were transfected with NiV-G and WT or mutant NiV-F and, 10 to 12 h later, infected with reverse-pseudotyped virions in phosphate-buffered saline–1% FBS for 2 h at 37°C over a 5-log viral dilution range (10^{-2} to 10^{-6}). After 2 h, cells were washed and 293T cell growth medium was added. At 24 h postinfection, cells were lysed and luciferase activity was measured as relative light units (RLU) with a *Renilla* luciferase detection system (Promega, Madison, WI) and a Veritas microplate luminometer (Turner Biosystems, Sunnyvale, CA). Quantitation of viral genome copies for the ephrinB2 VSV-pseudotyped viral prep was performed exactly as previously described (3). For quantitation of neutralization of viral entry, the reverse-pseudotyped viral entry assay was performed as described above, except in the presence of the indicated amounts of the specified Abs. For the mixed heterotrimer experiments with the K1A and K2A or R3A mutant proteins, the indicated DNA ratios of the expression plasmids for the indicated proteins were transfected into 293T cells 18 h before infection with the reverse-pseudotyped virions.

Western blot analysis of surface NiV-F and NiV-G proteins. Codon-optimized NiV-F and/or NiV-G expression plasmids (1:1 ratios when in combination) were transfected into 293T cells plated in six-well plates (total of 2 μg F and/or G plasmids with 3 μg PCDNA3.1 plasmid as filler DNA/well), as indicated. Cells were either cell surface biotinylated or not (EZ link Sulfo-NHS-LC-Biotin; Pierce, Rockford, IL), as specified, and biotinylated proteins were precipitated with streptavidin-agarose beads (Pierce, Rockford, IL). Twenty percent of the biotinylated cell lysate was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and subsequently detected by Western blotting with anti-tag (HA or AU1), anti-F_2, or anti-G peptide Abs, as indicated. Primary and secondary Abs were used at 1:1,000 and 1:20,000 dilutions, respectively, followed by ECL Plus detection (Amersham Biosciences, Piscataway, NJ). For quantification of relative processing levels for the various NiV-F proteins, the ratio of the densitometric units of the F_1 subunit over those of the sum of the precursor F_0 and the F_1 subunits was calculated.

Lipid raft association of NiV-F proteins. 293T cells transfected with WT or mutant NiV-F proteins (as described above) were washed with phosphate-buffered saline and resuspended in TNE buffer (25 mM Tris [pH 7.5], 150 mM NaCl, 5 mM EDTA) containing a protease inhibitor cocktail (Roche, Indianapolis, IN). Cells were Dounce homogenized, and their nuclei were isolated and discarded. Postnuclear supernatants were treated with 1% Triton X-100 for 30 min at 4°C. Cell lysates were then brought up to a 40% OptiPrep (Sigma-Aldrich, St. Louis MO) concentration in 1.2 ml, placed at the bottom of an ultracentrifuge tube, and layered sequentially with 30% (3 ml) and 5% (0.8 ml) OptiPrep layers (in TNE buffer plus protease inhibitors). These discontinuous gradients were centrifuged at 45,000 rpm for 16 h at 4°C in an SW50.1 rotor. After centrifugation, 12 equal fractions were manually collected from the top, protein from 200 μl of each fraction was precipitated by a methanol-chloroform extraction method (69), and each fraction was analyzed by Western blotting. The overall lipid raft domain isolation procedure was similar to that used by Fleming et al. (22).

Production of NiV-HR2-Fc immunoadhesin and fusion inhibition. The NiV-HR2-Fc expression plasmid was transfected into 293T cells, and at 24 h posttransfection, supernatants were collected and concentrated with a Centriplus YM-10 filter (Millipore, Bedford, MA). Protein concentrations were measured by an Fc-specific enzyme-linked immunosorbent assay as previously described (44). For NiV fusion inhibition, the indicated amounts of NiV-HR2-Fc were added to 293T cells transfected with NiV-G and WT or N-glycan mutant NiV-F expression plasmids. Fusion was quantified after overnight incubation as described above.

Fusion kinetics of WT or mutant NiV-F proteins. The fusion kinetics of WT and mutant NiV-F proteins were determined in a β-lactamase reporter cell-cell fusion assay as previously described (3, 34, 53). For better sensitivity, the β-lactamase gene was also codon optimized for mammalian cell expression (Geneart, Inc., Toronto, Ontario, Canada). Fusion-nonpermissive PK13 effector cells were cotransfected with β-lactamase, NiV-G, and WT or mutant NiV-F expression constructs with Lipofectamine 2000. These were then added to 293T target cells labeled with CCF2-AM dye. Effector and target cells were mixed and incubated at 37°C, and cell-cell fusion was detected by analyzing the shift from green to blue fluorescence, indicating β-lactamase cleavage of CCF2. Fluorescence was quantified every 3 min with a CytoFluor Series 4000 Fluorescence multiwell plate reader (PerSeptive Biosystems, Framingham, MA). The results are expressed as the ratio of blue to green fluorescence obtained with NiV-G- and NiV-F-transfected effectors minus the background blue and green fluorescence obtained with empty-vector-transfected cells.

NiV-F–NiV-G coimmunoprecipitation. 293T cells in 10-cm plates were transfected with 20 μg of the indicated NiV-F-G plasmids at a 1:1 ratio with Lipofectamine 2000. At 24 h posttransfection, cells were lysed and cell lysates were subjected to immunoprecipitation as previously described (3, 33), with a 1:100 dilution of anti-NiV-G peptide serum. Coimmunoprecipitated (co-IP) proteins were analyzed by Western blotting with the appropriate anti-tag Ab as described above and then quantified by densitometry with a VersaDoc Imaging System (Bio-Rad, Hercules, CA).

RESULTS

The membrane-proximal region in the CT of the NiV fusion protein plays a role in membrane fusion. The CT of NiV-F can be conveniently divided into four distinct regions, i.e., a membrane-proximal polybasic region (T1), a functional tyrosine-based endocytic motif (YSRL) (18, 39), a highly charged region (T3), and a C-terminal region that is rich in polar residues (T4). To investigate the potential roles that these cytoplasmic regions may play in membrane fusion, protein expression, processing, and transport, we made a series of deletion mutants that lack various regions of the CT, as illustrated in Fig. 1A. The first amino acid of the CT is a glutamic acid and is likely required to demarcate the membrane-spanning domain. Thus, we kept this amino acid in every deletion mutant in order to maximize the likelihood of correct protein folding and expression. We then analyzed the relative levels of CSE and processing of such deletion mutants and compared them to those of WT NiV-F.

Briefly, 293T cells transfected with expression plasmids for WT NiV-F or the indicated mutant proteins were cell surface biotinylated and lysed and cell surface proteins were precipitated with streptavidin beads and then NiV-F detected by Western blotting with the specified Abs (Fig. 1B). Alternatively, we performed flow cytometric analysis on parallel samples of 293T cells expressing WT NiV-F or the deletion mutants with polyclonal anti-NiV-F antiserum 834, which was previously described (3, 33, 45) (Fig. 1D). Both biotinylation and flow cytometric CSE analyses indicated that the deletion mutants were expressed to at least 50% of the WT level and some were even expressed at levels higher than that of the WT. The cell surface biotinylation experiments also showed that the deletion mutants were cleaved and processed more or less at WT levels, with the exception of the deletion mutant missing the entire CT (Fig. 1B, bottom). Interestingly, although this deletion mutant (−T) did not include removal of the C-terminal AU1 tag, the anti-AU1 MAb was not able to detect this protein by Western blotting, perhaps because of the close proximity of the AU1 tag to the detergent-lipid micelles (Fig. 1B, top left part). However, the −T mutant was clearly expressed, as shown by blotting with an anti-F_2 peptide antiserum previously described (3, 33) (Fig. 1B, right part), as well as by flow cytometry (Fig. 1D). Notably, the −T mutant was also not processed efficiently despite being expressed on the cell surface (Fig. 1B, right part).

Cleavage of NiV-F requires active endocytosis and processing by endosomal cathepsin L, which is in part mediated by the YXXΦ endocytic motif in the T2 region (18, 39). Since the AU1 tag contains a putative YXXΦ endocytic motif, we sought to determine if our AU1 tag had any inadvertent effects on the expression or processing of NiV-F. Figure 1C shows that there were no differences in cleavage or processing efficiency between tagged and untagged WT NiV-F (F and F_{NA}, respectively). Interestingly, the untagged version of the T2 mutant ($-T2_{NA}$), which lacks the endogenous YXXΦ motif, also showed no differences from untagged WT NiV-F (F_{NA}), similar to what has been found with the tagged versions (compare Fig. 1B and C).

Next, we asked whether the CT deletion mutations affected the fusogenicity of the NiV-F fusion protein. To normalize for the differences in CSE, we compared the fusion-to-CSE ratios induced by WT NiV-F and the indicated deletion mutants (Fig. 1D). We performed our syncytium-forming assays by transfecting in 0.3 μg of NiV-F and -G per 12-well plate, which was previously determined to result in CSE and fusion with the WT NiV-F protein in the linear range of measurement (3). CSE was measured by flow cytometry as described above, and fusion was determined by counting nuclei inside syncytia (more than

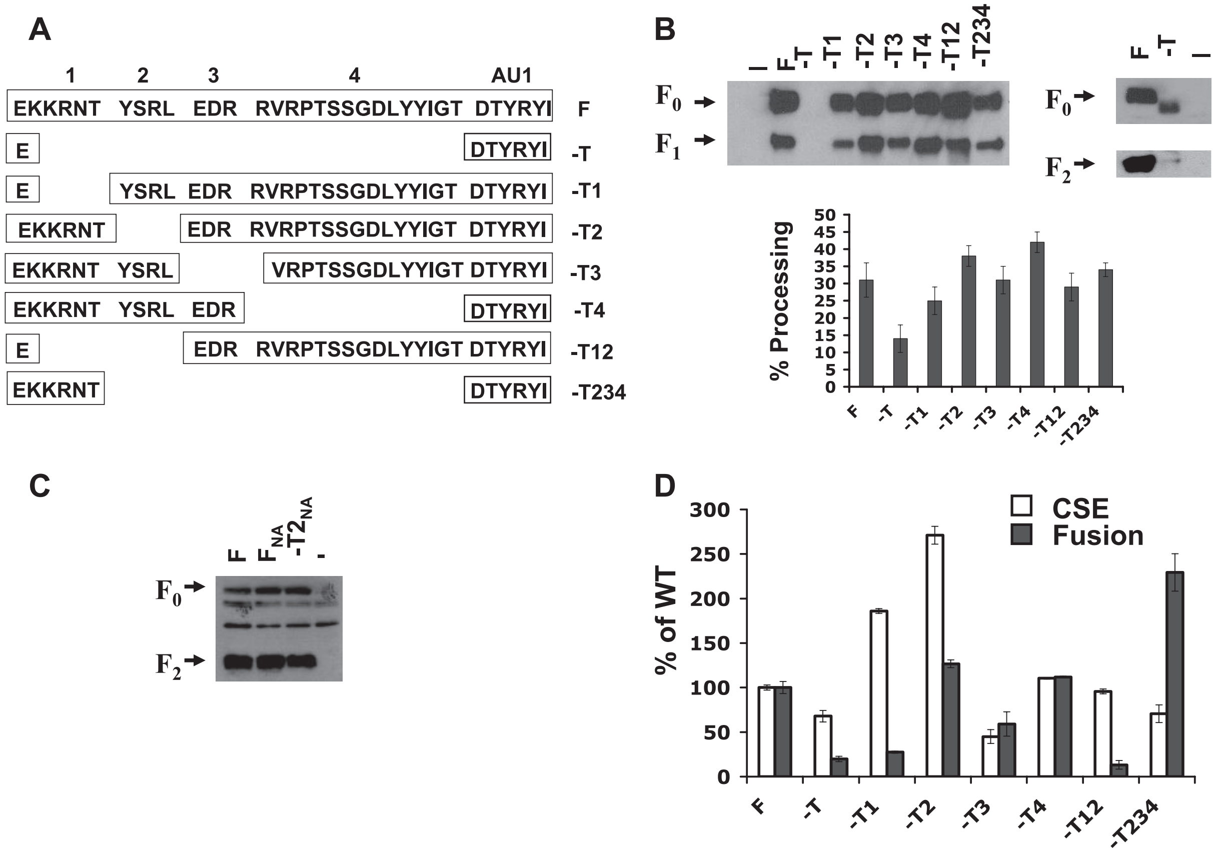


FIG. 1. Analysis of NiV-F CT deletion mutants. (A) Schematic of the NiV-F CT deletion mutants. NiV-F CT was divided into four regions (numbered 1, 2, 3, and 4) as described in the text, and the names of the deletion mutants examined are indicated. (B) Western blot analysis of immunoprecipitated surface WT and mutant NiV-F proteins. Briefly, biotinylated cells were lysed, cell surface biotinylated proteins were precipitated with streptavidin agarose beads, and NiV-F was detected in the biotinylated precipitates by Western blotting with either a monoclonal anti-AU1 tag Ab (left part) or a rabbit anti NiV-F_2 antipeptide Ab (3) (right part). Percent processing was calculated as the densitometric units of the F_1 subunit over those of the sum of the precursor F_0 and the F_1 subunits (bottom part) ($n = 3$). (C) The AU1 tag does not affect cleavage and processing of F. Identical cell surface biotinylation experiments were performed with tagged (F) and untagged versions of WT F (F_{NA}) and the −T2 mutant ($-T2_{NA}$). A rabbit anti NiV-F_2 antipeptide Ab (3) was used to detect NiV-F. (D) Relative levels of CSE and fusion obtained for WT NiV-F and the indicated CT deletion mutants. Fusion was determined by counting nuclei in syncytia per field. At least 10 fields were counted per condition. CSE was determined by flow cytometry with polyclonal anti-NiV-F specific antiserum as described previously (3). Both CSE and fusion levels were separately normalized to levels of WT NiV-F protein, set at 100%. Data shown are averages ± standard errors from three independent experiments.

four nuclei per cell) per microscopic field, respectively. Figure 1D shows the relative CSE and fusogenicity of WT NiV-F and the indicated mutants, and Table 1 (top) shows their corresponding fusion/CSE ratios. Interestingly, all mutants that lacked the membrane-proximal T1 region (−T1 and −T12) were hypofusogenic and had fusion/CSE ratios of less than 0.5 (by definition, that of WT NiV-F is 1.0), while all mutants that retained the T1 region (−T3, −T4, and −T234) had fusion/CSE ratios equal to or higher than that of WT NiV-F, with the exception of the −T2 mutant, which had a fusion/CSE ratio of 0.5. Since the fusion defect in the tailless mutant (−T) was likely due at least partially to its processing defect, it was not included in Table 1 for comparison. These results indicate that the CT, in particular, the membrane-proximal T1 region of the CT, plays an important role in membrane fusion and syncytium formation.

Polybasic KKR motif in the membrane-proximal region of the NiV-F CT modulates NiV-F-induced membrane fusion. To

TABLE 1. Fusion/CSE ratios

Env fusion protein	Fusion/CSE ratio[a]
F	1.0
−T1	0.1
−T2	0.5
−T3	1.3
−T4	1.0
−T12	0.1
−T234	3.2
K1A	5.5
K2A	0.2
R3A	0.3
N4A	0.9
T5A	0.9

[a] The ratio of the normalized fusion and CSE values for each mutant was calculated from the data in Fig. 1D and 2C. By definition, the fusion/CSE ratio for WT NiV-F would be 1.0 (100%/100%). Ratios of >1 indicate increased fusogenicity, while mutants with decreased fusogenicity would have ratios of <1.

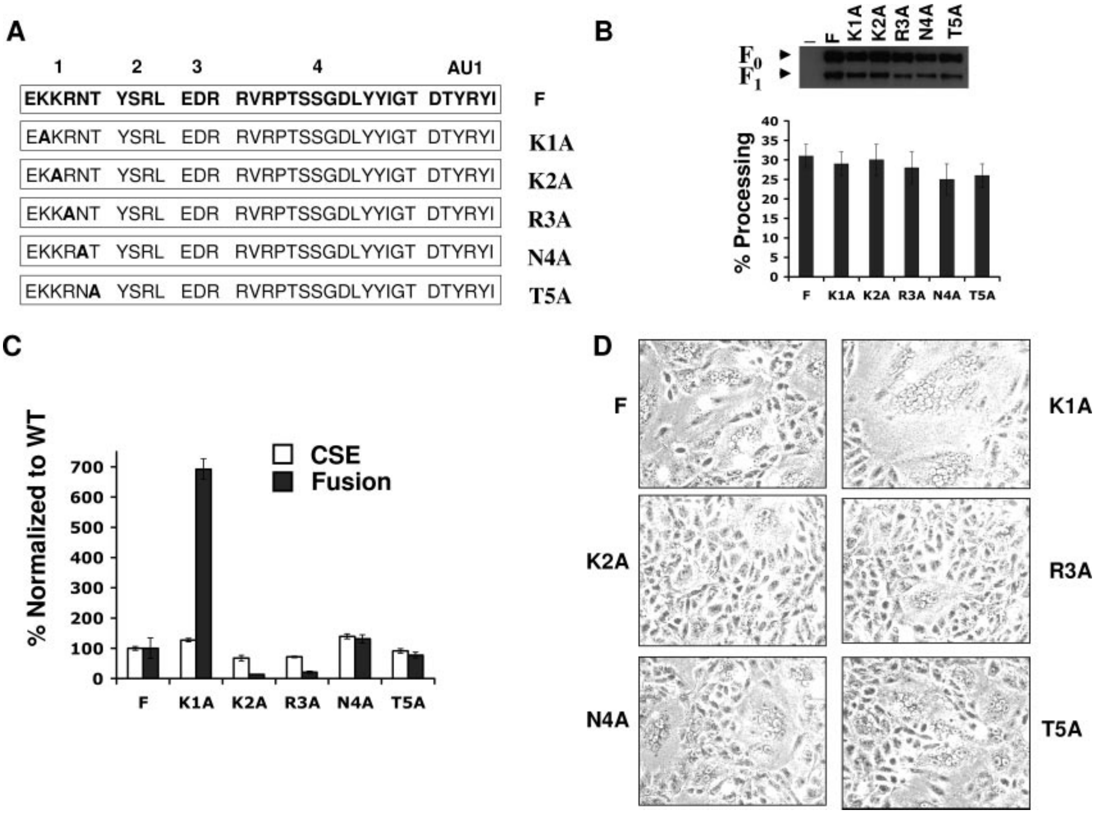


FIG. 2. Analysis of membrane-proximal point mutations in the CTs of NiV-F. (A) Schematic of the NiV-F CT point mutants, showing the sequence of the whole CT, and the positions of the five alanine substitutions in the membrane-proximal region, designated K1A, K2A, R3A, N4A, and T5A. (B) Western blot analysis of immunoprecipitated cell surface biotinylated WT and mutant NiV-F proteins. Surface proteins were analyzed exactly as described in Fig. 1B. Percent processing was also analyzed as described in the legend to Fig. 1B, and the densitometric results are shown graphically. (C) Relative levels of CSE and fusion obtained for WT NiV-F or CT point mutant proteins in 293T cells. Fusion and CSE were determined exactly as for Fig. 1C. Data shown are averages ± standard errors from three independent experiments. (D) Pictures of syncytium formation by the WT NiV-F or the various NiV-F point mutants and WT NiV-G in Vero cells. Representative ×100 fields are shown.

finely map the particular residues within the T1 region that can modulate NiV-F-mediated fusion, we individually mutated each amino acid of the T1 sequence (KKRNT) to an alanine, as depicted in Fig. 2A. 293T cells transfected with an expression plasmid encoding each of the alanine scan mutants were cell surface biotinylated, lysed, precipitated with streptavidin, and subjected to Western blotting to detect NiV-F as described above. All of the alanine scan mutants had levels of CSE and processing similar to those of WT NiV-F (Fig. 2B). Similar levels of CSE of WT and mutant NiV-F proteins were also observed by flow cytometric analyses (Fig. 2C). Next, we determined the fusogenicity of these mutants by quantifying syncytium formation. Representative pictures of syncytia formed by each mutant are shown in Fig. 2D, and the fusion/CSE ratios for WT NiV-F and the indicated mutant were determined (Table 1, bottom). Interestingly, despite WT levels of CSE, mutation of the K1 residue resulted in hyperfusogenicity (fusion/CSE ratio of 5.5) while mutation of the K2 or R3 residue resulted in hypofusogenicity (fusion/CSE ratios of 0.2 and 0.3, respectively) (Table 1, bottom). Mutation of the N4 or T5 residue did not result in any significant change in CSE or fusion (ratio of 0.9) relative to the WT NiV-F protein (Fig. 2C and D and Table 1, bottom). Similar but less dramatic effects on fusogenicity were observed in Vero cells. In summary, these results indicate that the membrane-proximal polybasic KKR sequence in the CT of NiV-F protein can up- or downmodulate its fusogenicity.

Fusion of membrane-proximal NiV-F mutants correlates with entry of ephrinB2-reverse-pseudotyped virus-like particles. Next, we sought to determine if the differences in cell-cell fusion exhibited by the NiV-F CT mutant proteins corresponded to viral entry differences. However, some of these CT mutants were very inefficiently incorporated into our pseudotyped VSV-*Renilla* luciferase (VSVrLuc) reporter viruses, a previously established method for examining NiV envelope-mediated entry (3). To circumvent the problem of variable envelope protein incorporation into VSVrLuc, we developed a novel reverse-pseudotype VSVrLuc entry assay, for which we reverse pseudotyped VSVrLuc with the NiV receptor ephrinB2 (B2-VSVrLuc). We then used these B2-VSVrLuc virions to infect 293T cells previously transfected with equal amounts of mutant or WT NiV-F along with WT NiV-G in a 96-well plate format. Infection of cells expressing HIV Env glycoproteins with viral particles reverse pseudotyped with CD4 and the corresponding coreceptor has been previously reported (36, 56). Figure 3A shows that B2-VSVrLuc viral entry only occurs when cells express both the NiV-F and NiV-G glycoproteins (Fig. 3A). In addition, reverse-

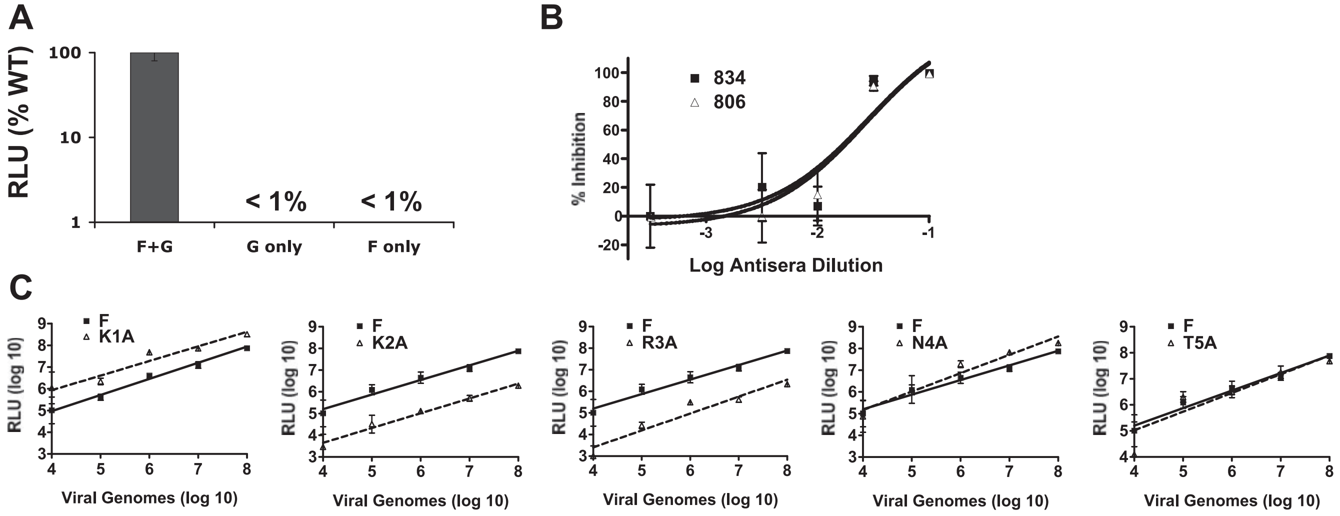


FIG. 3. Reverse-pseudotyped viral entry assay for membrane-proximal CT point mutants. (A) An ephrinB2-pseudotyped VSV-*Renilla* luciferase reporter virus (B2-VSV-rLuc) was used to infect 293T cells previously transfected with expression plasmids for NiV-F–NiV-G, NiV-G alone, or NiV-F alone. Numbers of RLU are shown on a logarithmic scale. (B) Reverse-pseudotyped viral entry into NiV-F- or NiV-G-transfected 293T cells was inhibited by anti-NiV-F and anti-NiV-G specific antisera 834 and 806, respectively. Data are presented as percent inhibition, where 0% represents infection in the absence of any antiserum. The data were normalized as follows. The number of RLU obtained at each serum dilution was calculated as a percentage of the average number of RLU obtained in the absence of any antiserum. Percent inhibition was then calculated as 100% minus the percent infection at each serum dilution. The percent inhibition values were regressed and graphed with GraphPad PRISM. An average of two experiments is shown, with four independent wells per datum point (serum dilution) ± the standard deviation. (C) Relative entry levels of B2-VSV-rLuc virus into 293T cells expressing the WT NiV-G protein and the WT or mutant NiV-F protein. RLU were quantified 24 h postinfection and graphed against the number of viral genomes per milliliter. A single preparation of B2-VSV-rLuc was used for all of the experiments shown. The number of genome copies in the viral preparation was analyzed by reverse transcription-PCR as described in Materials and Methods. The data shown are averages from three independent experiments ± the standard deviations.

pseudotyped viral entry was specifically blocked by previously characterized anti-NiV-F or anti-NiV-G antiserum (3, 33, 44) (Fig. 3B).

We then determined the entry of these B2-VSVrLuc virions into cells expressing WT NiV-G and WT NiV-F or the indicated NiV-F CT point mutants. Entry into K1A-expressing cells was about 8- to 10-fold higher than that of WT NiV-F over several logs of viral input. Conversely, entry into K2A- and R3A-expressing cells was 8- to 30-fold lower than that of WT NiV-F over the same range of viral inputs. Entry levels obtained for the cells expressing the N4A or T5A mutant protein were similar to those expressing WT NiV-F. Thus, our reverse-pseudotype B2-VSVrLuc entry assay results are consistent with our cell-cell fusion results and further demonstrate that the membrane-proximal polybasic KKR motif in the NiV-F CT can modulate virus-cell membrane fusion.

Differential binding and neutralization of hyper- and hypofusogenic NiV-F mutants by distinct novel anti-NiV-F rabbit MAbs. We then asked how specific residues in the KKR region might be modulating fusion. Inside-out signaling from the CT has been reported for other class I enveloped viruses (2, 61), including at least one paramyxovirus (67). We first asked whether any of the KKR alanine mutations affected the overall ectodomain conformation of the NiV-F protein.

We had previously produced conformational polyclonal and monoclonal rabbit Abs by genetically immunizing rabbits with codon-optimized NiV-F and NiV-G and NiV-M expression plasmids (4). We screened a panel of our rabbit MAbs and found two (MAbs 92 and 66) whose epitopes were conformational and distinct. They were conformational because they detected the NiV-F protein in its native state by flow cytometry (Fig. 4A) but not in its denatured form, for example, by Western blot analysis on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown). They were distinct because Ab 66 bound to NiV-F and HeV-F equivalently while Ab 92 bound to NiV-F approximately10-fold more efficiently than to HeV-F at the same Ab concentrations (Fig. 4A). As a control, polyclonal Ab 834 (3, 33, 44) was used to show that the levels of NiV-F and HeV-F were approximately equally recognized by flow cytometry in the same experiment (Fig. 4A).

Then we measured the relative binding of these Abs to the various WT or mutant NiV-F proteins by flow cytometry. We reasoned that conformational differences in the ectodomain might be revealed by differential binding of these Abs. In order to quantitatively correct for variations in the transfection efficiencies and cell surface protein expression levels of the various mutants from experiment to experiment, we analyzed the binding data obtained by calculating the ratios of the mean fluorescence intensities of pairs of Abs (92-66, 92-834, and 66-834). Figure 4B shows the relative binding ratios of thee Ab combinations for WT NiV-F and the indicated mutants. There was a modest but significant decrease in binding of MAb 92 to the hyperfusogenic K1A mutant, as the 92/834 and 92/66 ratios, but not the 66/834 ratio, were lower than those of the WT NiV-F protein ($P = 0.015$, $P = 0.0005$, and $P = 0.94$, respectively) (Fig. 4B). We also detected an increase in binding of MAb 66 to the hypofusogenic R3A mutant protein, as the 66/834 ratio for this mutant was increased, the 92/66 ratio was decreased, and the 92/834 ratio was unchanged compared to those obtained with the WT NiV-F protein ($P = 0.048$, $P =$

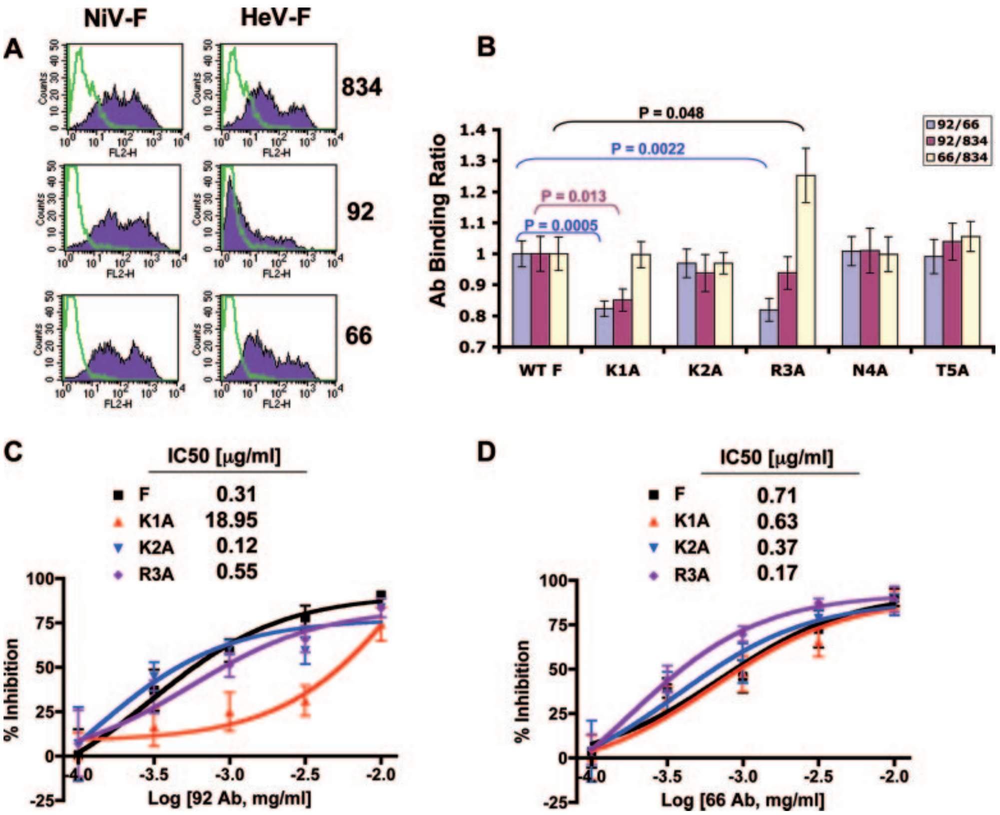


FIG. 4. Specific CT mutants affect the ectodomain conformation as exhibited by differential MAb binding and neutralization. (A) Flow cytometry histograms showing binding of polyclonal anti-NiV-F antiserum 834 or anti-NiV-F MAb 92 or 66 to 293T cells expressing either NiV-F, HeV-F, or neither (pcDNA3 control). Green contours indicate binding of Abs to 293T cells transfected with the pcDNA3.1 backbone only. Overlaid filled purple histograms indicate binding of Abs to NiV-F- or HeV-F-expressing cells, as indicated. (B) MAb binding ratios of pairs of anti-NiV-F Abs. Polyclonal (antiserum 834) or monoclonal (antisera 492 and 66) rabbit Abs were used to stain 293T cells transfected with WT NiV-F or the indicated CT point mutants at a concentration previously determined to be in the linear range of the binding curve. To compare data from repeat experiments and to control for transfection efficiency and differential expression, a set of binding ratios was calculated by dividing the mean fluorescence intensities obtained for the various Abs (92/66, 92/834, and 66/834). The Ab binding ratios for WT NiV-F is necessarily defined as 1. *P* values were calculated with a nonpaired Student *t* test and multiplied by five, which takes into account the Bonferroni correction for the multiple pairwise comparisons (WT versus the five mutants). (C and D) Neutralization of CT mutant proteins by anti-NiV-F Abs. 293T cells expressing the WT NiV-G protein and the WT or mutant NiV-F protein were infected with B2-VSV-rLuc reverse-pseudotyped virus 8 h posttransfection in the presence of increasing amounts of MAb 92 (C) or 66 (D). The amount of viral entry obtained in the absence of anti-NiV-F MAb (artificially represented by the [MAb] = −4.0 datum point) was normalized to 100%, which is equivalent to 0% inhibition. The percent inhibition was then plotted against the logarithm of the Ab concentration. Inhibition curves were regressed, and IC$_{50}$s were calculated with GraphPad PRISM. The data shown are normalized averages from three separate experiments ± the standard deviations.

0.0022, and $P = 0.84$, respectively) (Fig. 4B). All other point mutant proteins did not display a change in Ab binding relative to that of the WT NiV-F protein (*P* values of >0.5) (Fig. 4B). Similar Ab binding ratios were obtained over an Ab concentration range of 0.03 to 3 μg/ml, and the data shown are for 1 μg/ml.

Next, we measured the neutralization capabilities of MAbs 92 and 66 against the various mutant proteins with our reverse-pseudotyped viral entry assay. In general, our neutralization data were consistent with the above-mentioned binding data. For example, since MAb 92 bound relatively less to mutant K1A, we expected that mutant K1A may also be less sensitive to neutralization by MAb 92, and that was indeed the case. Figure 4C shows that the K1A protein was more than 10-fold less sensitive than WT NiV-F to neutralization by MAb 92 (the 50% inhibitory concentrations [IC$_{50}$s] for K1A and WT NiV-F were approximately 19 and 0.3 μg/ml, respectively). In addition, since MAb 66 bound more strongly to mutant R3A, we also expected that the R3A mutant might also be more sensitive to neutralization by MAb 66 than the WT NiV-F. Indeed, we observed that the R3A mutant protein was about fourfold more sensitive to neutralization by MAb 66 than was the WT NiV-F protein, as the IC$_{50}$s for the R3A and WT proteins were approximately 0.17 and 0.71 μg/ml, respectively (Fig. 4D). In toto, our MAb binding and neutralization data show that specific residues in the CT of NiV-F can affect the conformation of its ectodomain.

Association of NiV-F and the hyper- and hypofusogenic mutants with lipid raft domains. Viral envelope glycoproteins are often associated with lipid raft microdomains (22, 26, 43, 47, 62). Such membrane domains are known to have membrane cross-thicknesses greater than those of non-lipid raft cell membrane domains (23, 30) and are enriched in cholesterol and glycosphingolipids. Thus, differential association of WT or mu-

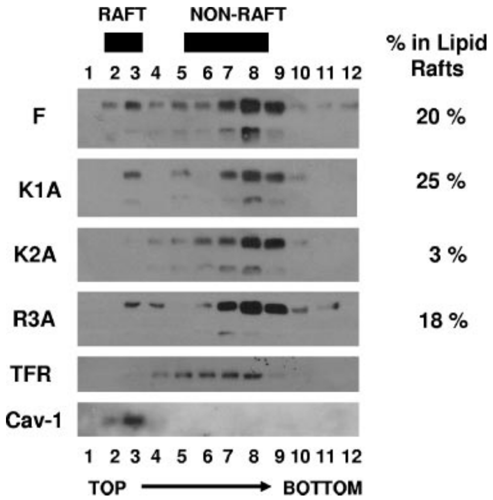


FIG. 5. Association of NiV-F and the hyper- and hypofusogenic mutants with lipid raft domains. Lipid raft fractionations were performed as described in Materials and Methods. Caveolin-1 (Cav-1) and transferrin receptor (TFR) were used as markers for raft (top) and nonraft (bottom) domains, respectively. NiV-F and the indicated mutants were detected by Western blotting with the AU1 Ab. The blots were then stripped and reprobed for Cav-1 and TFR to ensure the integrity of each lipid raft fractionation. Percent NiV-F in lipid rafts was calculated as the percentage of the NiV-F signal observed in the peak Cav-1 fractions (lanes 2 and 3 in most cases) over the sum of signals in the peak Cav-1 and peak TFR fractions (lanes 7 and 8 in most cases) for each sample. This controls for any slight variations between tubes. Representative Cav-1 and TFR blots are shown. The experiment was repeated twice with similar results. Band intensities were quantified by densitometry with a VersaDoc Imaging System (Bio-Rad).

tant NiV-F proteins with lipid raft domains may formally influence the conformation of their ectodomain epitopes, potentially affecting the conformational data in Fig. 4. Therefore, we assessed the relative association of WT and mutant NiV-F proteins with lipid raft domains. First, we observed that a distinct portion (~20%) of the total WT NiV-F protein was associated with lipid raft fractions (Fig. 5), as demonstrated by cofractionation with caveolin-1, a standard marker for lipid raft domains. However, the most of the NiV-F was in nonraft fractions, which were demarcated by the transferrin receptor, a membrane protein known not to be associated with lipid rafts (Fig. 5). With the exception of mutant K2A, all WT and mutant NiV-F proteins were found in both lipid raft and non-lipid raft domains at approximately equal distributions (18 to 25% in lipid raft fractions), indicating that, at least for mutants K1A and R3A, association with lipid rafts did not account for the differences in conformational MAb binding seen in Fig. 4. Interestingly, the hypofusogenic K2A mutant was almost completely absent from the lipid raft fractions, raising the possibility that altered association with lipid raft domains may contribute to its hypofusogenic phenotype and suggesting that mechanistic differences may underlie the hypofusogenic phenotypes of the K2A and R3A mutants.

NiV-F CT fusion mutants are differentially resistant to fusion inhibition by a reagent that prevents 6HB formation and exhibit corresponding rates of fusion kinetics relative to WT NiV-F. Having determined that the specific residues in the CT can affect the ectodomain conformation of NiV-F, we then asked whether the hyper- and hypofusogenic phenotypes exhibited by the NiV-F CT point mutants are mediated by fusion determinants in the ectodomain such as 6HB formation. We have previously shown that a soluble NiV-HR2-Fc protein (HR2 region of NiV-F linked to the Fc constant region of human immunoglobulin G1) inhibits NiV fusion specifically and that the sensitivity of inhibition by this protein inversely correlated with the fusion kinetics of the hyperfusogenic NiV-F N-glycan fusion proteins (3). With the same NiV-HR2-Fc inhibitory reagent, we tested the sensitivity of NiV-F CT mutants or WT NiV-F to fusion inhibition. We observed that the K1A mutant exhibited significantly greater resistance to NiV-HR2-Fc than WT NiV-F for all three concentrations of HR2-Fc tested (Fig. 6A). On the other hand, the K2A mutant exhibited a significantly lower resistance to NiV-HR2-Fc (Fig. 6A), especially when subsaturating amounts of HR2-Fc were

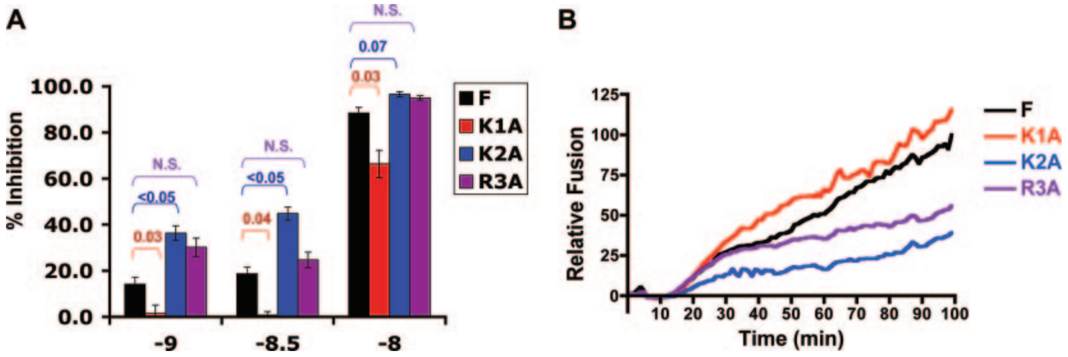
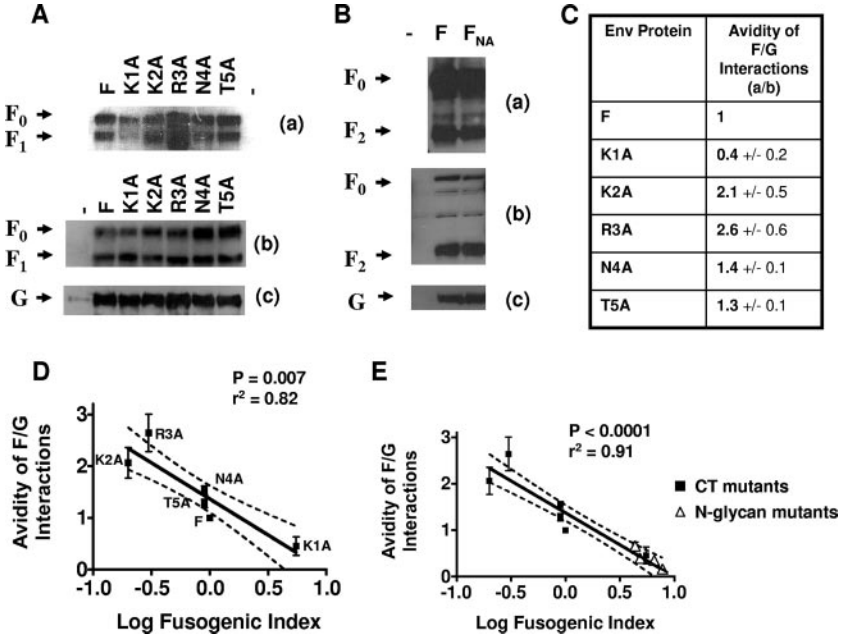


FIG. 6. NiV-F CT fusion mutants are differentially resistant to fusion inhibition by NiV-F HR2-Fc and exhibit corresponding rates of fusion kinetics relative to WT NiV-F. (A) The sensitivity of NiV envelope-mediated fusion to inhibition by NiV-HR2-Fc is shown for WT NiV-F and the indicated CT mutants. For each fusion protein, the amount of fusion in the absence of any inhibitor is set at 0% inhibition. One representative experiment out of two is shown. Error bars indicate standard deviations. *P* values were calculated with the Student *t* test and the Bonferroni correction to account for the multiple pairwise comparisons of significance (F versus K1A, F versus K2A, and F versus R3A). (B) Fusion kinetics of WT or mutant NiV-F protein. NiV-G was expressed with WT NiV-F or the indicated mutants in effector PK13 cells, and the relative rate of fusion was assessed with target 293T cells loaded with CCF2 dye (see Materials and Methods). Relative fusion is the ratio of blue to green fluorescence obtained with NiV-G- and NiV-F-transfected effectors minus the ratio of background blue and green fluorescence obtained with empty-vector (pcDNA3)-transfected cells. Each datum point is an average from three independent experiments.



Env Protein	Avidity of F/G Interactions (a/b)
F	1
K1A	0.4 +/- 0.2
K2A	2.1 +/- 0.5
R3A	2.6 +/- 0.6
N4A	1.4 +/- 0.1
T5A	1.3 +/- 0.1

FIG. 7. Fusogenicity of WT NiV-F and the CT mutants inversely correlates with the avidity of F-G interactions. (A) Western blot analysis of co-IP F_0 and F_1 (top part a), immunoprecipitated G (bottom part c), and the relative amounts of F_0 and F_1 present in total cell lysate (middle part b). Cell lysates of 293T cells transfected with WT NiV-G and NiV-F or the indicated CT mutants were immunoprecipitated with rabbit anti-NiV-G specific antisera. The top and middle parts were blotted with mouse anti-AU1 to detect NiV-F, and the bottom part was blotted with mouse anti-HA to detect NiV-G. (B) A coimmunoprecipitation experiment identical to that in panel A was performed with tagged and untagged NiV-F (F and F_{NA}, respectively) but with a rabbit anti-F_2 peptide Ab for detection. Parts a, b, and c are as in panel A. (C) Relative avidities of NiV-F–NiV-G interactions for WT NiV-F and the indicated CT mutants. The amounts of co-IP NiV fusion proteins in panel A were quantified by densitometry as described in the text, with a VersaDoc Imaging System (Bio-Rad). The avidity of F-G interactions is represented by the ratio of the amount of NiV-F protein co-IP with anti-NiV-G antiserum to the relative amount of NiV-F expressed in cell lysates (parts a and b, respectively). The data presented are averages ± standard errors from three experiments. (D) Avidity of the F-G interactions from panel C plotted against the fusion/CSE ratios from Table 1. Pearson correlation analysis was performed with GraphPad PRISM. (E) The avidities of F-G interactions for the multiple N-glycan mutants previously reported by Aguilar et al. (3) were overlaid with the datum points from panel C and plotted together against their respective fusogenic indexes. CT mutants and N-glycan mutants are represented by closed and open symbols, respectively. Pearson correlation analysis was performed with GraphPad PRISM.

used. These results suggested that the rate of 6HB formation contributed to the hyper- and hypofusogenicity of the K1A and K2A mutants, respectively. Interestingly, mutant R3A did not reveal a significant difference in resistance to inhibition by the NiV-HR2-Fc molecule relative to the WT NiV-F protein, suggesting that the hypofusogenic phenotype of the K2A and R3A mutants may be mediated via distinct mechanisms. This is also consistent with our Ab binding data, which suggest that the K2A and R3A mutants differentially affect ectodomain conformation (Fig. 4B).

In order to determine if sensitivity to NiV-HR2-Fc inhibition is actually due to the rate of 6HB formation and, hence, fusion pore formation, we measured fusion kinetics mediated by NiV-F or the indicated CT mutants and WT NiV-G. Real-time fusion kinetics can be measured and quantified with a β-lactamase reporter cell-cell fusion assay that we previously described for analysis of our hyperfusogenic N-glycan NiV-F mutants (3). We found that cells expressing the hyperfusogenic K1A fusion mutant showed faster fusion kinetics and fused to a greater extent than cells expressing WT NiV-F (Fig. 6B). In contrast, the hypofusogenic K2A mutant showed slower fusion kinetics and fused to a lesser extent than cells expressing the WT NiV-F protein (Fig. 6B). Interestingly, although the cells expressing the R3A mutant fused at the same rate as the WT NiV-F protein for the first 40 min, thereafter, their rates of fusion diverged, with the R3A mutant slowing down significantly such that at 100 min, it had fused at less than 50% of the WT NiV-F level (Fig. 6B). The results in Fig. 6A and B strongly suggest a mechanistic difference between the hypofusogenic phenotypes exhibited by the K2A and R3A mutants.

Fusogenicity of NiV-F inversely correlates to the avidity of F-G interactions for the CT mutant proteins. We had previously provided evidence for the attachment protein displacement model for paramyxoviral entry. At least for NiV, the hyperfusogenic N-glycan mutants appear to have weaker interactions between the NiV-F mutants and NiV-G, allowing greater NiV-F–NiV-G dissociation after receptor binding. Thus, fusogenicity inversely correlated to the avidity of F-G interactions for the hyperfusogenic N-glycan NiV-F mutants (3). Here, we asked if the relative avidity of NiV-F–NiV-G associations correlated with the fusogenicity of the CT mutants.

We coexpressed NiV-G with WT NiV-F or the aforementioned mutants in permissive 293T cells and determined the relative avidity of NiV-F and NiV-G interactions by immunoprecipitating whole cell lysates with anti-NiV-G antiserum and detecting the amount of co-IP NiV-F by Western blotting with an AU1 epitope tag Ab (Fig. 7A, part a). The relative amounts of WT and mutant NiV-F were also determined in total cell lysates (Fig. 7A, part b). To normalize for the various expression levels of WT or mutant NiV-F in any single experiment, we calculated the ratio of the level of co-IP NiV-F to the corresponding amount of NiV-F in the total cell lysate. For example, if the amount of co-IP NiV-F was densitometrically quantified at 160 U and the corresponding amount of NiV-F in the cell lysate was 100 U, the F-G co-IP ratio would be 1.6. This ratiometric value was arbitrarily set to 1.0 to indicate the relative avidity of the WT NiV-F–NiV-G interactions (Fig. 7C). On this scale, a value of greater or less than 1.0 would indicate a corresponding increased or decreased avidity in F-G interaction relative to the WT proteins, respectively. Also, we note that the AU1 tag did not affect NiV-F's interaction with G, as the same experiment performed with tagged and untagged NiV-F revealed no difference in the amount of F that can be co-IP with G (Fig. 7B).

When we plotted the relative avidity of NiV-G interactions with WT NiV-F or the indicated CT mutants (Fig. 7C) against their fusogenicities (fusion/CSE ratio) as determined in Table 1, we obtained a significant negative correlation ($r^2 = 0.82$, $P = 0.007$) between the avidity of F-G interaction and the fusogenicity of the NiV-F protein (Fig. 7D). Thus, for example, the NiV-F mutant (K1A) with the lowest relative avidity of F-G interaction (0.4) was also the most fusogenic NiV-F CT mutant examined (fusion/CSE ratio of 5.5), and mutants (K2A and R3A) with the highest relative avidities of F-G interaction (2.1 and 2.6) were the least fusogenic (fusion/CSE ratios of 0.2 and 0.3). These results suggest that the effects of the CT mutants on modulating fusogenicity were linked to the increasing or decreasing avidity of F-G interactions and provide further support for the model (3, 63, 75) where dissociation of the attachment protein from the fusion protein is a rate-limiting step required for fusion peptide exposure and subsequent membrane fusion.

DISCUSSION

Our results implicate the cytoplasmic domain of the NiV fusion protein in modulating fusion through its membrane-proximal polybasic KKR motif in an inside-out signaling manner. Our data also shed some light on the mechanisms by which the KKR motif modulates fusion; specific residues within the KKR motif can modulate the conformation of NiV-F's ectodomain and thus have an effect on fusion kinetics by regulating the rate of 6HB formation and the avidity of the F-G interactions.

The CTs of other paramyxovirus fusion proteins are known to be required for various protein functions, including proper surface expression, membrane fusion, fusion pore enlargement, transition from hemifusion to complete fusion, and/or budding (7, 19, 59, 65, 68), although removal of the CT has resulted in quite distinct phenotypes in different paramyxoviruses, ranging from no effect (12) to fusion pore formation (65) to fusion pore enlargement (19) to syncytium formation (59, 65). In this report, we show that relatively large deletions in the NiV-F CT did not significantly compromise conformational integrity or CSE but can either reduce or enhance fusion (Fig. 1). In addition, while point mutations in the membrane-proximal region had no significant effect on conformational integrity, processing, or CSE, they variably affected fusogenicity (Fig. 2). Indeed, we identified a membrane-proximal polybasic KKR patch in the CT of NiV-F as having the ability to up- or downmodulate fusogenicity. Polybasic residues can also be found near the membrane-spanning region in the CTs of most other paramyxoviruses, but to our knowledge, their function in modulating fusion has not been reported.

Our data show that specific CT mutants with changes in the KKR motif mediate their hyper- or hypofusogenic phenotypes through common mechanisms that have been defined for other class I fusion proteins. For example, the hyperfusogenic V3 loop and CT mutants of the HIV-1 envelope glycoprotein also show faster fusion kinetics and display increased resistance to heptad repeat peptide inhibition (1, 52). In the case of NiV, our results suggest that the hyper- and hypofusogenicity phenotypes of the K1A and K2A mutants are governed by the rate of 6HB formation (Fig. 6A) during fusion pore formation, resulting in increased or decreased fusion kinetics, respectively (Fig. 6B). However, since we did not detect any apparent differences between the R3A mutant and WT NiV-F during 6HB formation and its hypofusogenic phenotype was only manifested in slower fusion kinetics at later time points (Fig. 6B), we speculate that a step post 6HB formation, perhaps fusion pore enlargement, may be affected by the hypofusogenic mutation R3A. The CT of at least one other paramyxovirus, SV5, has been implicated in fusion pore enlargement (19). Our lipid raft results also highlight the mechanistic differences observed between the hypofusogenic K2A and R3A mutants observed in Fig. 4 and 6. The K2A, but not the R3A, mutant displayed differences in lipid raft association compared to the WT NiV-F protein. While many hyper- or hypofusogenic phenotypes in class I viral fusion proteins have been identified, it is uncommon to find a contiguous series of residues within a small patch that have such contrasting contributory roles in fusogenicity.

It remains to be determined how these three basic residues in NiV-F CT actually modulate the kinetics of fusion. Do the KKR mutants stabilize or destabilize the metastable prefusogenic conformation of NiV-F, and/or do they affect subsequent steps in the fusion process? It is also possible that the KKR basic motif may interact with cellular proteins that directly or indirectly modulate the actin cortical cytoskeleton, which is intimately involved in membrane dynamics and curvature during fusion and syncytium formation (37, 49, 51). Dutch and colleagues have previously reported that various transdominant Rho-GTPases can up- or down-regulate HeV fusion (57). Since the ERM (ezrin-radixin-monesin) family of proteins is known to connect the CTs of various membrane proteins to the actin cortical cytoskeleton and the ERM proteins themselves are known to be activated and inactivated by distinct Rho GTPases (21, 29), we speculate that the ERM proteins may connect the CT of NiV-F to the actin cortical cytoskeleton and that modulation of the CT's attachment to the cortical cytoskeleton by the Rho GTPases, or by our various KKR mutants, is what accounts for the hyper- or hypofusogenic phenotypes seen. Intriguingly, ERM proteins preferentially bind CTs of membrane proteins that have isoelectric points higher than 9.0 and that have basic amino acid clusters (29, 74). They also prefer to bind CTs that contain phosphorylated serines and tyrosine motifs (17, 58). NiV-F's CT has an isoelectric point of

9.88, contains a C-terminal tyrosine-rich motif, and contains two basic clusters, i.e., the membrane-proximal KKR cluster that we know affects fusion and an RRVR cluster between regions T3 and T4. In addition, preliminary mass spectrometry analysis indicated that the two C-terminal serines in NiV-F are phosphorylated (unpublished observations). Therefore, it seems plausible that cellular factors such as ERM proteins may connect the CT of NiV-F or HeV-F to the actin cortical cytoskeleton of cells, and the strength and stability of this connection may modulate fusogenicity.

Our data also suggest that the KKR motif modulates fusogenicity via an inside-out signaling mechanism. Differential MAb recognition of the ectodomain correlating with differential neutralization (Fig. 4), faster or slower rates of fusion kinetics affected by the rate of 6HB formation (Fig. 6), and differential effects on the avidity of F-G interactions (Fig. 7) all argue that mutations of these cytoplasmic residues can affect the conformation and subsequent fusogenic function of the ectodomain. Interestingly, data from differential MAb binding, rate of 6HB formation, and fusion kinetics experiments also reveal that distinct mechanisms underlie the similar hypofusogenic phenotypes of the K2A and R3A mutants. For example, while MAbs 92 and 66 clearly bound differentially to the R3A mutant, no difference in K2A binding was observed (Fig. 4B). On the other hand, while K2A was significantly more sensitive to inhibition by HR2-Fc compared to WT NiV-F, R3A was similar in sensitivity to WT NiV-F (Fig. 6A). This equivalent sensitivity to HR2-Fc inhibition is consistent with our real-time fusion kinetics data showing that for the first 40 min, R3A fused at the same rate and to the same extent as WT NiV-F, while K2A fused much more slowly from the very beginning (Fig. 6B). However, after 40 min, R3A began to exhibit slower fusion kinetics and eventually fused to a much lesser extent than WT NiV-F at 100 min. As mentioned above, it is likely that the defect in fusion in the R3A mutant is manifested at a stage post 6HB formation, such as fusion pore enlargement.

We previously suggested that a critical parameter that governs NiV envelope-mediated fusion is the avidity of F and G association, which we quantified by a rigorous coimmunoprecipitation assay (3). Our published data showed a strong and significant negative correlation between the degree of hyperfusogenicity exhibited by a variety of ectodomain N-glycan mutants and the avidity of F and G association. We had therefore favored the attachment protein displacement model of paramyxovirus fusion where the dissociation of G from F after receptor engagement better allows for the conformational changes in F that lead to fusion peptide exposure and membrane fusion. We now provide data to further expand and support this model with both hyper- and hypofusogenic mutants (Fig. 7D). Indeed, as shown in Fig. 7E, when we added our present datum points to the datum points from our hyperfusogenic N-glycan mutants (3), the Pearson correlation became even stronger ($r^2 = 0.91$) and more significant ($P < 0.0001$). These data suggest that F and G dissociation can be a common pathway for the triggering of F regardless of the determinants of fusion in F involved. However, since we performed these studies with receptor-containing 293T cells, we are not able to distinguish whether the differences in F-G association between WT and mutant fusion proteins we have observed occur pre or post receptor binding. True avidity measurements would have to be done with truly receptor-negative cells. In addition, it remains to be determined whether the KKR motif affects the interaction of NiV-F with NiV-G directly and/or via modification of the NiV-F ectodomain's overall conformation. At least for one other paramyxovirus F protein (NDV), the ectodomain HR2 region has been implicated in binding to the attachment protein HN (24); therefore, the effects of NiV-F CT mutants on the avidities of NiV-F–NiV-G interactions observed here may be due to inside-out signaling.

We also note that NiV-F processing is usually increased when G is cotransfected (compare Fig. 7A with Fig. 1B and 2B). Since both F and G are encoded by codon-optimized genes, a potential explanation is that expression of G competes for transcriptional or translational resources, resulting in less overexpression of F; overexpression of F in the absence of G can overwhelm the proteolytic machinery required for F cleavage. However, it would be interesting to determine whether the presence of G, and its association with F, can intrinsically affect F processing, either by modulating its endosomal recycling behavior or changing the conformation of F to make it more accessible to cathepsin L cleavage.

Finally, it remains to be determined how the NiV-F CT actually stabilizes or destabilizes F-G interactions and whether the fusion-modulatory role played by the polybasic motif in the CT of NiV-F is unique for NiV (or the henipaviruses). The studies presented in this report point to the many determinants of fusion in NiV-F and underscore the complexities that regulate the "proper" amount of fusion mediated by NiV-F, which has both fusion-promoting and fusion-inhibiting determinants. Further studies of these determinants will enhance our understanding of the pathobiology of this deadly emerging virus and may reveal more targets for therapeutic intervention.

ACKNOWLEDGMENTS

This study was supported by NIH grants AI059051, AI060694, and AI069317 to B.L. Additional support was provided by a Charles E. Culpepper Medical Scholarship from the Rockefeller Brothers Fund and a Burroughs Wellcome Fund Career Development Award. We acknowledge support from the UCLA AIDS Institute and the CFAR flow cytometry core supported by NIH grants CA16042 and AI28697.

REFERENCES

1. **Abrahamyan, L. G., S. R. Mkrtchyan, J. Binley, M. Lu, G. B. Melikyan, and F. S. Cohen.** 2005. The cytoplasmic tail slows the folding of human immunodeficiency virus type 1 Env from a late prebundle configuration into the six-helix bundle. J. Virol. **79:**106–115.
2. **Aguilar, H. C., W. F. Anderson, and P. M. Cannon.** 2003. Cytoplasmic tail of Moloney murine leukemia virus envelope protein influences the conformation of the extracellular domain: implications for mechanism of action of the R Peptide. J. Virol. **77:**1281–1291.
3. **Aguilar, H. C., K. A. Matreyek, C. M. Filone, S. T. Hashimi, E. L. Levroney, O. A. Negrete, A. Bertolotti-Ciarlet, D. Y. Choi, I. McHardy, J. A. Fulcher, S. V. Su, M. C. Wolf, L. Kohatsu, L. G. Baum, and B. Lee.** 2006. N-glycans on Nipah virus fusion protein protect against neutralization but reduce membrane fusion and viral entry. J. Virol. **80:**4878–4889.
4. **Aguilar, H. C., O. A. Negrete, K. A. Matreyek, D. Y. Choi, and B. Lee.** 2006. Novel rabbit monoclonal antibodies against the henipavirus envelope glycoproteins reveal conformational epitopes related to fusogenicity and receptor binding, abstr. P19-4. *In* American Society for Virology, 25th Annual Meeting. American Society for Virology, Madison, WI.
5. **Anonymous.** 28 April 2004, posting date. NIPAH virus breaks out in Bangladesh: mortality rates of 60% to 74%. Human to-human transmission may be implicated. Wildlife Trust. www.ewire.com/display.cfm/WirelowemID/2117. [Online.]
6. **Anonymous.** 2004. Nipah virus outbreak(s) in Bangladesh, January-April 2004. Wkly. Epidemiol. Rec. **79:**168–171.
7. **Bagai, S., and R. A. Lamb.** 1996. Truncation of the COOH-terminal region

of the paramyxovirus SV5 fusion protein leads to hemifusion but not complete fusion. J. Cell Biol. **135:**73–84.

8. **Baker, K. A., R. E. Dutch, R. A. Lamb, and T. S. Jardetzky.** 1999. Structural basis for paramyxovirus-mediated membrane fusion. Mol. Cell **3:**309–319.
9. **Bonaparte, M. I., A. S. Dimitrov, K. N. Bossart, G. Crameri, B. A. Mungall, K. A. Bishop, V. Choudhry, D. S. Dimitrov, L. F. Wang, B. T. Eaton, and C. C. Broder.** 2005. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. Proc. Natl. Acad. Sci. USA **102:**10652–10657.
10. **Bossart, K. N., L. F. Wang, B. T. Eaton, and C. C. Broder.** 2001. Functional expression and membrane fusion tropism of the envelope glycoproteins of Hendra virus. Virology **290:**121–135.
11. Reference deleted.
12. **Branigan, P. J., N. D. Day, C. Liu, L. L. Gutshall, J. A. Melero, R. T. Sarisky, and A. M. Del Vecchio.** 2006. The cytoplasmic domain of the F protein of human respiratory syncytial virus is not required for cell fusion. J. Gen. Virol. **87:**395–398.
13. **Brody, B. A., S. S. Rhee, and E. Hunter.** 1994. Postassembly cleavage of a retroviral glycoprotein cytoplasmic domain removes a necessary incorporation signal and activates fusion activity. J. Virol. **68:**4620–4627.
14. **Carr, C. M., and P. S. Kim.** 1993. A spring-loaded mechanism for the conformational change of influenza hemagglutinin. Cell **73:**823–832.
15. **Carter, J. R., C. T. Pager, S. D. Fowler, and R. E. Dutch.** 2005. Role of N-linked glycosylation of the Hendra virus fusion protein. J. Virol. **79:**7922–7925.
16. **Chakrabarti, L., M. Emerman, P. Tiollais, and P. Sonigo.** 1989. The cytoplasmic domain of simian immunodeficiency virus transmembrane protein modulates infectivity. J. Virol. **63:**4395–4403.
17. **Dickson, T. C., C. D. Mintz, D. L. Benson, and S. R. Salton.** 2002. Functional binding interaction identified between the axonal CAM L1 and members of the ERM family. J. Cell Biol. **157:**1105–1112.
18. **Diederich, S., M. Moll, H. D. Klenk, and A. Maisner.** 2005. The Nipah virus fusion protein is cleaved within the endosomal compartment. J. Biol. Chem. **280:**29899–29903.
19. **Dutch, R. E., and R. A. Lamb.** 2001. Deletion of the cytoplasmic tail of the fusion protein of the paramyxovirus simian virus 5 affects fusion pore enlargement. J. Virol. **75:**5363–5369.
20. **Fass, D., S. C. Harrison, and P. S. Kim.** 1996. Retrovirus envelope domain at 1.7 Angstrom resolution. Nat. Struct. Biol. **3:**465–469.
21. **Faure, S., L. I. Salazar-Fontana, M. Semichon, V. L. Tybulewicz, G. Bismuth, A. Trautmann, R. N. Germain, and J. Delon.** 2004. ERM proteins regulate cytoskeleton relaxation promoting T cell-APC conjugation. Nat. Immunol. **5:**272–279.
22. **Fleming, E. H., A. A. Kolokoltsov, R. A. Davey, J. E. Nichols, and N. J. Roberts, Jr.** 2006. Respiratory syncytial virus F envelope protein associates with lipid rafts without a requirement for other virus proteins. J. Virol. **80:**12160–12170.
23. **Gandhavadi, M., D. Allende, A. Vidal, S. A. Simon, and T. J. McIntosh.** 2002. Structure, composition, and peptide binding properties of detergent soluble bilayers and detergent resistant rafts. Biophys. J. **82:**1469–1482.
24. **Gravel, K. A., and T. G. Morrison.** 2003. Interacting domains of the HN and F proteins of Newcastle disease virus. J. Virol. **77:**11040–11049.
25. **Green, N., T. M. Shinnick, O. Witte, A. Ponticelli, J. G. Sutcliffe, and R. A. Lerner.** 1981. Sequence-specific antibodies show that maturation of Moloney leukemia virus envelope polyprotein involves removal of a COOH-terminal peptide. Proc. Natl. Acad. Sci. USA **78:**6023–6027.
26. **Hawkes, D. J., and J. Mak.** 2006. Lipid membrane; a novel target for viral and bacterial pathogens. Curr. Drug Targets **7:**1615–1621.
27. **Horvath, C. M., R. G. Paterson, M. A. Shaughnessy, R. Wood, and R. A. Lamb.** 1992. Biological activity of paramyxovirus fusion proteins: factors influencing formation of syncytia. J. Virol. **66:**4564–4569.
28. **Ito, M., M. Nishio, H. Komada, Y. Ito, and M. Tsurudome.** 2000. An amino acid in the heptad repeat 1 domain is important for the haemagglutinin-neuraminidase-independent fusing activity of simian virus 5 fusion protein. J. Gen. Virol. **81:**719–727.
29. **Ivetic, A., and A. J. Ridley.** 2004. Ezrin/radixin/moesin proteins and Rho GTPase signalling in leucocytes. Immunology **112:**165–176.
30. **Jacobson, K., O. G. Mouritsen, and R. G. Anderson.** 2007. Lipid rafts: at a crossroad between cell biology and physics. Nat. Cell Biol. **9:**7–14.
31. **Kodama, T., D. P. Burns, H. W. Kestler III, M. D. Daniel, and R. C. Desrosiers.** 1990. Molecular changes associated with replication of simian immunodeficiency virus in human cells. J. Med. Primatol. **19:**431–437.
32. **Lam, S. K.** 2003. Nipah virus—a potential agent of bioterrorism? Antiviral Res. **57:**113–119.
33. **Levroney, E. L., H. C. Aguilar, J. A. Fulcher, L. Kohatsu, K. E. Pace, M. Pang, K. B. Gurney, L. G. Baum, and B. Lee.** 2005. Novel innate immune functions for galectin-1: galectin-1 inhibits cell fusion by Nipah virus envelope glycoproteins and augments dendritic cell secretion of proinflammatory cytokines. J. Immunol. **175:**413–420.
34. **Lineberger, J. E., R. Danzeisen, D. J. Hazuda, A. J. Simon, and M. D. Miller.** 2002. Altering expression levels of human immunodeficiency virus type 1 gp120-gp41 affects efficiency but not kinetics of cell-cell fusion. J. Virol. **76:**3522–3533.
35. **Lou, Z., Y. Xu, K. Xiang, N. Su, L. Qin, X. Li, G. F. Gao, M. Bartlam, and Z. Rao.** 2006. Crystal structures of Nipah and Hendra virus fusion core proteins. FEBS J. **273:**4538–4547.
36. **Mebatsion, T., S. Finke, F. Weiland, and K. K. Conzelmann.** 1997. A CXCR4/CD4 pseudotype rhabdovirus that selectively infects HIV-1 envelope protein-expressing cells. Cell **90:**841–847.
37. **Melikyan, G. B., S. A. Brener, D. C. Ok, and F. S. Cohen.** 1997. Inner but not outer membrane leaflets control the transition from glycosylphosphatidylinositol-anchored influenza hemagglutinin-induced hemifusion to full fusion. J. Cell Biol. **136:**995–1005.
38. **Melikyan, G. B., R. M. Markosyan, H. Hemmati, M. K. Delmedico, D. M. Lambert, and F. S. Cohen.** 2000. Evidence that the transition of HIV-1 gp41 into a six-helix bundle, not the bundle configuration, induces membrane fusion. J. Cell Biol. **151:**413–423.
39. **Meulendyke, K. A., M. A. Wurth, R. O. McCann, and R. E. Dutch.** 2005. Endocytosis plays a critical role in proteolytic processing of the Hendra virus fusion protein. J. Virol. **79:**12643–12649.
40. **Moll, M., A. Kaufmann, and A. Maisner.** 2004. Influence of N-glycans on processing and biological activity of the Nipah virus fusion protein. J. Virol. **78:**7274–7278.
41. **Mulligan, M. J., G. V. Yamshchikov, G. D. Ritter, Jr., F. Gao, M. J. Jin, C. D. Nail, C. P. Spies, B. H. Hahn, and R. W. Compans.** 1992. Cytoplasmic domain truncation enhances fusion activity by the exterior glycoprotein complex of human immunodeficiency virus type 2 in selected cell types. J. Virol. **66:**3971–3975.
42. **Murakami, T., S. Ablan, E. O. Freed, and Y. Tanaka.** 2004. Regulation of human immunodeficiency virus type 1 Env-mediated membrane fusion by viral protease activity. J. Virol. **78:**1026–1031.
43. **Nayak, D. P., and S. Barman.** 2002. Role of lipid rafts in virus assembly and budding. Adv. Virus Res. **58:**1–28.
44. **Negrete, O. A., E. L. Levroney, H. C. Aguilar, A. Bertolotti-Ciarlet, R. Nazarian, S. Tajyar, and B. Lee.** 2005. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. Nature **436:**401–405.
45. **Negrete, O. A., M. C. Wolf, H. C. Aguilar, S. Enterlein, W. Wang, E. Muhlberger, S. V. Su, A. Bertolotti-Ciarlet, R. Flick, and B. Lee.** 2006. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. PLoS Pathogens **2:**e7.
46. **Olson, J. G., C. Rupprecht, P. E. Rollin, U. S. An, M. Niezgoda, T. Clemins, J. Walston, and T. G. Ksiazek.** 2002. Antibodies to Nipah-like virus in bats (Pteropus lylei), Cambodia. Emerg. Infect. Dis. **8:**987–988.
47. **Ono, A., and E. O. Freed.** 2001. Plasma membrane rafts play a critical role in HIV-1 assembly and release. Proc. Natl. Acad. Sci. USA **98:**13925–13930.
48. **Paterson, R. G., S. W. Hiebert, and R. A. Lamb.** 1985. Expression at the cell surface of biologically active fusion and hemagglutinin/neuraminidase proteins of the paramyxovirus simian virus 5 from cloned cDNA. Proc. Natl. Acad. Sci. USA **82:**7520–7524.
49. **Pontow, S. E., N. V. Heyden, S. Wei, and L. Ratner.** 2004. Actin cytoskeletal reorganizations and coreceptor-mediated activation of Rac during human immunodeficiency virus-induced cell fusion. J. Virol. **78:**7138–7147.
50. **Ragheb, J. A., and W. F. Anderson.** 1994. pH-independent murine leukemia virus ecotropic envelope-mediated cell fusion: implications for the role of the R peptide and p12E TM in viral entry. J. Virol. **68:**3220–3231.
51. **Razinkov, V. I., G. B. Melikyan, R. M. Epand, R. F. Epand, and F. S. Cohen.** 1998. Effects of spontaneous bilayer curvature on influenza virus-mediated fusion pores. J. Gen. Physiol. **112:**409–422.
52. **Reeves, J. D., S. A. Gallo, N. Ahmad, J. L. Miamidian, P. E. Harvey, M. Sharron, S. Pohlmann, J. N. Sfakianos, C. A. Derdeyn, R. Blumenthal, E. Hunter, and R. W. Doms.** 2002. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics. Proc. Natl. Acad. Sci. USA **99:**16249–16254.
53. **Reeves, J. D., J. L. Miamidian, M. J. Biscone, F. H. Lee, N. Ahmad, T. C. Pierson, and R. W. Doms.** 2004. Impact of mutations in the coreceptor binding site on human immunodeficiency virus type 1 fusion, infection, and entry inhibitor sensitivity. J. Virol. **78:**5476–5485.
54. **Rein, A., J. Mirro, J. G. Haynes, S. M. Ernst, and K. Nagashima.** 1994. Function of the cytoplasmic domain of a retroviral transmembrane protein: p15E-p2E cleavage activates the membrane fusion capability of the murine leukemia virus Env protein. J. Virol. **68:**1773–1781.
55. **Russell, C. J., T. S. Jardetzky, and R. A. Lamb.** 2001. Membrane fusion machines of paramyxoviruses: capture of intermediates of fusion. EMBO J. **20:**4024–4034.
56. **Schnell, M. J., J. E. Johnson, L. Buonocore, and J. K. Rose.** 1997. Construction of a novel virus that targets HIV-1-infected cells and controls HIV-1 infection. Cell **90:**849–857.
57. **Schowalter, R. M., M. A. Wurth, H. C. Aguilar, B. Lee, C. L. Moncman, R. O. McCann, and R. E. Dutch.** 2006. Rho GTPase activity modulates paramyxovirus fusion protein-mediated cell-cell fusion. Virology **350:**323–334.
58. **Serrador, J. M., M. Vicente-Manzanares, J. Calvo, O. Barreiro, M. C. Montoya, R. Schwartz-Albiez, H. Furthmayr, F. Lozano, and F. Sanchez-Madrid.** 2002. A novel serine-rich motif in the intercellular adhesion molecule 3 is critical for its ezrin/radixin/moesin-directed subcellular targeting. J. Biol. Chem. **277:**10400–10409.

59. **Seth, S., A. Vincent, and R. W. Compans.** 2003. Mutations in the cytoplasmic domain of a paramyxovirus fusion glycoprotein rescue syncytium formation and eliminate the hemagglutinin-neuraminidase protein requirement for membrane fusion. J. Virol. **77:**167–178.
60. **Shimizu, H., F. Hasebe, H. Tsuchie, S. Morikawa, H. Ushijima, and T. Kitamura.** 1992. Analysis of a human immunodeficiency virus type 1 isolate carrying a truncated transmembrane glycoprotein. Virology **189:**534–546.
61. **Spies, C. P., G. D. Ritter, Jr., M. J. Mulligan, and R. W. Compans.** 1994. Truncation of the cytoplasmic domain of the simian immunodeficiency virus envelope glycoprotein alters the conformation of the external domain. J. Virol. **68:**585–591.
62. **Takeda, M., G. P. Leser, C. J. Russell, and R. A. Lamb.** 2003. Influenza virus hemagglutinin concentrates in lipid raft microdomains for efficient viral fusion. Proc. Natl. Acad. Sci. USA **100:**14610–14617.
63. **Takimoto, T., G. L. Taylor, H. C. Connaris, S. J. Crennell, and A. Portner.** 2002. Role of the hemagglutinin-neuraminidase protein in the mechanism of paramyxovirus-cell membrane fusion. J. Virol. **76:**13028–13033.
64. **Tan, C. T., and K. T. Wong.** 2003. Nipah encephalitis outbreak in Malaysia. Ann. Acad. Med. Singapore **32:**112–117.
65. **Tong, S., M. Li, A. Vincent, R. W. Compans, E. Fritsch, R. Beier, C. Klenk, M. Ohuchi, and H. D. Klenk.** 2002. Regulation of fusion activity by the cytoplasmic domain of a paramyxovirus F protein. Virology **301:**322–333.
66. **Wang, L. F., M. Yu, E. Hansson, L. I. Pritchard, B. Shiell, W. P. Michalski, and B. T. Eaton.** 2000. The exceptionally large genome of Hendra virus: support for creation of a new genus within the family *Paramyxoviridae*. J. Virol. **74:**9972–9979.
67. **Waning, D. L., C. J. Russell, T. S. Jardetzky, and R. A. Lamb.** 2004. Activation of a paramyxovirus fusion protein is modulated by inside-out signaling from the cytoplasmic tail. Proc. Natl. Acad. Sci. USA **101:**9217–9222.
68. **Waning, D. L., A. P. Schmitt, G. P. Leser, and R. A. Lamb.** 2002. Roles for the cytoplasmic tails of the fusion and hemagglutinin-neuraminidase proteins in budding of the paramyxovirus simian virus 5. J. Virol. **76:**9284–9297.
69. **Wessel, D., and U. I. Flugge.** 1984. A method for the quantitative recovery of protein in dilute solution in the presence of detergents and lipids. Anal. Biochem. **138:**141–143.
70. **Wong, K. T., W. J. Shieh, S. Kumar, K. Norain, W. Abdullah, J. Guarner, C. S. Goldsmith, K. B. Chua, S. K. Lam, C. T. Tan, K. J. Goh, H. T. Chong, R. Jusoh, P. E. Rollin, T. G. Ksiazek, and S. R. Zaki.** 2002. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am. J. Pathol. **161:**2153–2167.
71. **Wyss, S., A. S. Dimitrov, F. Baribaud, T. G. Edwards, R. Blumenthal, and J. A. Hoxie.** 2005. Regulation of human immunodeficiency virus type 1 envelope glycoprotein fusion by a membrane-interactive domain in the gp41 cytoplasmic tail. J. Virol. **79:**12231–12241.
72. **Yin, H. S., R. G. Paterson, X. Wen, R. A. Lamb, and T. S. Jardetzky.** 2005. Structure of the uncleaved ectodomain of the paramyxovirus (hPIV3) fusion protein. Proc. Natl. Acad. Sci. USA **102:**9288–9293.
73. **Yin, H. S., X. Wen, R. G. Paterson, R. A. Lamb, and T. S. Jardetzky.** 2006. Structure of the parainfluenza virus 5 F protein in its metastable, prefusion conformation. Nature **439:**38–44.
74. **Yonemura, S., M. Hirao, Y. Doi, N. Takahashi, T. Kondo, and S. Tsukita.** 1998. Ezrin/radixin/moesin (ERM) proteins bind to a positively charged amino acid cluster in the juxta-membrane cytoplasmic domain of CD44, CD43, and ICAM-2. J. Cell Biol. **140:**885–895.
75. **Zaitsev, V., M. von Itzstein, D. Groves, M. Kiefel, T. Takimoto, A. Portner, and G. Taylor.** 2004. Second sialic acid binding site in Newcastle disease virus hemagglutinin-neuraminidase: implications for fusion. J. Virol. **78:** 3733–3741.
76. **Zingler, K., and D. R. Littman.** 1993. Truncation of the cytoplasmic domain of the simian immunodeficiency virus envelope glycoprotein increases Env incorporation into particles and fusogenicity and infectivity. J. Virol. **67:** 2824–2831.

doi:10.1038/nature14428

Single-dose attenuated Vesiculovax vaccines protect primates against Ebola Makona virus

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The family *Filoviridae* contains three genera, Ebolavirus, Marburgvirus, and Cuevavirus[1]. Some members of the genus, including Zaire ebolavirus (ZEBOV), can cause lethal haemorrhagic fever in humans. During 2014 an unprecedented ZEBOV outbreak occurred in West Africa and is still ongoing, resulting in over 10,000 deaths, and causing global concern of uncontrolled disease. To meet this challenge a rapid-acting vaccine is needed. Many vaccine approaches have shown promise in being able to protect nonhuman primates against ZEBOV[2]. In response to the current ZEBOV outbreak several of these vaccines have been fast tracked for human use. However, it is not known whether any of these vaccines can provide protection against the new outbreak Makona strain of ZEBOV. One of these approaches is a first-generation recombinant vesicular stomatitis virus (rVSV)-based vaccine expressing the ZEBOV glycoprotein (GP) (rVSV/ZEBOV). To address safety concerns associated with this vector, we developed two candidate, further-attenuated rVSV/ZEBOV vaccines. Both attenuated vaccines produced an approximately tenfold lower vaccine-associated viraemia compared to the first-generation vaccine and both provided complete, single-dose protection of macaques from lethal challenge with the Makona outbreak strain of ZEBOV.

Since discovery of the virus in 1976, outbreaks of ZEBOV have been detected sporadically in Africa. With increasing population growth the frequency of human contact with natural virus reservoirs[3] is likely to rise, potentially leading to more catastrophic outbreaks such as the current epidemic in West Africa, thus increasing the need for effective antiviral strategies. A highly effective countermeasure would be a preventive vaccine that can be simply and widely administered to people in regions of virus zoonosis and provide a 'blanket immunity' curtailing any future outbreaks. Also important will be the ability to rapidly combat deliberate misuse of these deadly viruses. Therefore, a preventive vaccine should ideally confer rapid, single-dose protection.

There are currently no licensed filovirus vaccines or post-exposure treatments available for human use. However, there are at least ten different vaccine approaches that have shown the potential to protect nonhuman primates (NHPs) from lethal ZEBOV infection, including platforms based on recombinant adenovirus serotype 5 (rAd5) vectors, combined DNA/rAd5 vectors, combined rAd serotype 26 and 35 vectors, recombinant chimpanzee adenovirus serotype 3 (rChAd3) vectors, combined rChAd3 and modified vaccinia Ankara (MVA) vectors, virus-like particles (VLPs), alphavirus replicons, recombinant human parainfluenza virus 3 (rHPIV3), rabies virus, and recombinant vesicular stomatitis virus (rVSV)[2]. Of the vaccines advancing to phase I trials, the rChAd3 and rVSV vectored vaccines have shown success in single-dose protection of NHPs against ZEBOV challenge; with the caveat that the rChAd3/ZEBOV vaccine requires a boost with an MVA/ZEBOV vector for protection past 6 months[4]. Also, NHPs inoculated with the rChAd3/ZEBOV vaccine were challenged with a ZEBOV seed stock containing a large virus population encoding 8 uridines (U) at a critical transcription editing site in the GP gene[4]. This specific genetic feature typically arises following prolonged passage of ZEBOV in Vero E6 cells and results in higher levels of expression of full-length GP. In contrast, low-passage ZEBOV isolates retain 7U at the GP editing site, resulting in higher levels of secreted GP (sGP) expression, which is associated with greater viral virulence[5–7]. Importantly, studies have shown that rAd-based ZEBOV vaccines that completely protect NHPs against ZEBOV stocks containing high populations of 8U virus are not able to completely protect vaccinated macaques challenged with ZEBOV stocks containing high populations of 7U virus[8].

The first generation rVSV/ZEBOV vaccine that replaces the VSV glycoprotein G with the ZEBOV GP (rVSV/ZEBOVΔG), originally developed by Drs Feldmann and Geisbert[9] and currently licensed by Merck, has demonstrated solid single-dose NHP protection against a low-passage 7U ZEBOV stock[8]. The rVSV/ZEBOVΔG vector has also protected 50% of NHPs when administered shortly after ZEBOV challenge[10], and has demonstrated safety in a NHP neurovirulence model[11]. However, there is a robust post-vaccination viraemia in macaques and a recent phase I trial of the rVSV/ZEBOVΔG vaccine in Geneva was halted due to temporary joint pain in some patients. The level of vaccine-associated viraemia and frequency of adverse events will be more fully documented as data from ongoing phase 3 trials become available for this vector; but the early observation suggest that a further-attenuated rVSV vector may be more desirable for widespread administration in endemic regions of Africa.

To address this possible safety concern we have developed and tested two further-attenuated rVSV/ZEBOV vaccine candidates for efficacy. One of these vaccines is based on an rVSV vector that has advanced through clinical evaluation. It was attenuated by translocating the VSV nucleoprotein (N) gene from position 1 to position 4 in the genome (N4) and truncating the cytoplasmic tail (CT) of the VSV G protein from 29 to 1 amino acids (CT1)[12]. This rVSVN4CT1 vector was modified to maximally express HIV-1 gag from position 1 in the genome (rVSVN4CT1gag1) by positioning the *gag* gene immediately adjacent to the single strong 3′ VSV transcription promoter. The rVSVN4CT1gag1 vector has demonstrated safety in mouse and NHP neurovirulence studies[12,13], and replication is restricted to the IM inoculation site and draining lymph node following vaccination of mice[14]. The rVSVN4CT1gag1 vector has demonstrated safety and immunogenicity in two phase I clinical trials (HVTN 090 and HVTN 087: http://clinicaltrials.gov/) and no post-vaccination viraemia was detected in urine, saliva, and blood of vaccine recipients. The rVSVN4CT1GP1 vector described here (Fig. 1a, N4) is analogous in design to that of the rVSVN4CT1gag1 vaccine and expresses ZEBOV GP from genome position 1. The other attenuated rVSV/ZEBOV

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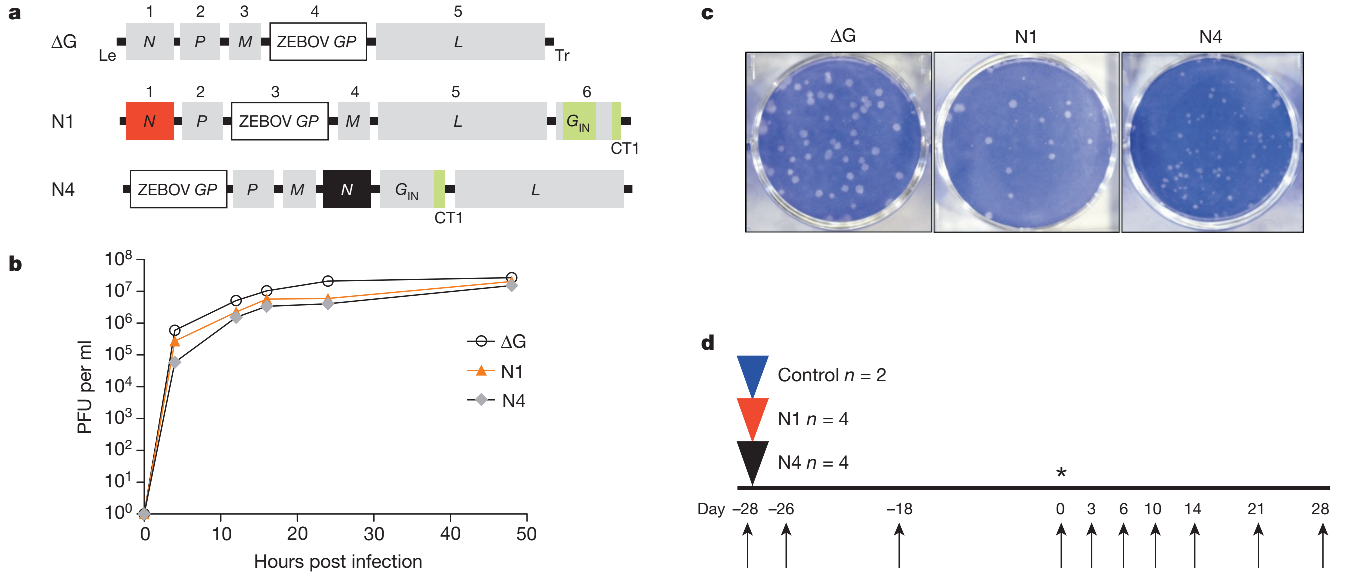


Figure 1 | rVSV/ZEBOV vector design, growth kinetics and vaccine study strategy. **a**, Genome organization comparing ZEBOV GP (Mayinga strain)-expressing rVSV vectors as described in methods. The rVSV/ZEBOVΔG (ΔG) vector had the natural VSV *G* gene replaced with the ZEBOV *GP* at position 4 within the genome. rVSVN1CT1GP3 (N1) vector retained the position of VSV *N* in position 1 (orange box), insertion of ZEBOV *GP* at position 3 and a truncated form of VSV *G* containing the CT1 truncation was inserted at position 6. The rVSVN4CT1GP1 (N4) vector had the insertion of ZEBOV *GP* in position 1, attenuating *N* gene translocation (N4) (black box) and truncated G protein cytoplasmic tail (CT1). Numbers above vector constructs designate genome positions. Virus leader (Le), trailer (Tr), and intergenic regions are shown in black. Shaded regions represent deleted amino acid regions. **b**, Single-cycle growth kinetics comparing the ΔG, N1, and N4 vectors depicted in **a**. Data shown are mean ± s.d. from two biological replicates titrated by plaque assay in triplicate. Titre differences between ΔG and N1 vectors were statistically significant at 4 ($P = 0.0001$), 12 ($P = 0.0055$), and 24 h post infection ($P = 0.0001$). Likewise, ΔG and N4 vector titres were significantly different at 4 ($P = 0.0001$), 12 ($P = 0.0005$), 24 ($P = 0.0001$), and 48 h post infection ($P = 0.0068$). Unpaired *t*-test, $P = 0.05$. **c**, Crystal violet-stained Vero cell monolayers showing plaques generated by the ΔG, N1, and N4 vectors at 48 h post infection. **d**, Flow chart showing the day of vaccination (triangles), days of sampling (arrows), day of challenge (*). Blue triangle, unvaccinated cohort; orange triangle, N1-vaccinated cohort; black triangle, N4-vaccinated cohort.

vaccine described here (rVSVN1CT1GP3), expressing a truncated form of VSV G, was designed to be of intermediate attenuation between rVSVN4CT1GP1 and the first generation rVSV/ZEBOVΔG vaccine (Fig. 1a, N1). Both attenuated rVSV/ZEBOV vectors express GP from the ZEBOV Mayinga strain, as do most other candidate ZEBOV vaccines currently under evaluation. Sequence homology between GPs from the new West African Makona strains analysed to date and the 1976 Mayinga strain is approximately 97%. Although this difference is not likely to affect the protective efficacy of the current ZEBOV vaccines against the heterologous West African strains, it is possible that small changes in sequence could lead to reduced efficacy of a vaccine[15]. It is well established that small variations in sequence and even single amino acid changes in sequence for other viruses including influenza, respiratory syncytial virus, polio, equine infectious anaemia virus, and SIV can reduce vaccine efficacy. Here we assessed the ability of our newly developed next-generation rVSV-based vaccines expressing ZEBOV Mayinga GP to protect against heterologous challenge with the new outbreak Makona strain of ZEBOV in cynomolgus monkeys.

Table 1 | Clinical findings for NHPs challenged with ZEBOV-Guinea

Animal	Vaccine	Day −26*	$PRNT_{50}$†	Clinical signs observed‡	Final outcome
129	N/A	–	0/0	Fever (6), anorexia (5–8), depression (6–8), mild rash (6–8), lymphopenia (3, 6), thrombocytopenia (6), ALT→(6), ALP→→→ (6), AST→→→ (6), GGT→→→ (6), CRP increase (6)	Expired day 8
276	N/A	–	0/0	Fever (6), anorexia (6–7), depression (6–7), mild rash (6–7), thrombocytopenia (6, 10), ALT→(6), ALP→→→ (6), AST→→→ (6), GGT→ (6), CRP increase (6)	Expired day 7
0910078	N1	+	0/40	Ø§	Survived
1001100	N1	–	0/160	CRP increase (6)	Survived
117	N1	–	0/80	Lymphopenia (6), CRP increase (6, 10)	Survived
0907095	N1	–	0/160	Lymphopenia (6, 10), CRP increase (6, 10), ALT→→(6), ALP→(6), AST→→(6)	Survived
0807174	N4	+	0/160	Lymphopenia (6), CRP increase (6, 10)	Survived
0901014	N4	–	0/80	Ø	Survived
119	N4	+	0/80	Ø	Survived
0811013	N4	+	0/20	Ø	Survived

* rVSV viraemia 2 days post vaccination. –, below limit of detection (25 PFU per ml); +, up to 3×10^2 PFU per ml.

† 50% plaque reduction neutralization titre at day of challenge and terminal day presented as day of challenge/terminal day.

‡ Days after ZEBOV challenge are in parentheses. Fever is defined as a temperature more than 1.4 °C above baseline or at least 0.9 °C above baseline and ≥ 39.7 °C. Lymphopenia and thrombocytopenia are defined by a ≥ 35% drop in numbers of lymphocytes and platelets, respectively. ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma glutamyltransferase. CRP, C-reactive protein: two- to threefold increase,→; 4- to fivefold increase, →→; > 5 fold increase, →→→.

§ No symptoms observed.

Results from an *in vitro* growth kinetics study (Fig. 1b) indicate an approximate tenfold reduction in growth rate early in infection for rVSVN4CT1GP1 relative to rVSV/ZEBOVΔG. Also noted during virus plaque assay were the larger more rapidly forming plaques generated by rVSV/ZEBOVΔG compared to rVSVN4CT1GP1, with rVSVN1CT1GP3 showing intermediate growth and plaque size (Fig. 1c).

We next tested if the further-attenuated rVSV/ZEBOV vaccines could provide NHPs with single-dose protection against challenge with ZEBOV isolated from the current outbreak in Guinea[16]. Groups of four cynomolgus macaques were inoculated intramuscularly with 2×10^{7} plaque-forming units (PFU) of either rVSVN4CT1GP1 or rVSVN1CT1GP3; a group of two control macaques were unvaccinated (Fig. 1d, arrow heads). None of the macaques showed any sign of illness or distress following vaccine administration. Consistent with the statistically significant growth differences between rVSV/ZEBOVΔG and the more attenuated vectors seen during *in vitro* growth kinetics studies, levels of both attenuated vaccine viruses detected in the blood of vaccinated macaques (500 PFU per ml) were 10- to 50-fold lower than those detected for the more replication competent rVSV/ZEBOVΔG[9] (Table 1, day −26). The ZEBOV GP-specific humoral immune response was assessed for all animals before vaccination (Fig. 2a, −28) and after vaccination (Fig. 2a, −18 and 0) by IgG capture ELISA and neutralizing antibody titres (Table 1, plaque 50% reduction neutralization test (PRNT)$_{50}$). Results showed neutralizing titres at terminal days for vaccinated cohorts and detectable circulating levels of anti-ZEBOV GP IgG for both vaccine cohorts after vaccination and before challenge with no detectable levels for the unvaccinated control animals (Fig. 2a). A cell-mediated immune response was also detected in all vaccinated animals by ZEBOV GP-specific interferon gamma (IFN-γ) ELISpot assay 10 days after vaccination (Extended Data Fig. 1a and b).

The eight vaccinated and two unvaccinated control macaques were challenged by intramuscular injection with 1,000 PFU of a low passage 100% 7U Makona strain stock of ZEBOV[16] 28 days after the single injection vaccination (Fig. 1d, asterisk). None of the animals vaccinated with either of the two further-attenuated rVSV/ZEBOV vectors showed any severe signs of illness following challenge with ZEBOV (Table 1), whereas the two unvaccinated control macaques succumbed to disease on days 7 and 8 (Fig. 2b). Circulating infectious ZEBOV was isolated from both of the unvaccinated control macaques on days 3 and 6 post challenge (Fig. 2c, blue) but no circulating infectious ZEBOV could be detected in any of the vaccinated animals. Examination of tissues by immunohistochemistry showed abundant ZEBOV antigen in tissues of the unvaccinated control animals (129 and 276) (Fig. 3a–d) whereas ZEBOV antigen was not detected in tissues of the macaques vaccinated with rVSVN1CT1GP3 (1001100) or rVSVN4CT1GP1 (0807174) (Fig. 3e–h).

Here we show protection against a new West African Makona strain of ZEBOV using a novel filovirus vaccine platform. The large reduction in vaccine-associated viraemia indicates a significant increase of *in vivo* attenuation for these next-generation rVSV/ZEBOV vaccine vectors, which should translate into greater safety and reduced adverse

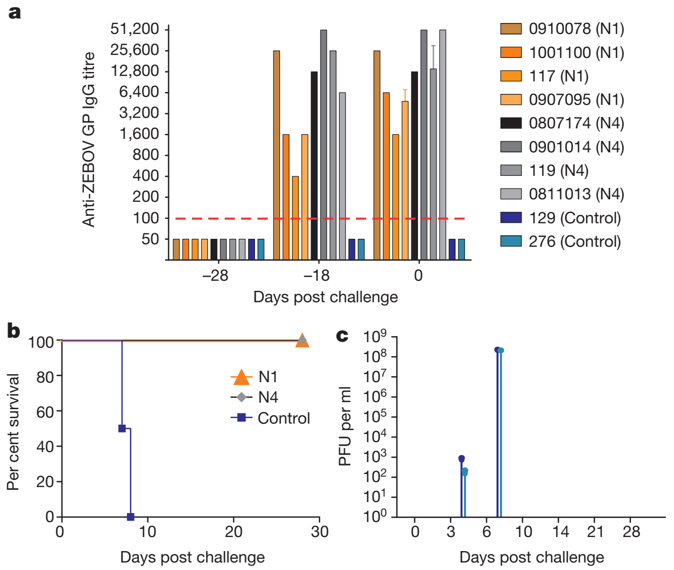


Figure 2 | N1 and N4 vaccination results in circulating anti-ZEBOV GP IgG and protection in cynomolgus macaques. **a**, Reciprocal endpoint dilution titres for circulating IgG against ZEBOV GP for control (blue), N1 (orange), and N4 cohorts (black-grey) on day of vaccination (−28), 10 days post vaccination (−18), and on day of challenge (0). Red dashed line depicts limit of detection for ELISA assay. Error bars represent s.e.m. **b**, Kaplan–Meier survival curve for each cohort post ZEBOV challenge. **c**, Circulating infectious virus load displayed as plaque forming units per ml. Data shown are from individual animals. Lower limit of detection is 25 PFU per ml.

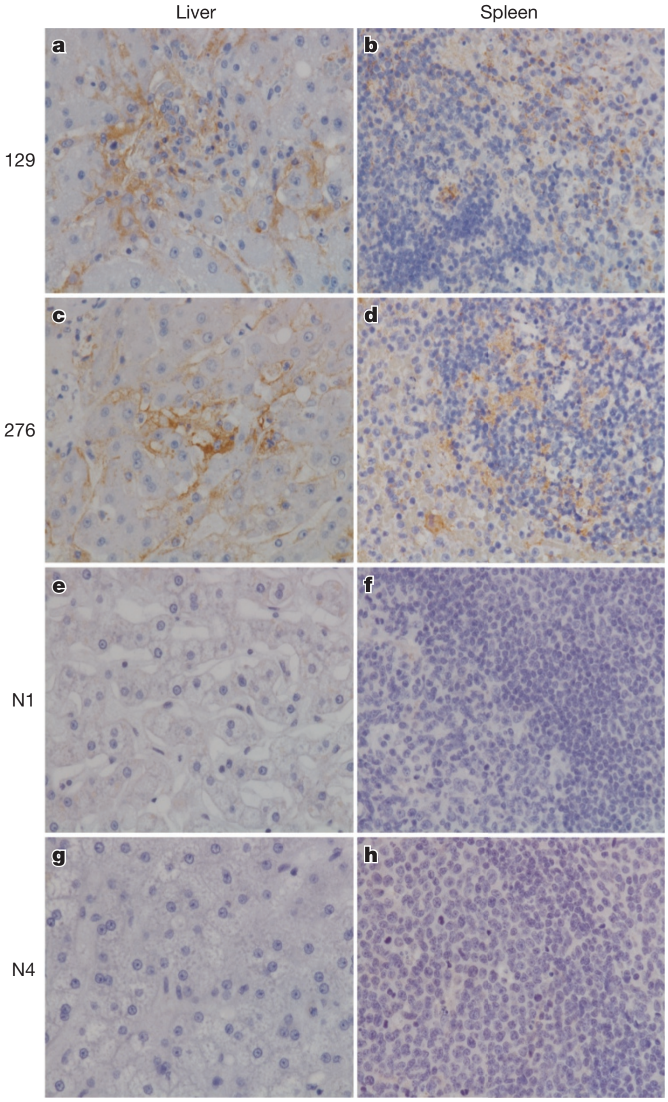


Figure 3 | Comparison of ZEBOV antigen in tissues of cynomolgus macaques either vaccinated or unvaccinated. **a**, **c**, Liver, diffuse cytoplasmic immunolabelling (brown) of sinusoidal lining cells in both ZEBOV-infected control animals. **b**, **d**, Spleen, diffuse cytoplasmic immunolabelling of dendriform mononuclear cells in the red and white pulp of ZEBOV-infected control animals. **e**, **f**, Liver and spleen, respectively, with a lack of immunolabelling from N1 cohort animal 0910078. **g**, **h**, Liver and spleen, respectively, with a lack of immunolabelling from N4 cohort animal 0807174.

events in humans. Importantly, single-dose vaccination of NHPs with highly attenuated forms of rVSV expressing ZEBOV Mayinga GP provides complete protection from heterologous challenge with a highly virulent 7U ZEBOV isolated early during the current West African outbreak[16]. ZEBOV genome sequencing from cases later during the West Africa outbreak has revealed little drift in the GP gene[17,18], suggesting that this vaccine platform could also be efficacious against currently circulating ZEBOV. These findings pave the way for the identification and manufacture of safer, single-dose, high efficacy vaccine(s) to combat current and future filovirus outbreaks in Africa and their potential use as biological weapons.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 12 February; accepted 31 March 2015.

1. Feldman, H., Sanchez, A. & Geisbert, T. W. in *Fields Virology* Ch. 32 (Lippincott Williams and Wilkins, 2013).
2. Mire, C. E. & Geisbert, T. W. in *Biology and Pathology of Rhabdo- and Filoviruses* Ch. 22 (World Scientific Publishing, 2014).
3. Leroy, E. M. *et al.* Fruit bats as reservoirs of Ebola virus. *Nature* **438,** 575–576 (2005).
4. Stanley, D. A. *et al.* Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nature Med.* **20,** 1126–1129 (2014).
5. Volchkova, V. A., Dolnik, O., Martinez, M. J., Reynard, O. & Volchkov, V. E. Genomic RNA editing and its impact on Ebola virus adaptation during serial passages in cell culture and infection of guinea pigs. *J. Infect. Dis.* **204** (Suppl 3), S941–S946 (2011).
6. Kugelman, J. R. *et al.* Ebola virus genome plasticity as a marker of its passaging history: a comparison of *in vitro* passaging to non-human primate infection. *PLoS ONE* **7,** e50316 (2012).
7. Mohan, G. S., Li, W., Ye, L., Compans, R. W. & Yang, C. Antigenic subversion: a novel mechanism of host immune evasion by Ebola virus. *PLoS Pathog.* **8,** e1003065 (2012).
8. Hirschberg, R. *et al.* Challenges, progress, and opportunities: proceedings of the filovirus medical countermeasures workshop. *Viruses* **6,** 2673–2697 (2014).
9. Jones, S. M. *et al.* Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nature Med.* **11,** 786–790 (2005).
10. Feldmann, H. *et al.* Effective post-exposure treatment of Ebola infection. *PLoS Pathog.* **3,** e2 (2007).
11. Mire, C. E. *et al.* Recombinant vesicular stomatitis virus vaccine vectors expressing filovirus glycoproteins lack neurovirulence in nonhuman primates. *PLoS Negl. Trop. Dis.* **6,** e1567 (2012).
12. Clarke, D. K. *et al.* Synergistic attenuation of vesicular stomatitis virus by combination of specific G gene truncations and N gene translocations. *J. Virol.* **81,** 2056–2064 (2007).
13. Clarke, D. K. *et al.* Neurovirulence and immunogenicity of attenuated recombinant vesicular stomatitis viruses in nonhuman primates. *J. Virol.* **88,** 6690–6701 (2014).
14. Johnson, J. E. *et al. In vivo* biodistribution of a highly attenuated recombinant vesicular stomatitis virus expressing HIV-1 Gag following intramuscular, intranasal, or intravenous inoculation. *Vaccine* **27,** 2930–2939 (2009).
15. Kugelman, J. R. *et al.* Evaluation of the potential impact of Ebola virus genomic drift on the efficacy of sequence-based candidate therapeutics. *MBio* **6,** e02227–14 (2015).
16. Baize, S. *et al.* Emergence of Zaire Ebola virus disease in guinea. *N. Engl. J. Med.* **371,** 1418–1425 (2014).
17. Gire, S. K. *et al.* Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. *Science* **345,** 1369–1372 (2014).
18. Hoenen, T. *et al.* Mutation rate and genotype variation of Ebola virus from Mali case sequences. *Science* http://dx.doi.org/10.1126/science.aaa5646 (2015).

Acknowledgements We thank V. Borisevich and D. Deer for assistance with clinical pathology assays performed in the GNL BSL-4 laboratory, and the staff of the UTMB Animal Resources Center for animal care. This study was supported by the funds from the Department of Microbiology and Immunology at UTMB to T.W.G. and in part by the Department of Health and Human Services, National Institutes of Health grant R01AI09881701 to J.H.E. and T.W.G.

Author Contributions D.K.C., D.M. and T.E.L. designed the vaccine vectors and did preparative work. J.H.E. M.A.E., A.O.-S. and R.X. designed, conducted, and analysed the *in vitro* vaccine characterization studies. C.E.M., J.H.E., and T.W.G. conceived and designed the NHP study. C.E.M., J.B.G. and T.W.G. performed the NHP vaccination and challenge experiments, and conducted clinical observations of the animals. J.B.G. and K.N.A. performed the clinical pathology assays. J.B.G. performed the ZEBOV infectivity assays. C.E.M., D.M., J.B.G., K.N.A., M.A.E., K.A.F., D.K.C., J.H.E. and T.W.G. analysed the data. K.A.F. performed histologic and immunohistochemical analysis of the data. C.E.M., D.M., D.K.C. and T.W.G. wrote the paper. All authors had access to all of the data and approved the final version of the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Readers are welcome to comment on the online version of the paper. The authors declare competing financial interests: details are available in the online version of the paper. Correspondence and requests for materials should be addressed to T.W.G. (twgeisbe@utmb.edu)

METHODS

No statistical methods were used to predetermine sample size.

Generation of N4 and N1 ZEBOV vectors. As described previously[12,19] an rVSV$_{IN}$N4CT1gag1 vector (Indian serotype) expressing HIV-1 gag was used as the backbone for generating the attenuated rVSVN4CT1 vector expressing the Zaire ebolavirus (ZEBOV) glycoprotein (GP). The corresponding rVSV$_{IN}$N4CT1gag1 genomic cDNA was modified by exchanging the gag gene expression cassette via XhoI/NotI restriction sites with an expression cassette encoding a full length ZEBOV GP [1976, Mayinga strain], generating the rVSV$_{IN}$N4CT1-ZEBOVGP1 cDNA (Fig. 1a, N4). The N1 vector was generated by first inserting ZEBOV GP into a VSV-N1ΔG backbone via XhoI/NotI restriction sites within a transcriptional cassette located at position 3 in the genome; followed by the insertion of a PCR fragment containing a portion of VSV *L*, a modified VSV *G* CT1 gene and trailer into the N1 genome at position 6 via the HindIII/RsrII sites, generating the rVSV$_{IN}$N1(G CT1)6-ZEBOVGP3 cDNA (Fig. 1a, N1).

The rVSV-ZEBOV vectors were rescued from genomic cDNA as previously described[20]. Rescued virus was plaque purified and amplified on Vero cell monolayers (ATCC, CCL-81). For animal studies, virus vectors were purified from infected BHK-21(ATCC CCL-10) cell supernatants by centrifugation through a 10% sucrose cushion. Purified virus was resuspended in PBS, pH 7.0, mixed with a sucrose phosphate (SP) stabilizer (7 mM K_2HPO_4, 4 mM KH_2PO_4, 218 mM sucrose), snap frozen in ethanol/dry ice and stored at −80 °C until ready for use.

Growth kinetics study of ΔG, N4 and N1 ZEBOV vectors. Single-step growth curves were performed by adsorbing the N4, N1 and a ΔG control virus to duplicate monolayers of Vero cells (ATCC, CCL-81) in six-well plates at a multiplicity of infection (MOI) of 10 for 15 min at room temperature with continued rocking followed by incubation at 37 °C with 5% CO_2 for 30 min without agitation. The inoculum was aspirated, the cells washed 3× with serum-free Dulbecco's minimal Eagle's medium (DMEM) and then DMEM containing 5% fetal bovine serum (FBS) was added to the plates, which were placed at 32 °C with 5% CO_2. Samples for titration were taken at 4, 8, 12, 16, 24 and 48 h post infection and replaced with the same volume of fresh media. Virus titres were determined in duplicate by plaque assay on Vero cells. Growth curves were performed in triplicate for each virus. Plaque images for each vector were taken at 48 h post infection, after staining with a 1% crystal violet solution. Statistical analysis of rVSV titres were performed using unpaired *t*-test with a 95% confidence level ($P < 0.05$) with the GraphPad Prism program.

Challenge virus. The ZEBOV Makona strain seed stock originated from serum from a fatal case early during the 2014 outbreak in Guékédou, Guinea[16] (NCBI accession number KJ660347) and was passaged twice in Vero E6 cells (ATCC, CRL-1586). The virus stock was deep sequenced as 100% 7U at the GP editing site in the viral genome (see below).

Deep sequencing. Approximately 1 ml of the ZEBOV Makona strain seed stock was removed from the seed stock vial and placed in 5 ml of TRIzol LS and vortexed three times and allowed to sit for 10 min. The 6 ml were then placed into two separate 3 ml Nunc cryo-vials for removal from the BSL-4. RNA was isolated from the TRIzol LS/sample mixture using Zymo Research Direct-zol RNA mini-prep per manufacturer's instructions. Approximately 150 ng of purified RNA were used to make cDNA using the NuGen Ovation RNA-seq 2.0 kit ultimately for the preparation of the double-stranded DNA library using Encore Ion Torrent library prep kit. Sequencing was performed by the UTMB Molecular Core on the Ion Torrent using 318-v2 deep sequencing chips. Sequence analysis was performed using DNA Star Seqman NGen software based on paired-end analysis of 100 base pairs overlaps.

Vaccination and animal challenge. Ten, healthy, filovirus-naive, adult (~3 to 9.5 kg, 7 female and 3 male), Chinese origin cynomolgus macaques (*Macaca fascicularis*) were randomized with Microsoft Excel into two experiment groups of four animals each and a control group of two animals. Animals in one experimental group were vaccinated by intramuscular injection of approximately 2×10^7 PFU of the rVSVN4CT1GP1vaccine while animals in the other experimental group were vaccinated with approximately 2×10^7 PFU of the VSV-N1CT1 ZEBOVGP vaccine. The two control animals were not vaccinated. Four weeks after the single injection vaccination all ten animals were challenged by intramuscular injection with 1,000 PFU of the ZEBOV Makona strain virus. All animals were given physical exams and blood was collected before vaccination, at day 10 after vaccination, at the time of ZEBOV challenge and on days 3, 6, 10, 14, 21 and 28 after ZEBOV challenge (Fig. 1d, arrows). Animals were monitored daily and scored for disease progression with an internal filovirus scoring protocol approved by the UTMB Institutional Animal Care and Use Committee. The scoring changes measured from baseline included posture/activity level, attitude/behaviour, food and water intake, weight, respiration, and disease manifestations such as visible rash, haemorrhage, ecchymosis, or flushed skin. A score of ≥ 9 indicated that an animal met criteria for euthanasia. This study was not blinded.

Anti-ZEBOV GP IgG ELISA. Serum collected at indicated time points was tested for immunoglobulin G (IgG) antibodies against ZEBOV. Enzyme-linked immunosorbent assay (ELISA) using recombinant ZEBOV GPdTM purified protein (Integrated BioTherapeutics, Inc.) was used to detect cross-reactive IgG. ZEBOV GPdTM was diluted to an optimal working concentration of 100 ng per well in 0.1 ml carbonate/bicarbonate buffer (carbonate/bicarbonate buffer with azide tablets from Sigma catalogue number 08058-50TAB-F) and used to coat Immulon 2HB flat bottom ELISA plates (Thermo Labsystem catalogue number 3455) for 18 h at 4 °C. Coated plates were blocked (10% FBS + 1× PBS) for at least 2 h. The serum samples were assayed at twofold dilutions starting at a 1:100 dilution in ELISA diluent (1% heat inactivated fetal bovine serum (HI-FBS), 1× PBS, and 0.2% Tween-20). Samples were incubated for 1 h at room temperature, removed, and plates were washed. Wells were then incubated at room temperature for 1 h with anti-monkey IgG conjugated to horseradish peroxidase (Fitzgerald Industries International) at a 1:2,500 dilution. These wells were washed and then incubated with 2,2'-azine-di(3ethylbenzthiazoline-6-sulfonate) peroxidase substrate system (KPL) at room temperature for approximately 10 min. Reaction was stopped with 1% SDS and read for dilution endpoints at 405 nm on a microplate reader (Molecular Devices Emax system). Absorbance values were normalized by subtraction of background $A_{405\,\mathrm{nm}}$ from uncoated wells for each serum dilution. Antigen-specific serum IgG end-point titres were defined as the reciprocal of the last normalized serum dilution giving an $A_{405\,\mathrm{nm}}$ greater than 0.1.

ZEBOV neutralization assay. Neutralization assays were performed by measuring plaque reduction in a constant virus:serum dilution format as previously described[9]. Briefly, a standard amount of ZEBOV (~100 PFU) was incubated with serial twofold dilutions of the serum sample for 60 min. The mixture was used to inoculate Vero E6 cells (ATCC, CRL-1586) for 60 min. Cells were overlaid with an agar medium, incubated for 7 days, and plaques were counted 48 h after neutral red staining. Endpoint titres were determined by the dilution of serum, which neutralized 50% of the plaques ($PRNT_{50}$).

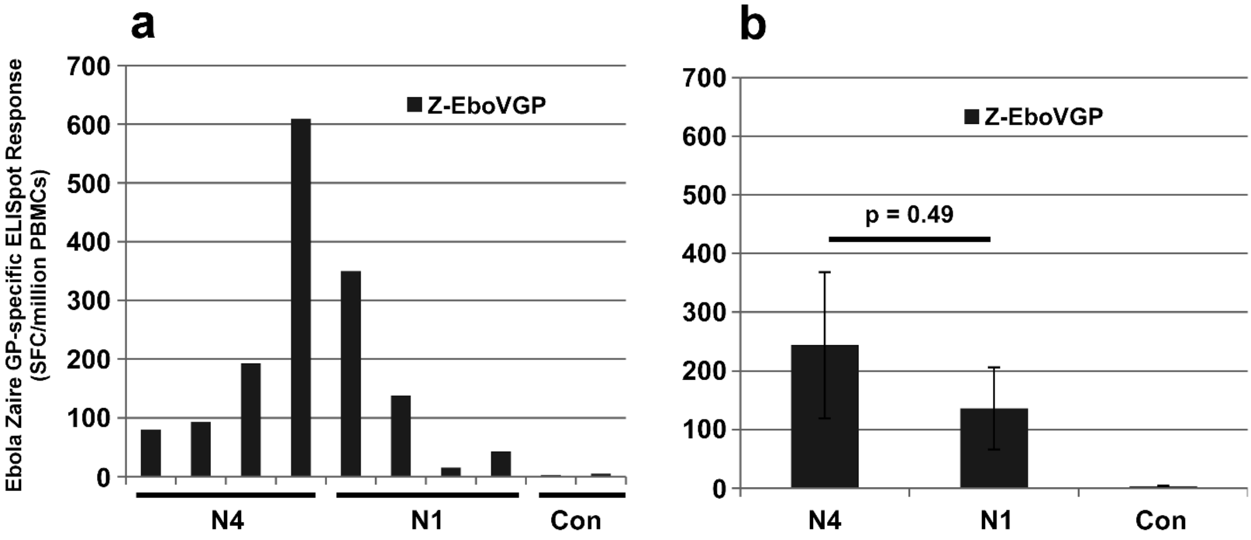
IFN-γ ELISpot assay. Ninety-six-well flat-bottomed ELISpot plates (Millipore) were coated overnight with a mouse anti-human IFN-γ monoclonal antibody (clone 27; BD-Pharmingen) at a concentration of 10 μg ml^{-1}, after which the plates were washed three times with 1× PBS and then blocked for 2 h with PBS containing 5% heat-inactivated FBS. Heparinized whole blood was collected 10 days after immunization of macaques, and peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by Ficoll-Hypaque density gradient centrifugation, and resuspended in complete R05 culture medium. The isolated macaque PBMCs were washed once with complete R05 culture medium and resuspended in complete R05 culture medium containing either 5 μg ml^{-1} phytohaemagglutinin mucoprotein (Sigma), peptide pools (15-mers overlapping by 11 amino acids; final peptide concentration, 1 μM each) spanning the ZEBOV Mayinga strain GP, or medium alone. The input cell number was 2×10^5 PBMCs per well (2×10^6 PBMCs per ml), and cells were assayed in duplicate wells. Cells were incubated for 18 to 24 h at 37 °C and then removed from the ELISpot plate by first being washed with deionized water and then being washed six times with 1× PBS containing 0.25% Tween 20. Thereafter, plates were treated with a rabbit polyclonal anti-human IFN-γ biotinylated detection antibody (0.65 μg per well; Life Technologies) diluted with 1× PBS containing 1% bovine serum albumin (BSA) and were incubated at 37 °C for 2 h. ELISpot plates were then washed 6 times with 1× PBS containing 0.25% Tween 20, treated with 100 μl per well of streptavidin–horseradish peroxidase conjugate (BD Biosciences) diluted 1:250 with 1× PBS containing 10% FBS and 0.005% Tween 20, and incubated for an additional 1 h at room temperature. Unbound conjugate was removed by rinsing the plate six times with 1× PBS containing 0.25% Tween 20 and three times with 1× PBS. A chromogenic substrate (100 μl per well) (one-step nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate (NBT/BCIP); Pierce) was then added for 3 to 5 min before being rinsed away with water, after which the plates were air dried and the resulting spots counted using an ImmunoSpot reader (CTL Inc.). Peptide-specific IFN-γ ELISpot responses were considered positive if the responses (minus the medium background) were threefold above the medium response and 50 spot-forming cells (SFC) per 10^6 PBMCs. Unpaired *t*-test analysis of IFN-γ ELISpot data was performed on GraphPad Prism version 5.02 software. Two-tailed *P* values less than 0.05 indicated that the tests were statistically significant.

Detection of viraemia. Virus titration of the rVSV vaccine vectors and ZEBOV was performed by plaque assay with Vero E6 cells (ATCC, CRL-1586) from cell culture or serum samples as previously described[9]. Briefly, increasing tenfold dilutions of the samples were adsorbed to Vero E6 monolayers in duplicate wells (200 μl); the limit of detection was 25 PFU per ml.

Haematology and serum biochemistry. Total white blood cell counts, white blood cell differentials, red blood cell counts, platelet counts, haematocrit values, total haemoglobin concentrations, mean cell volumes, mean corpuscular volumes, and mean corpuscular haemoglobin concentrations were analysed from blood collected in tubes containing EDTA using a laser based haematologic analyser (Beckman Coulter). Serum samples were tested for concentrations of albumin, amylase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, glucose, cholesterol, total protein, total bilirubin, blood urea nitrogen, creatine, and C-reactive protein by using a Piccolo point-of-care analyser and Biochemistry Panel Plus analyser discs (Abaxis).

Histopathology and immunohistochemistry. Necropsy was performed on all subjects. Tissue samples of all major organs were collected for histopathological and immunohistochemical examination, immersion-fixed in 10% neutral buffered formalin, and processed for histopathology as previously described[21]. For immunohistochemistry, specific anti-ZEBOV immunoreactivity was detected using an anti-ZEBOV VP40 protein rabbit primary antibody (Integrated BioTherapeutics, Inc.) at a 1:4,000 dilution. In brief, tissue sections were processed for immunohistochemistry using the Dako Autostainer (Dako). Secondary antibody used was biotinylated goat anti-rabbit IgG (Vector Laboratories) at 1:200 followed by Dako LSAB2 streptavidin–horseradish peroxidase (Dako). Slides were developed with Dako DAB chromagen (Dako) and counterstained with haematoxylin. Non-immune rabbit IgG was used as a negative control.

19. Cooper, D. *et al.* Attenuation of recombinant vesicular stomatitis virus HIV-1 vaccine vectors by gene translocations and G gene truncation reduces neurovirulence and enhances immunogenicity in mice. *J. Virol.* **82,** 207–219 (2008). Medline CrossRef.
20. Witko, S. E. *et al.* An efficient helper-virus-free method for rescue of recombinant paramyxoviruses and rhadoviruses from a cell line suitable for vaccine development. *J. Virol. Methods* **135,** 91–101 (2006). Medline CrossRef.
21. Thi, E. P. *et al.* Marburg virus infection in nonhuman primates: Therapeutic treatment by lipid-encapsulated siRNA. *Sci. Transl. Med.* **6,** 250ra116 (2014).



Extended Data Figure 1 | Relative immunogenicity of rVSV/ZEBOV vectors in cynomolgus macaques. At study day −28, cynomolgus macaques were immunized intramuscularly with 2×10^7 PFU of either N4 or N1 vectors. Ten days after a single immunization, PBMCs were prepared and ZEBOV GP-specific T-cell responses were quantified by IFN-γ ELISpot assay. **a**, ZEBOV GP-specific IFN-γ ELISpot responses in individual macaques. **b**, Average ZEBOV GP-specific IFN-γ ELISpot responses with s.e.m. indicated.

From: Garry, Robert F on behalf of Garry, Robert F <rfgarry@tulane.edu>
To: Geisbert, Thomas W.; Cross, Robert W.; Broder, Christopher; Luis Branco - ZalgenLabs. com
Subject: rVSV constructs - gen3?
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Some food for thought - would appreciate some feedback.

I really like the idea of focusing on the N4CT1 constructs to attenuate the rVSV constructs, particularly for the virus families we're dealing with where neruovirulence of the rVSVs has been shown or could be a limitation.

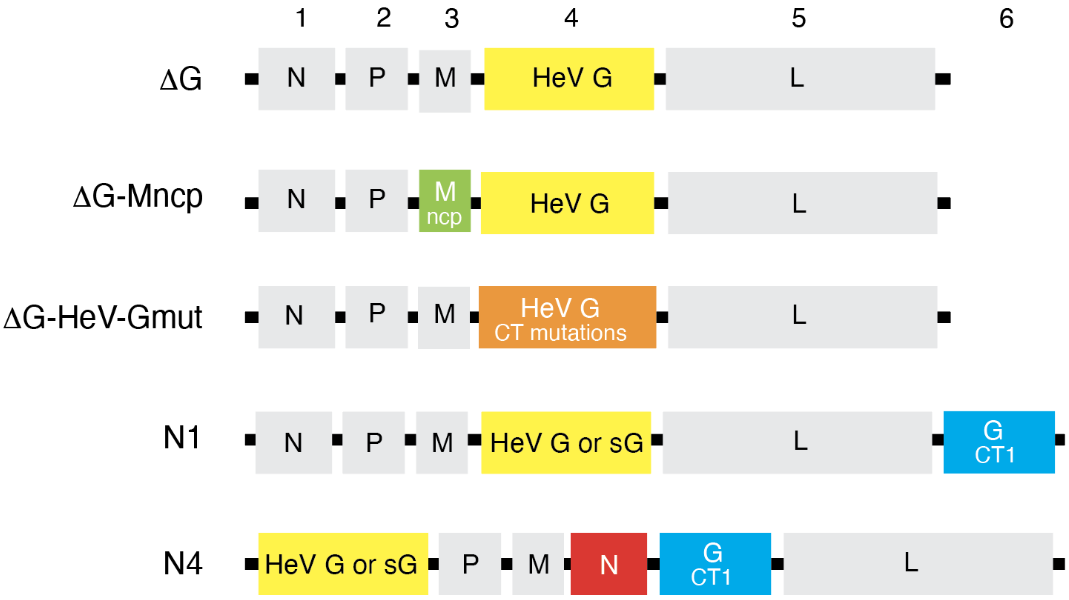
I coming to think that we should also propose testing other attenuating mutations as in Clarke et al. [attached]

I also like inserting sG constructs.

Additionally, I think it's possible that we could both increase expression of our viral glycoproteins AND attenuate them by truncating and mutating the cytoplasmic tails. IOWS mutate not only the VSV G cytoplasmic tail but also or mutate the cytoplasmic tail of the inserted viral glycoprotein.

This makes a lot of sense [in my mind] for all our prototype pathogens but especially for JUNV - check out the Nunberg paper attached. By truncating the CT of JUNC GPC you eliminate the need for the SSP but more importantly drive the GP to the cell surface.

This draft figure is derived from Tom's Makona paper and shows what I think might be a good grantsmanship approach of not putting all the eggs in one rVSV basket for any one virus.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Article

A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses

Moushimi Amaya [1,*], Randy Yin [1,2], Lianying Yan [1,2], Viktoriya Borisevich [3,4], Bishwo N. Adhikari [5,6], Andrew Bennett [6,7], Francisco Malagon [6,7], Regina Z. Cer [5], Kimberly A. Bishop-Lilly [5], Antony S. Dimitrov [1,2], Robert W. Cross [3,4], Thomas W. Geisbert [3,4] and Christopher C. Broder [1,*]

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Abstract: The henipaviruses, Nipah virus (NiV), and Hendra virus (HeV) can cause fatal diseases in humans and animals, whereas Cedar virus is a nonpathogenic henipavirus. Here, using a recombinant Cedar virus (rCedV) reverse genetics platform, the fusion (F) and attachment (G) glycoprotein genes of rCedV were replaced with those of NiV-Bangladesh (NiV-B) or HeV, generating replication-competent chimeric viruses (rCedV-NiV-B and rCedV-HeV), both with and without green fluorescent protein (GFP) or luciferase protein genes. The rCedV chimeras induced a Type I interferon response and utilized only ephrin-B2 and ephrin-B3 as entry receptors compared to rCedV. The neutralizing potencies of well-characterized cross-reactive NiV/HeV F and G specific monoclonal antibodies against rCedV-NiV-B-GFP and rCedV-HeV-GFP highly correlated with measurements obtained using authentic NiV-B and HeV when tested in parallel by plaque reduction neutralization tests (PRNT). A rapid, high-throughput, and quantitative fluorescence reduction neutralization test (FRNT) using the GFP-encoding chimeras was established, and monoclonal antibody neutralization data derived by FRNT highly correlated with data derived by PRNT. The FRNT assay could also measure serum neutralization titers from henipavirus G glycoprotein immunized animals. These rCedV chimeras are an authentic henipavirus-based surrogate neutralization assay that is rapid, cost-effective, and can be utilized outside high containment.

Keywords: Hendra virus; Nipah virus; Cedar virus; henipavirus; chimera; reverse genetics; virus neutralization; vaccine; virus-host cell interaction; antibody; serum

check for updates

Citation: Amaya, M.; Yin, R.; Yan, L.; Borisevich, V.; Adhikari, B.N.; Bennett, A.; Malagon, F.; Cer, R.Z.; Bishop-Lilly, K.A.; Dimitrov, A.S.; et al. A Recombinant Chimeric Cedar Virus-Based Surrogate Neutralization Assay Platform for Pathogenic Henipaviruses. *Viruses* **2023**, *15*, 1077. https://doi.org/10.3390/v15051077

Academic Editors: Patrick C. Y. Woo and Kenneth Li

Received: 4 February 2023
Revised: 20 April 2023
Accepted: 22 April 2023
Published: 28 April 2023

1. Introduction

The bat-borne highly pathogenic Hendra virus (HeV) and Nipah virus (NiV) are the prototype members of the genus *Henipavirus* within the family *Paramyxoviridae* [1]. HeV and NiV are classified as Biosafety Level-4 (BSL-4) pathogens because of their high lethality and lack of approved vaccines or antivirals and are transboundary agents of significant disease threats to livestock and people in Australia and South and Southeast Asia, respectively. The genus now includes nine other reported henipaviruses; the four viral isolates of Cedar virus (CedV), Gamak virus, Daeryong virus, and Langya virus (LayV), [2–4] and five additional species known only from nucleic acid sequence information; Ghana bat virus (GhV), Mòjiāng virus, Melian virus, Denwin virus, and Angavokely virus (AngV) [5–8]. The recognized or apparent natural reservoir of all isolates of NiV, HeV, and CedV, along with the genomic data of GhV and AngV, are old-world fruit bats of the family *Pteropodidae*.

Whereas the six other reported henipaviruses are, or are likely, of rodent origins, including the isolate LayV. Only HeV and NiV are known to be associated with severe and often fatal henipaviral disease in humans and a number of animal species (reviewed in: [9,10]), while LayV was associated with nonfatal febrile illnesses in humans [4]. In contrast, CedV is the only henipavirus isolate demonstrated to be nonpathogenic in well-established animal models of NiV and HeV infection and disease, including guinea pigs, ferrets, hamsters [2,11], and African green monkeys (Geisbert, T.W. and Broder, C.C., unpublished). An important distinction between CedV and other henipaviruses lies within the *P* gene, which encodes the phosphoprotein (P), and the *P* gene transcripts of NiV and HeV undergo RNA editing to produce the V and W nonstructural proteins that are key interferon (IFN) antagonists (reviewed in [12,13]). The CedV *P* gene lacks both RNA editing and does not encode V or W [2,14]. Several studies with recombinant NiV variants have demonstrated the differential importance of the V and W proteins in the pathogenesis brought about by NiV infection in both the hamster and ferret models, and a lack of the V protein resulted in nonlethal infections [15–18]. All other recognized or proposed henipaviruses have the potential to express V and W proteins based on current genetic data. The absence of these proteins in CedV is hypothesized to be the key factor underlying its nonpathogenic nature in established NiV and HeV animal models. These data permitted the rescue and characterization of recombinant CedV (rCedV) by reverse genetics at BSL-2 containment, and CedV is now recognized as a BSL-2 restricted agent [19–21].

The development of effective countermeasures against NiV and HeV has been a research priority since their discovery [22]. NiV and henipaviral diseases are also included on the WHO's Blueprint List of Priority Pathogens [23], and NiV is among the Coalition for Epidemic Preparedness Innovations (CEPI) list of Priority Diseases needing urgent research and countermeasure development [24,25]. There are currently no licensed NiV/HeV vaccines or antivirals approved for human use, although a licensed vaccine to prevent HeV infection in horses, based on a soluble form of the attachment (G) glycoprotein (sG) from HeV (HeV-sG), was launched in Australia (Equivac® HeV) by Zoetis, Inc., in November 2012 [26]. A NiV vaccine formulated for human use with the HeV-sG immunogen is currently in Phase 1 human clinical trials [27]. As a therapeutic approach development for henipavirus human infection, the human monoclonal antibody (mAb) m102.4, specific to the NiV and HeV G glycoprotein receptor binding site, has completed a Phase I clinical trial in Australia [28]. To date, 18 individuals exposed to either HeV in Australia ($n = 17$) or NiV in the United States ($n = 1$) have received high-dose, post-exposure, m102.4 therapy (15–20 mg/kg) by emergency use protocols and no evidence of virus infection has been reported.

The G glycoprotein (also referred to as the receptor-binding protein (RBP)), together with the fusion (F) glycoprotein on the surface of the henipavirus virion, are the mediators of virus attachment and infection [29]. The RBP determines the cellular tropism of infection. The NiV and HeV G glycoproteins specifically bind to cells expressing the ephrin-B class ligands, ephrin-B2, and ephrin-B3 [30–33]. In contrast, CedV has a uniquely broad ephrin protein tropism and can utilize both B-class and A-class ephrins for cell entry and infection [21,34]. The binding of NiV or HeV G to their ephrin entry receptors on cells triggers a well-characterized activation and refolding of F from a pre- to post-fusion conformation that facilitates the merger of the virion and host cell membranes and subsequent delivery of the viral nucleocapsid into the cell cytoplasm (reviewed in [35]). Importantly, the F and G glycoproteins are also the major viral structural protein targets of neutralizing antibodies and the relevant antigens employed in all henipavirus vaccine strategies [22]. However, for pathogenic henipavirus vaccines or antibody-based countermeasure strategies, the assessment and quantification of neutralizing antibody responses or their potencies against authentic NiV and HeV, which requires BSL-4 containment, can be a major challenge.

Using a rCedV reverse genetics platform, the CedV F and G glycoprotein genes were replaced with those of NiV-B or HeV, and replication-competent chimeric henipaviruses (rCedV-NiV-B and rCedV-HeV) were rescued. Both non-reporter and two reporter gene versions, encoding a green fluorescent protein (GFP) or luciferase protein (Luc), of the rCedV chimeras were also produced. Characterization of the chimeric viruses revealed no significant differences in their replication kinetics or ability to induce a type I IFN response compared to rCedV and possessed the same ephrin B-class entry receptor tropisms as NiV-B and HeV. The neutralization potencies of several well-characterized cross-reactive NiV and HeV F and G specific mAbs against rCedV-NiV-B-GFP and rCedV-HeV-GFP were highly correlated with those measured using authentic NiV-B and HeV when tested in parallel by a plaque reduction neutralization test (PRNT). A rapid high-throughput and quantitative fluorescence reduction neutralization test (FRNT) using the GFP-encoding chimeras was established that also yielded highly correlated mAb neutralization potencies with those derived by PRNT. The FRNT assay was also suitable for measuring serum neutralization titers from animals immunized with recombinant HeV or NiV soluble G glycoproteins. Taken together, the rCedV chimera platform is an authentic henipavirus-based surrogate neutralization assay for pathogenic henipaviruses that is rapid, cost-effective and can be utilized outside BSL-4 containment.

2. Materials and Methods

2.1. Construction of pOLTV5-rCedV Chimeric Antigenomes

A full description of the synthesis of the rCedV antigenome clone (pOLTV5-rCedV) has previously been described [20]. Here, an optimized version of the pOLTV5-rCedV antigenome clone was designed, and the 3′ end of the T7 minimal promoter ($T7_{min}$) sequence (TAATACGACTCACTATA) was modified by the addition of nucleotides GGGAGA to generate a T7 optimal promoter ($T7_{opt}$) [36]. The $T7_{opt}$ sequence was then followed by the insertion of a self-cleaving autocatalytic hammerhead ribozyme A (HHRbzA) sequence (GGGAGATTGGTCTGATGAGTCCGTGAGGACGAAACGGAGTCTAGACTCCGTC) [36]. This synthesized gene fragment ($T7_{opt}$-HHRbzA) (Genscript; NJ, USA) was enzymatically inserted to precede the CedV 3′ Leader (3′ Le) sequence in the pOLTV5-rCedV plasmid to yield pOLTV5$_{opt}$-rCedV (Figure 1A).

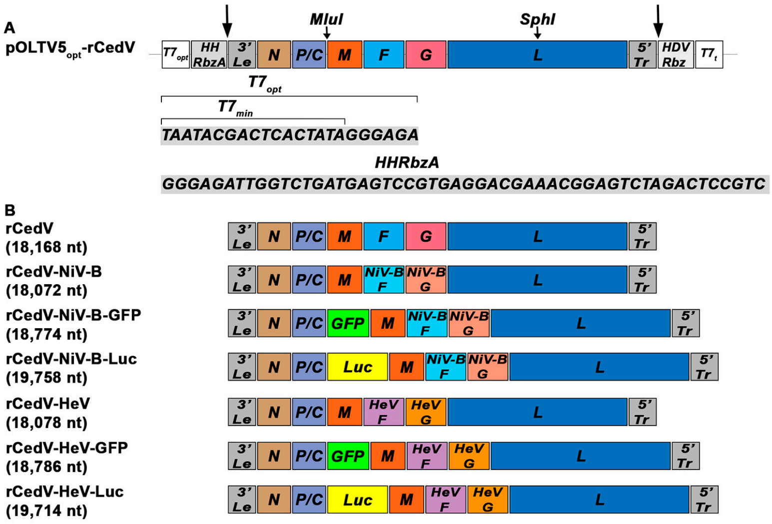

Figure 1. Schematic representation of the optimized rCedV plasmid and the genomes of the generated rCedV chimeric viruses. (**A**) The pOLTV5$_{opt}$-rCedV plasmid illustrates the location and sequences of the T7 optimal promoter ($T7_{opt}$) and the Hammerhead Ribozyme A (HHRbzA). The long arrows indicate regions of self-cleavage. Unique restriction sites MluI and SphI used to construct the rCedV chimeric plasmids are shown. (**B**) The genomes and the lengths of the generated chimeras are schematically diagrammed as rCedV-NiV-B, rCedV-NiV-B-GFP, rCedV-NiV-B-Luc, rCedV-HeV, rCedV-HeV-GFP, and rCedV-HeV-Luc. pOLTV5$_{opt}$-rCedV, optimized pOLTV5-rCedV plasmid; $T7_{min}$, T7 minimal promoter; $T7_{opt}$, T7 optimal promoter; HHRbzA, Hammerhead Ribozyme A; 3′Le, 3′ Leader; 5′Tr, 5′ Trailer; HDVRbz, hepatitis delta virus ribozyme; $T7_t$, T7 terminator.

Large gene cassettes comprising CedV F and G untranslated intergenic regions flanking the respective NiV-B or HeV F and G coding sequences were synthesized (Genscript). The NiV-B F and G coding sequences were based on the NiV-B 2010 Faridpur isolate (GenBank: JN808864.1). The HeV F and G protein sequences used here, HeV genome (GenBank: MN062017.1), are identical to that of the HeV 2008 Redlands isolate (GenBank: JN255805.1). The isolates and GenBank accession numbers for each F and G protein are listed in Table 1. Unique restriction enzyme sites facilitated the insertion of the NiV-B or HeV F and G gene cassettes into pOLTV5$_{opt}$-rCedV (Figure 1A) to ultimately generate non-reporter versions, pOLTV5$_{opt}$-rCedV-NiV-B or pOLTV5$_{opt}$-rCedV-HeV. The "rule-of-six" was maintained by removing the last three nucleotides (ACG; amino acid Threonine) from the NiV-B F coding sequence and adding a stop codon (TAA) to the end of the HeV F coding region. The insertion of a modified turbo Green Fluorescent Protein (*GFP*) gene (Lonza Inc., Allendale, NJ, USA) or firefly (*Photinus pyralis*) luciferase (*Luc*) gene (a kind gift from Dr. B Schaefer, USU) between CedV *P* and *M* genes of the newly constructed pOLTV5$_{opt}$-rCedV chimeric plasmids using standard molecular techniques was as previously described [20,37] and yielded pOLTV5$_{opt}$-rCedV chimeric reporter gene encoding versions of the rCedV antigenome clones. All cloning was performed with *Escherichia coli* Stbl2 cells (Invitrogen; Carlsbad, CA, USA). The insertions were sequenced to obtain at least 2-fold coverage.

Table 1. GenBank accession numbers of NiV-B and HeV envelope glycoproteins.

Henipavirus	Isolate	Protein	GenBank Accession Number
NiV-B	2010 Faridpur	F	AEZ01396.1
		G	AEZ01397.1
HeV	2008 Redlands	F	AEQ38070.1
		G	AEQ38071.1

2.2. Cells, Monoclonal Antibodies, Rhesus Macaque, and Rabbit Immune Sera

BSR-T7/5 cells, a BHK-derived cell line stably expressing T7 RNA polymerase [38], HeLa-USU, HeLa (ATCC CCL-2), Vero E6 (ATCC CCL-81), and Vero 76 (ATCC CRL-1587) cell lines were maintained at 37 °C, 5% CO_2 in Dulbecco's modified eagle media (DMEM) (Quality Biological; Gaithersburg, MD, USA) supplemented with 10% cosmic calf serum (CCS) and 1% L-glutamine (Quality Biological) (DMEM-10). HeLa-USU-ephrin-B2 and HeLa-USU-ephrin-B3 stable cell lines were maintained in DMEM-10% CCS, 1% L-glutamine supplemented with 0.4 mg/mL Hygromycin B (Invitrogen).

The neutralizing HeV and NiV cross-reactive human mAb, m102.4, is a G glycoprotein-specific IgG1 subclass antibody [28,39–43]. The humanized 5B3.1 (h5B3.1) mAb [44,45] and the murine mAbs 12B2 and 1F5 [46] are IgG1 mAbs cross-reactive to HeV and NiV F glycoprotein. Anti-NiV G glycoprotein-specific sera were from four rhesus macaques immunized at the University of Texas Medical Branch at Galveston, TX, on days 0, 28, and 56 with an equal mixture of 0.1 mg NiV-B and 0.1 mg NiV-M recombinant soluble G (sG) glycoproteins adjuvanted with aluminum hydroxide suspension (Auro Vaccines, LLC, Pearl River, NY, USA) [47]. Sera were collected on days 42 and 84 post-immunization and stored at −80 °C. Anti-HeV G glycoprotein-specific sera were prepared using the HeV/NiV recombinant soluble HeV G glycoprotein vaccine candidate (HeV-sG) [26,27,48–54] or HeV-sG$_{tet}$ [55], a tetrameric version of the HeV sG glycoprotein which was constructed similarly to other henipavirus sG glycoproteins as described in Cheliout Da Silva et al. [56]. Sera from rabbits immunized on days 0 and 28 with 0.1 mg HeV-sG or HeV-sG$_{tet}$ formulated with complete Freund's adjuvant (initial injection) and boosted with immunogen formulated with incomplete Freund's adjuvant (booster injection) were prepared by Noble Life Sciences; Woodbine, MD. Sera were collected on day 45 post-immunization and stored at −80 °C.

2.3. Rescue of Recombinant CedV Chimeras

BSR-T7/5 cells in a 12-well plate (2.5×10^5 cells/well) were co-transfected with pCMV-CedV helper plasmids pCMV-CedV-N (1.25 µg), pCMV-CedV-P (0.8 µg) and pCMV-CedV-L (0.4 µg) together with one of the pOLTV5$_{\text{opt}}$-rCedV chimera antigenome constructs (3.5 µg) using TransIT-LT1 transfection reagent (Mirus Bio; Madison, WI, USA) according to the manufacturer's recommendations. After 4–5 days, transfected cells were observed for syncytia formation and/or GFP expression. Supernatants from successful rescue wells were collected and passaged onto naïve Vero E6 cells in a T-75 flask to prepare a master stock of each of the rCedV chimeras. When maximal syncytia and/or GFP expression was observed (~2–3 days), viral supernatants were collected and clarified by centrifugation at 948× *g* (2400 rpm) for 10 min to pellet cell debris. The supernatant was transferred to screw-cap tubes as single-use aliquots and stored at −80 °C. All rCedV chimeras were deep sequenced using Illumina short reads, and variants were analyzed. Briefly, sequencing libraries were prepared from total RNA using the TruSeq Stranded Total RNA Sample Prep kit (Illumina, San Diego, CA, USA) and subjected to multiplexed sequencing on either the Illumina MiSeq platform using 600 cycles, V3 chemistry, or the Illumina NextSeq platform using 300 cycles, V2 chemistry. The resulting sequencing reads were analyzed using EDGE Bioinformatics tools [57] and an in-house metagenomics pipeline called MetaDetector (unpublished). EDGE Bioinformatics suite was used for read processing, and the host read subtraction, de novo assembly, taxonomic classification, and variant detection. Sequencing reads were also processed in parallel using MetaDetector, which checked for quality using FASTQC [58], trimmed for quality using BBDuk (Q20) [59], and removed incidental matching human genome reads using BBMAP [59]. The remaining reads were assembled using metaSPAdes and SPAdes [60]; the resulting contigs, along with all the cleaned singleton reads, were BLAST searched using Diamond [61] for taxonomic classification. The final assemblies were examined and constructs were identified to be annotated using BLASTn and BLASTx implemented in CLC Genomics Workbench (QIAGEN Bioinformatics; Redwood City, CA). Variant analysis was performed by mapping the reads to a publicly available reference Cedar virus genome from isolate CG1a (GenBank accession JQ001776) using EDGE Bioinformatics tools and iVar [62].

2.4. Viral Plaque Assay

Viral stocks were titrated by plaque assay as previously described [20,37,63]. Briefly, a ten-fold serial dilution of the virus stock was prepared in DMEM-10, 200 µL of which was applied to pre-seeded Vero E6 cells in duplicate (5×10^5 cells/well) in a 12-well plate and incubated for 1 h at 37 °C, 5% CO_2. A 2 mL overlay of a 1:1 mix of DMEM containing 5% CCS and 1% L-glutamine (DMEM-5) with 2% carboxymethylcellulose sodium salt (medium viscosity) (Sigma-Aldrich; St. Louis, MO, USA) was applied to all wells and incubated for 4 days at 37 °C, 5% CO_2. Cells were fixed with 4% Formaldehyde in 1× PBS for 1 h at room temperature and stained with 0.5% crystal violet in 80% methanol for 15 min at room temperature. The stain was removed and washed with diH_2O, and plaque-forming units (PFU) were counted and expressed as PFU/mL.

2.5. Virus Biosafety Procedures and Regulations

Laboratory manipulation guidelines and standard operating procedures for rCedV-NiV-B, rCedV-NiV-B-GFP, rCedV-NiV-B-Luc, rCedV-HeV, rCedV-HeV-GFP, rCedV-HeV-Luc, rCedV, rCedV-GFP and rCedV-Luc under BSL-2 conditions have been established, reviewed, and approved by the Uniformed Services University (USU), Institutional Biosafety Committee in accordance with NIH guidelines. The rCedV-HeV-GFP and/or rCedV-NiV-B-GFP chimeras have been previously used in mAb neutralization and mAb synergy neutralization studies [47,64,65]. A P2 stock of NiV-B was used in these studies. There were four mutations of sufficient frequency in comparison to the reference sequence GenBank Accession number AY988601.1. Of these, one was non-coding, and the other three led to single amino acid changes: one in the M protein and two in the F protein [66]. The HeV isolate used in these studies (GenBank Accession number NC_001906) was obtained from a patient from the 1994 outbreak in Australia and was provided by Dr. Thomas Ksiazek [45]. All studies with authentic NiV-B and HeV were performed within the BSL-4 facilities of the Galveston National Laboratory, The University of Texas Medical Branch at Galveston, TX, USA.

2.6. Western Blot Analysis

Vero E6 cells in a 6-well plate were infected at a density of 1×10^6 cells/well with rCedV-NiV-B, rCedV-NiV-B-GFP, rCedV-NiV-B-Luc, rCedV-HeV, rCedV-HeV-GFP, rCedV-HeV-Luc, rCedV, rCedV-GFP or rCedV-Luc at a multiplicity of infection (MOI) of 0.01. Simultaneously, the cells were co-transfected with a total of 2 µg of the plasmid to express both F and G glycoproteins from either NiV or HeV. A promoter-modified pcDNA3.1 vector with a hygromycin selection marker [67] encoding either NiV-F (pcDNA3.1-NiV-F) or NiV-G (pcDNA3.1-NiV-G) was used for the NiV-F and NiV-G expression. A promoter-modified pcDNA3.1 encoding either HeV-F (pcDNA3.1-HeV-F) or HeV-G (pcDNA3.1-HeV-G) was used for the HeV-F and HeV-G expression. At 24 h and 48 h post-infection, cells were collected and lysed with 1× RIPA (radioimmunoprecipitation assay) Lysis and Extraction Buffer (ThermoFisher Scientific; Waltham, MA, USA) containing a protein inhibitor cocktail (ThermoFisher Scientific). Total protein (~30 µg) in reducing sample buffer (2× lithium dodecyl sulfate (LDS) NuPage® sample buffer (Invitrogen), 5% β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA) was boiled for 10 min at 100 °C. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 4–12% Bis-Tris gel (ThermoFisher Scientific) and then transferred on nitrocellulose membranes (ThermoFisher Scientific). The membranes were blocked in 5% milk in 1× PBS with 0.1% Tween-20 at room temperature. Cross-reactive murine mAbs specific to NiV and HeV F (mAb 5G7) or to NiV and HeV G (mAb 48D3) glycoproteins, polyclonal rabbit sera to CedV-N (CSIRO, Victoria, Australia), and β-actin (ThermoFisher Scientific) were used as primary antibodies and subsequently probed with a corresponding secondary HRP-coupled antibody.

2.7. Virus Replication Kinetics

Vero E6 cells were seeded at a density of 2×10^4 cells/well in a 96-well cell culture plate. The next day, cells were infected at an MOI of 0.01 at 37 °C, 5% CO_2. After 1 h, the viral inoculum was removed, and fresh DMEM-10 was added to all wells. Supernatants were collected at 0, 8, 24, 48, and 72 h post-infection and stored at −80 °C until ready to analyze. Viral titers were determined by plaque assay as described in Section 2.4 and were expressed as plaque-forming units (PFU) per mL (PFU/mL). To determine intracellular luciferase activity, cells infected with either the non-reporter or Luc expressing viral chimeras were lysed with the Steady-Glo® Luciferase Assay System (Promega, Madison, WI, USA) in a 1:1 mixture with DMEM-10. After a 10 min incubation at room temperature, the homogenate was transferred to a white opaque 96-well cell culture plate, Nunc™ F96 MicroWell™ White Polystyrene Plate (ThermoFisher Scientific), and luminescence read using the GloMax®—Multi Detection System (Promega, Madison, WI, USA). Relative light units (RLU) were measured and normalized by subtracting the luminescence values of rCedV-NiV-B or rCedV-HeV infected cells from the luminescence values of rCedV-NiV-B-Luc or rCedV-HeV-Luc infected cells, respectively. Virus titers and luciferase activity levels at 0 h post-infection indicate the lower limit of detection for the plaque assay and the luminometer, respectively.

2.8. Ephrin Entry Receptor Tropism

HeLa-USU, HeLa-USU-ephrin-B2, and HeLa-USU-ephrin-B3 cell lines were seeded at a density of 2.5×10^5 cells/well in a 12-well cell culture plate. When confluent, the cell culture medium was removed, and cells were left uninfected (Mock) or infected at an MOI of 0.5 with either rCedV-NiV-B-GFP, rCedV-HeV-GFP, or rCedV-GFP individually diluted in DMEM-10. At 24 h post-infection, rCedV-NiV-B-GFP, rCedV-HeV-GFP, and rCedV-GFP infected cell cultures were monitored for GFP fluorescence and syncytia. Images were captured with a Zeiss Axio Observer A1 inverted microscope using the 5× objective.

2.9. Reverse Transcriptase Quantitative PCR (RT-qPCR) and Type I IFN Response

HeLa-CCL-2 cells were seeded at a density of 1.25×10^5 cells/well in a 24-well plate and incubated overnight. Cells were left intact (Mock), transfected with polyinosinic:polycytidylic acid (Poly I:C) (InvivoGen; San Diego, CA, USA) (10 μg/mL) using Lipofectamine LTX (ThermoFisher Scientific), or infected with rCedV-NiV-B, rCedV-HeV or rCedV at either an MOI of 0.5 or 1.0. At 24 h post-infection, total RNA was extracted using the RNeasy Mini Kit (Qiagen Sciences Inc., Germantown; MD, USA). An amount of 500 ng of DNase I digested RNA was converted to cDNA using the Superscript III First-Strand Synthesis System (ThermoFisher Scientific) with oligo(dT) primers. Quantitative PCR (qPCR) was then performed with the synthesized cDNA using the Power SYBR Green PCR Master Mix (ThermoFisher Scientific) and the Applied Biosystems 7500 Real-Time PCR System. PCR cycling conditions were: 95 °C, 10 min; 40× cycles of 95 °C, 15 s; 60 °C, 1 min; followed by a melt curve analysis at the completion of each experiment. Each sample was analyzed for IFN-α, IFN-β and 18S ribosomal RNA in triplicate, and fold changes were calculated relative to 18S ribosomal RNA and normalized to mock samples using the $2^{(-\Delta\Delta Ct)}$ method. IFN-α forward primer, 5′ TTTCTCCTGCCTGAAGGACAG 3′, IFN-α reverse primer, 5′ ACAGTCTCGTCTTTAGTACTCG 3′ [68]. IFN-β forward primer, 5′ GTCAGAGTGGAAATCCTAAG 3′, IFN-β reverse primer, 5′ ACAGCATCTGCTGGTTGAAG 3′ [69]. An 18S ribosomal RNA forward primer 5′ GGGCATTCGTATTTCATAGTCAGAG 3′, 18S ribosomal RNA reverse primer 5′ CGGTTCTTGATTAATGAAAACATCCT 3′ [70].

2.10. Plaque Reduction Neutralization Test (PRNT)

Vero 76 cells were seeded at a density of 6×10^5 cells/well in a 6-well plate and incubated overnight at 37 °C, 5% CO_2. The mAbs were serially diluted 3-fold in DMEM-10 such that an initial concentration of 10 μg/mL was used for the 9-point dose-response curve. The diluted mAbs were incubated with an equal volume of either rCedV-NiV-B-GFP, rCedV-HeV-GFP, NiV-B, or HeV at an MOI of 0.0001 for 1 h at 37 °C, 5% CO_2. MOI was calculated for a tentative 10^6 cells/well and 0.4 mL virus and antibody mixture per well. Each virus-mAb mixture (400 μL/well) was added to duplicate wells. Following a 1 h incubation at 37 °C, 5% CO_2, the wells were overlaid with a 1:1 mix of 0.8% agarose/DMEM-10 and incubated for 4 days. A neutral red solution was added to each well and incubated for 24 h, at which time plaques were counted. Neutralization percent (%) was calculated by subtracting the PFU_{mAb} for each virus from the respective PFU without the antibody, i.e., $Neutralization(\%) = 100 \times \frac{(PFU_0 - PFU_{mAb})}{PFU_0}$, where the PFU_{mAb} is the PFU at the respective mAb concentration, and PFU_0 is the PFU without the antibody. The 50% inhibitory concentration (IC_{50}) was determined as the antibody concentration at which there was a 50% reduction in plaque counts versus untreated control wells. The IC_{50} values were calculated by non-linear regression curve fitting with a variable slope using GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA). The limit of detection for this assay was 50 PFU.

2.11. Fluorescent Reduction Neutralization Test (FRNT)

Vero 76 cells were seeded at a density of 2×10^4 cells/well in black-walled clear bottom 96-well plates (Corning Life Sciences; Corning, NY, USA) and incubated for 24 h at 37 °C, 5% CO_2. m102.4, h5B3.1, 12B2, and 1F5 mAbs were serially diluted 3-fold such that an initial concentration of 1.1 μg/mL was used for the 7-point dose-response curve. Immunized sera were 3-fold serially diluted in DMEM-10 such that NiV-B and NiV-M sG immunized rhesus macaque sera were at a starting dilution of 1:200, and the HeV-sG and HeV-sG_{tet} rabbit sera were at a starting dilution of 1:400. An equal volume of DMEM-10 containing either rCedV-NiV-B-GFP or rCedV-HeV-GFP was added to each dilution for a final MOI of 0.05 and incubated for 2 h at 37 °C, 5% CO_2. Each of the virus-mAb or virus-sera mixtures (90 μL/well) was added to the pre-seeded Vero 76 cells in triplicate and incubated for an additional 24 h at 37 °C, 5% CO_2. The virus-mAb supernatants were removed, and the plates were fixed with 4% Formaldehyde in 1× PBS for 20 min at room temperature. The plates were then washed 3 times by hand with a slow stream of diH_2O, and the last wash was discarded before the plates were imaged using a CTL S6 analyzer (Cellular Technology Limited; Shaker Heights, OH, USA). Fluorescent foci were counted using the CTL Basic Count software. The 50% inhibitory concentration (IC_{50}) was determined as the antibody concentration or serum dilution at which there was a 50% reduction in fluorescent foci versus untreated control wells. The IC_{50} values were calculated by non-linear regression curve fitting with a variable slope using GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA). The limit of detection for this assay was 50 fluorescent foci.

2.12. Statistical Analysis

Data were analyzed and graphed using GraphPad Prism 9 (GraphPad Software Inc.). Unless otherwise stated, graphs and images are the average of three independent experiments and are expressed as the arithmetic mean. Standard deviations were calculated and represented accordingly. Statistical analyses for viral replication kinetics were performed with two-way ANOVA followed by the Tukey post hoc test ($\alpha = 0.05$). Statistical analyses for qPCR experiments were performed with the unpaired, two-tailed Student *t*-test using GraphPad Prism 9. Correlation analyses were performed using Pearson correlation coefficient analyses.

3. Results

3.1. Construction and Rescue of Recombinant Cedar Virus-Based Chimeras

To generate rCedV chimeric viruses encoding the NiV-B or HeV envelope glycoprotein genes, we first optimized the virus rescue efficiency of the previously described rCedV reverse genetics system [20]. A $pOLTV5_{opt}$-rCedV antigenome plasmid was constructed by inserting a DNA fragment containing sequences for a T7 optimal promoter ($T7_{opt}$) and an autocatalytic Hammerhead Ribozyme A (HHRbzA) sequence upstream of the rCedV 3′ leader antigenome sequence (see Materials and Methods) (Figure 1A). Next, a large fragment of the $pOLTV5_{opt}$-rCedV plasmid flanked by unique restriction enzyme sites MluI and SphI was replaced with synthesized DNA fragments containing the open reading frames of either the NiV-B or HeV F and G glycoproteins in place of the CedV F and G glycoprotein encoding region. Reporter genes containing antigenome plasmids encoding either *GFP* or *Luc* genes for each chimera were also generated (see Materials and Methods). The reverse genetics method was then used to rescue a panel of replication-competent rCedV chimeras as non-reporter gene versions (rCedV-NiV-B and rCedV-HeV) and reporter gene encoding versions expressing GFP (rCedV-NiV-B-GFP and rCedV-HeV-GFP) or Luc (rCedV-NiV-B-Luc and rCedV-HeV-Luc) proteins. A schematic representation of the genomes and genome lengths of all rescued viruses is illustrated in Figure 1B. Successful rescue of the viruses was confirmed by the detection of syncytia formation (cytopathic effect (CPE)) when supernatants from BSR-T7/5 cells transfected with the rCedV chimeric antigenome and CedV helper plasmids were then passaged onto Vero E6 cells. Stock virus preparations were subsequently prepared, and virus genomes were sequenced. When compared to the predicted genome sequences, the following mutations were detected within coding sequences in the chimera genomes (e.g., excluding intergenic regions) (Table S1). There were two mutations in the rCedV-HeV genome: one synonymous single nucleotide variation (SNV) and one SNV that resulted in a single amino acid change in the F protein. Three mutations were detected in the rCedV-HeV-GFP genome: one synonymous SNV and two that resulted in single amino acid changes: one in the N protein and the other in the M protein. There were four mutations in the rCedV-HeV-Luc genome, and all four were synonymous mutations. The rCedV-NiV-B genome contained four mutations, all resulting in single amino acid changes: one in the N protein, one in the M protein, and two in the L protein. There were eight mutations in the rCedV-NiV-B-GFP genome: six were synonymous SNVs, and two resulted in single amino acid changes in the N protein. There were nine mutations detected in the rCedV-NiV-B-Luc genome: five were synonymous SNVs, and four resulted in single amino acid changes: one in the N protein, one in the Luc protein, one in the M protein, and one in the L protein. No apparent loss of rCedV chimera reproductive capacity or reporter gene loss or integrity has been observed to date, probably owing to the requirement of these paramyxoviruses to the 'rule-of-six.'

3.2. Characterization of Recombinant Cedar Virus-Based Chimeric Viruses

We assessed the ability of the rCedV-NiV-B chimeras and the rCedV-HeV chimeras to facilitate membrane fusion and syncytia formation when used to infect cells (Figure 2). Vero E6 cells were either uninfected (Mock) or infected with either rCedV-NiV-B, rCedV-NiV-B-GFP, rCedV-NiV-B-Luc, rCedV-HeV, rCedV-HeV-GFP, or rCedV-HeV-Luc and comparisons then made to Vero E6 cells infected with rCedV, rCedV-GFP or rCedV-Luc. At 24 h post-infection, cells infected with the GFP-expressing viruses were imaged for fluorescence and syncytia (Figure 2A), while cells infected with the non-reporter or Luc expressing rCedV chimeras were imaged following fixation and crystal violet staining (Figure 2B). Fluorescence and/or syncytia (yellow arrows) were observed in all infected Vero E6 cells. The syncytia observed in cells infected with either the rCedV-NiV-B chimeras or the rCedV-HeV chimeras were noticeably larger and contained more nuclei than those syncytia observed in rCedV-infected cells (Figure 2). These data confirmed the functionality of the NiV-B and HeV F and G glycoproteins expressed in the context of rCedV.

We next evaluated the relative expression levels of NiV-B and HeV F and G envelope glycoproteins from cells infected with the rCedV chimeras in comparison to rCedV (comparative control). Vero E6 cells were uninfected (Mock) or infected with either rCedV-NiV-B, rCedV-NiV-B-GFP, rCedV-NiV-B-Luc, rCedV-HeV, rCedV-HeV-GFP, rCedV-HeV-Luc, rCedV, rCedV-GFP or rCedV-Luc. For additional comparative purposes, separate populations of Vero E6 cells were co-transfected with plasmids, pcDNA3.1-NiV-F and pcDNA3.1-NiV-G (pcDNA3.1-NiV F + G), or pcDNA3.1-HeV-F and pcDNA3.1-HeV-G (pcDNA3.1-HeV F + G). Representative western blot images for NiV-B and HeV F and G glycoproteins probed with cross-reactive NiV/HeV F or G specific mAbs are shown in Figure 3. We observed the precursor protein F_0 and the processed F_1 subunit in the lysates of cells infected with the rCedV-NiV-B chimeras (Figure 3A) or the rCedV-HeV chimeras (Figure 3B,C). A distinct band representing the G glycoprotein was detected in all rCedV-NiV-B (Figure 3A) and rCedV-HeV (Figure 3B,C) infected lysates. Furthermore, the NiV-B and HeV F and G glycoprotein SDS-PAGE gel migration profiles were similar to those observed in the pcDNA3.1-NiV F + G (Figure 3A) and pcDNA3.1-HeV F + G (Figure 3B,C) transfected cell lysates, respectively. Whereas, HeV/NiV-B F_0, $F_{1,}$ or G bands were not observed in any of the rCedV infected lysates (Figure 3). The lower levels of F_0, $F_{1,}$ and G observed in the rCedV-HeV-Luc infected lysates in comparison to the other rCedV-HeV chimera infected lysates at 24 h post-infection (Figure 3B) could be attributed to slower virus replication kinetics of rCedV-HeV-Luc (see Figure 4B). To address this, we analyzed lysates of all rCedV-HeV chimeras at 48 h post-infection by western blot. As shown in Figure 3C, the levels of HeV F_0, $F_{1,}$ and G glycoproteins in all rCedV-HeV chimeras were comparable at this later time point. The presence of CedV N protein served as an expressed viral protein control and was observed in all infected cell lysates, while β-actin served as a lysate loading control. These data confirm the expression of NiV-B and HeV F and G glycoproteins in infected cells and indicate functional compatibility between rCedV and NiV-B and HeV envelope glycoproteins in a relevant viral context.

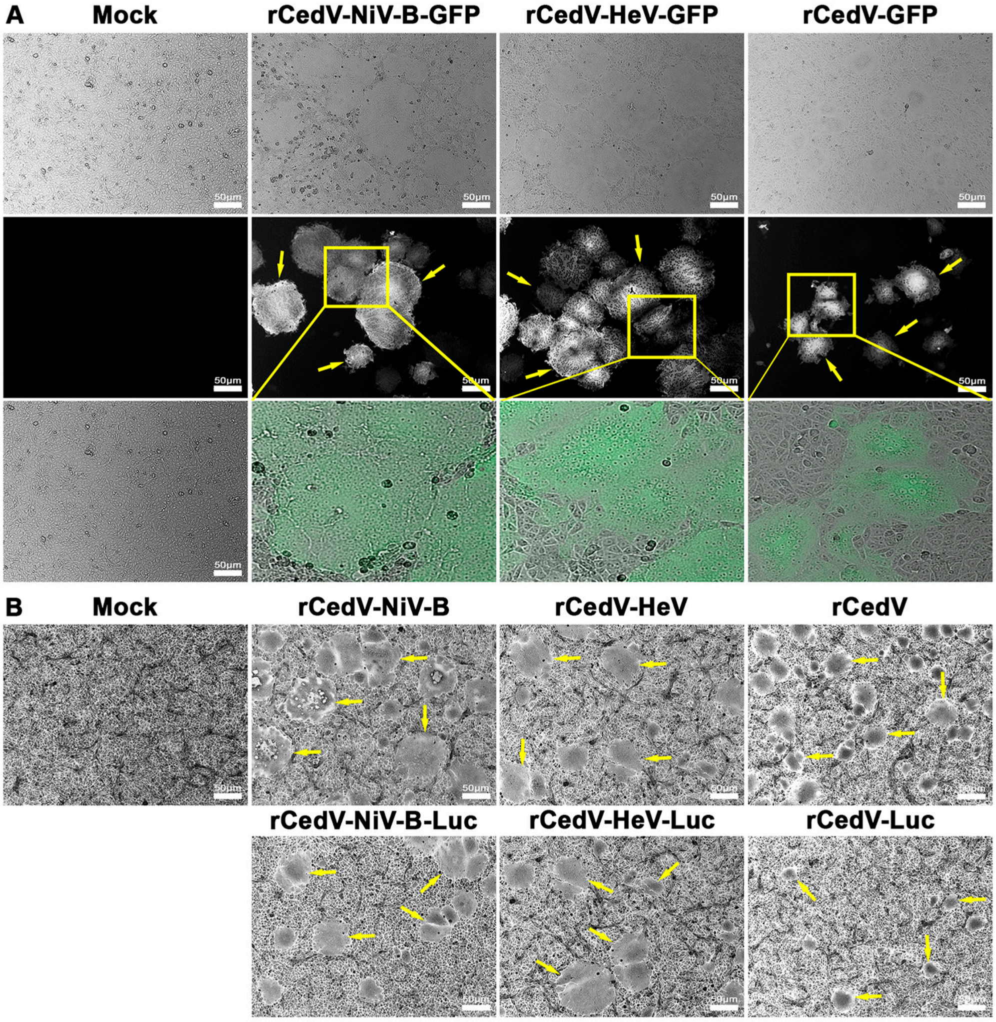

Figure 2. Syncytia induced by rCedV expressing NiV-B or HeV envelope glycoproteins. Vero E6 cells were uninfected (Mock) or infected with either rCedV-NiV-B, rCedV-NiV-B-GFP, rCedV-NiV-B-Luc, rCedV-HeV, rCedV-HeV-GFP, rCedV-HeV-Luc, rCedV, rCedV-GFP or rCedV-Luc at a MOI of 0.01. All images were taken 24 h post-infection. (**A**) Cells infected with GFP-expressing viruses. Transmitted light (**top** row), fluorescence (**middle** row), and merged (**bottom** row) images are shown. The respective zoomed-in fluorescence images (3rd row) are regions from the yellow boxes. (**B**) Cells infected with non-reporter or Luc expressing rCedV chimeras were fixed, stained, and then imaged for syncytia. The images taken with transmitted light are shown. Images were captured with a Zeiss Axio Observer A1 inverted microscope using a 5× objective. Arrows indicate giant multinucleated cells (syncytia). Representative images from three independent experiments are shown. Scale bar, 50 µm.

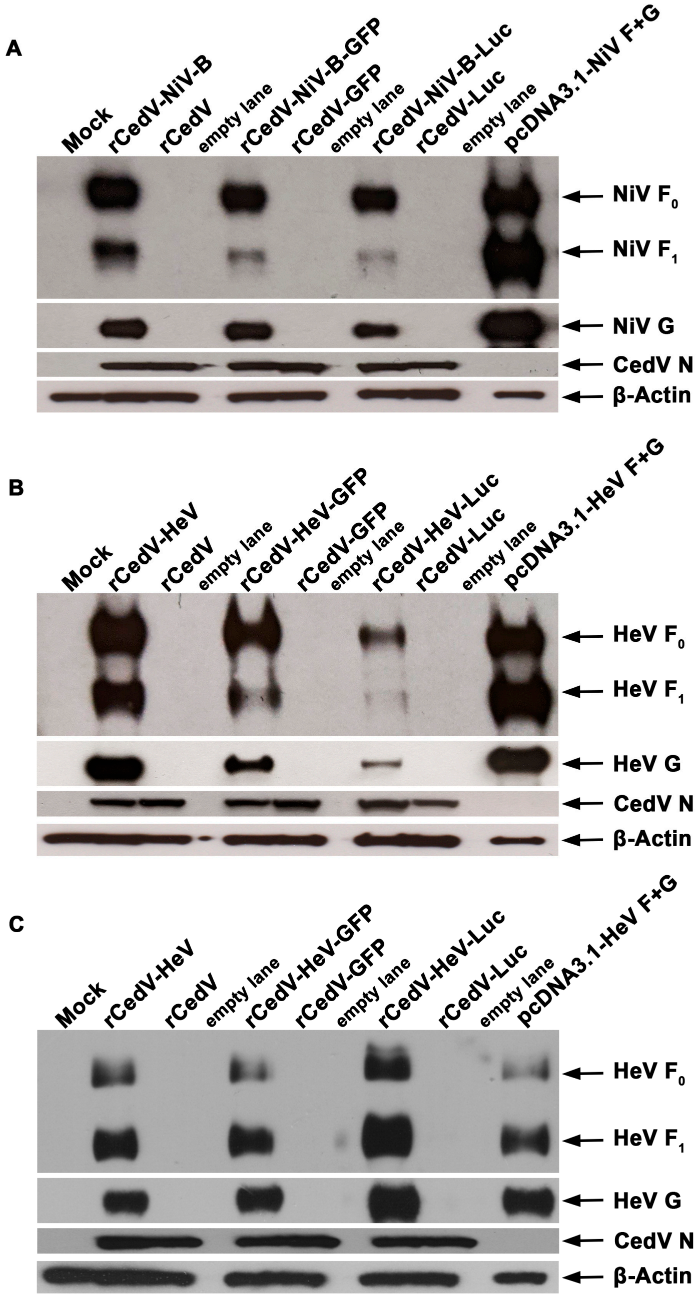

Figure 3. Expression of NiV-B and HeV envelope glycoproteins in infected cells. Vero E6 cells were uninfected (Mock) or infected at a MOI of 0.01 with either rCedV-NiV-B, rCedV-NiV-B-GFP, rCedV-NiV-B-Luc (**A**), rCedV-HeV, rCedV-HeV-GFP, rCedV-HeV-Luc (**B**,**C**), rCedV, rCedV-GFP or rCedV-Luc. As a reference, cells were co-transfected with a total of 2 µg of pcDNA3.1-NiV-F and pcDNA3.1-NiV-G (pcDNA3.1-NiV F + G), or pcDNA3.1-HeV-F and pcDNA3.1-HeV-G (pcDNA3.1-HeV F + G). Cells were harvested at 24 h post-infection (**A**,**B**) (rCedV-NiV-B and rCedV-HeV chimeras) or 48 h post-infection (**C**) (rCedV-HeV chimeras only), lysates were prepared and total protein (~30 µg) resolved by SDS-PAGE followed by western blot assay. The subsequent membrane was probed with HeV and NiV cross-reactive monoclonal antibodies (mAbs) against F glycoprotein (mAb 5G7) and G glycoprotein (mAb 48D3), polyclonal rabbit serum to CedV-N and β-actin. Representative images from two independent experiments are shown.

The replication kinetics of rCedV-NiV-B, rCedV-NiV-B-GFP, and rCedV-NiV-B-Luc (Figure 4A) and rCedV-HeV, rCedV-HeV-GFP, and rCedV-HeV-Luc (Figure 4B) were also compared to rCedV. Plaque assays were performed on harvested viral supernatants, and infectious virus titers were determined. We observed a gradual increase in infectious virus titers of all rCedV chimeric viruses that peaked 48 h post-infection (Figure 4). Specifically, the rCedV-NiV-B chimeras reached maximum virus titers of ~4–7 × 10^5 PFU/mL, while the rCedV-HeV chimeras peaked at ~2–9 × 10^5 PFU/mL. No statistically significant differences in replicated virus titers were observed between any of the rCedV-NiV-B chimeras or the rCedV-HeV chimeras or when compared to rCedV. In parallel, luciferase activity in rCedV-NiV-B-Luc and rCedV-HeV-Luc infected cells was measured. Figure 4 (right *y*-axes, black dashed lines) shows an increase in luminescence signal for both rCedV-NiV-B-Luc (Figure 4A) and rCedV-HeV-Luc (Figure 4B), which corresponded to the increase in infectious virus titers of their respective chimeras. Maximum luminescence signal was measured at 3.5 × 10^7 RLU at 48 h post-infection for rCedV-HeV-Luc and at ~8 × 10^6 RLU at 24 h post-infection for rCedV-NiV-B-Luc. The latter is likely due to extensive syncytia, and CPE observed in rCedV-NiV-B infected cells. These data illustrate that the rCedV chimeric viruses replicated efficiently and were comparable to rCedV and that luciferase activity is an indicator of viral genome expression.

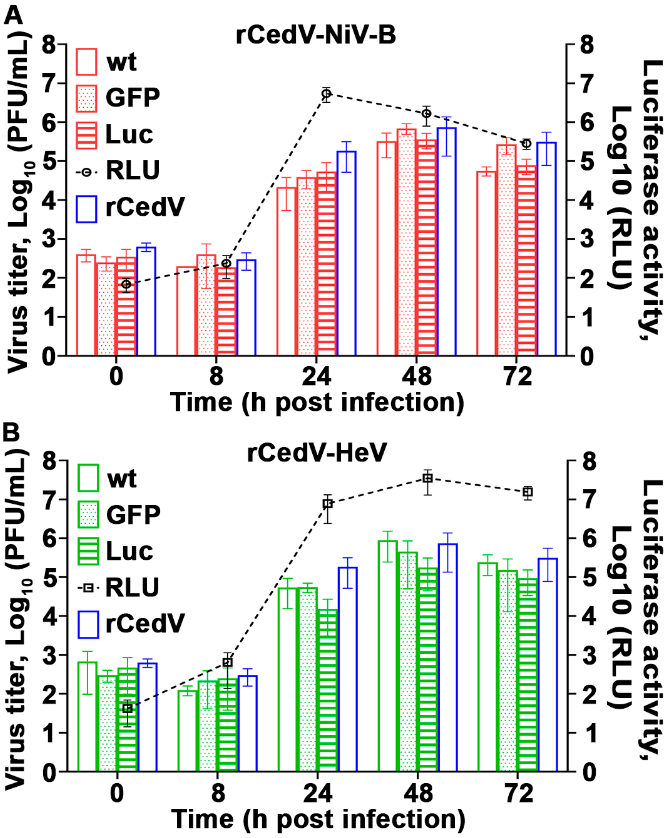

Figure 4. Replication kinetics of rCedV chimeras. Infectious virus titers (PFU/mL) determined from supernatants harvested at the indicated time points from Vero E6 cells infected at a MOI of 0.01 with rCedV-NiV-B (clear red bar), rCedV-NiV-B-GFP (dotted red bar), rCedV-NiV-B-Luc (striped red bar) (**A**), rCedV-HeV (clear green bar), rCedV-HeV-GFP (dotted green bar) or rCedV-HeV-Luc (striped green bar) (**B**). As a reference, separate populations of Vero E6 cells were also infected with rCedV (blue bar) (**A**,**B**). Normalized relative light units (RLU) for CedV-NiV-B-Luc (**A**) and rCedV-HeV-Luc (**B**) infected cells are represented on the right *y*-axes as black dashed lines. These data represent mean ± standard deviation from three independent experiments. Virus titers and luciferase activity levels at 0 h post-infection indicate the lower limit of detection for the plaque assay and the luminometer, respectively. Statistical analysis was performed in GraphPad Prism 9 by two-way ANOVA followed by Tukey's post hoc test ($\alpha = 0.05$).

3.3. Ephrin Entry Receptor Tropism of Recombinant Cedar Virus Chimeras

To define the receptor tropism of the newly generated rCedV chimeras, we used the NiV and HeV non-permissive cell line, HeLa-USU [31] and HeLa-USU cells stably expressing either ephrin-B2 (HeLa-USU-ephrin-B2) or ephrin-B3 (HeLa-USU-ephrin-B3) [20]. Here, all cells were either uninfected (Mock) or infected with rCedV-NiV-B-GFP, rCedV-HeV-GFP, or rCedV-GFP and at 24 h post-infection imaged for GFP expression. We observed fluorescence and/or syncytia in rCedV-NiV-B-GFP, and rCedV-HeV-GFP infected HeLa-USU-ephrin-B2 (Figure 5A) and HeLa-USU-ephrin-B3 (Figure 5B) cells, but not in the HeLa-USU infected cells (Figure 5C). In addition, GFP expression was detected in all rCedV-GFP infected cells, although syncytia were only observed in HeLa-USU-ephrin-B2 infected cells and were consistent with prior observations [20].

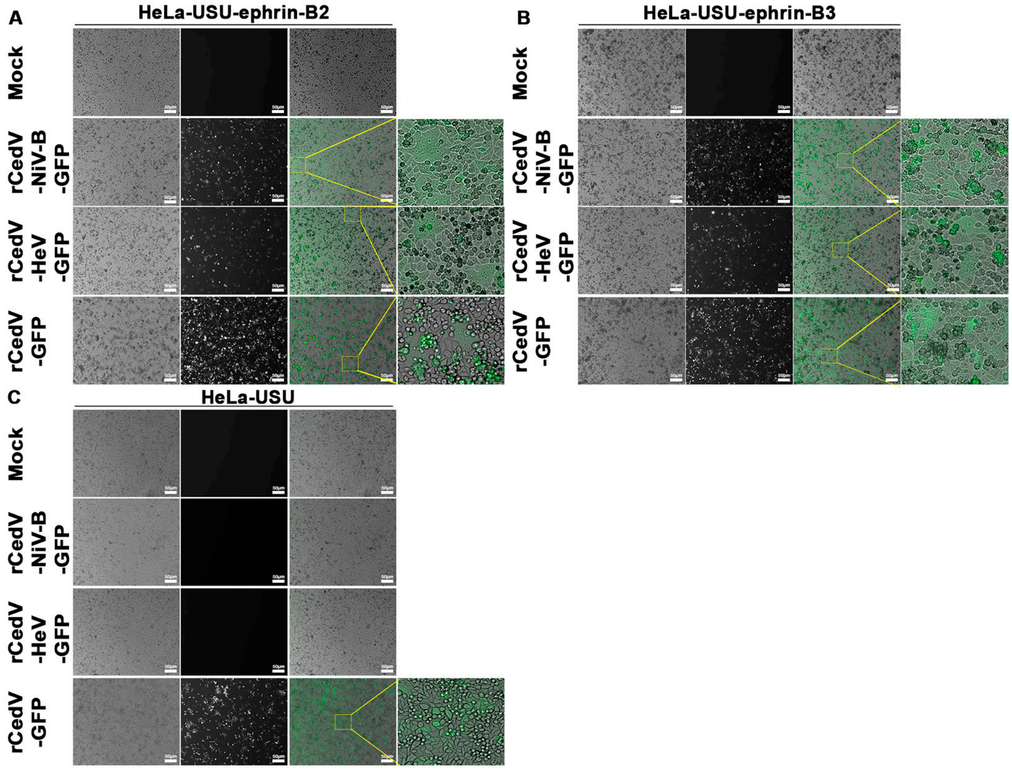

Figure 5. Ephrin-B2 and ephrin-B3 receptors facilitate rCedV-NiV-B-GFP and rCedV-HeV-GFP infection. Confluent HeLa-USU-ephrin-B2 (**A**), HeLa-USU-ephrin-B3 (**B**), and HeLa-USU (**C**) cells were uninfected (Mock) or infected with rCedV-NiV-B-GFP, rCedV-HeV-GFP, or rCedV-GFP at a MOI of 0.5. Infected cells were imaged for fluorescence and syncytia at 24 h post-infection. In each panel, transmitted light (1st column), fluorescence (2nd column), and merged (3rd column) images are shown. Zoomed-in regions are from the yellow boxes. Images were captured with a Zeiss Axio Observer A1 inverted microscope using a 5× objective. Representative images from two independent experiments are shown. Scale bar, 50 µm.

3.4. Recombinant Cedar Virus Chimeras Induce an Interferon Response

We next evaluated the induction of a type I IFN response in cells infected with the rCedV chimeras. HeLa-CCL-2 cells were uninfected (Mock) or infected with rCedV-NiV-B, rCedV-HeV, or rCedV. Additional HeLa-CCL-2 cells were transfected with Poly I:C to verify the induction of IFN-β (positive control). At 24 h post-infection, total RNA was extracted from all samples, and IFN-α and IFN-β mRNA levels were quantified by qPCR. As shown in Figure 6, in contrast to the mock samples, we observed a significant dose-dependent increase in IFN-β mRNA expression levels following rCedV-NiV-B, rCedV-HeV, and rCedV infection. A significant increase in the expression levels of IFN-α mRNA was not observed in any of the infected samples. These data demonstrate that rCedV chimeric viruses induced a robust and dose-dependent IFN-β response similar to rCedV [20] and also CedV [2].

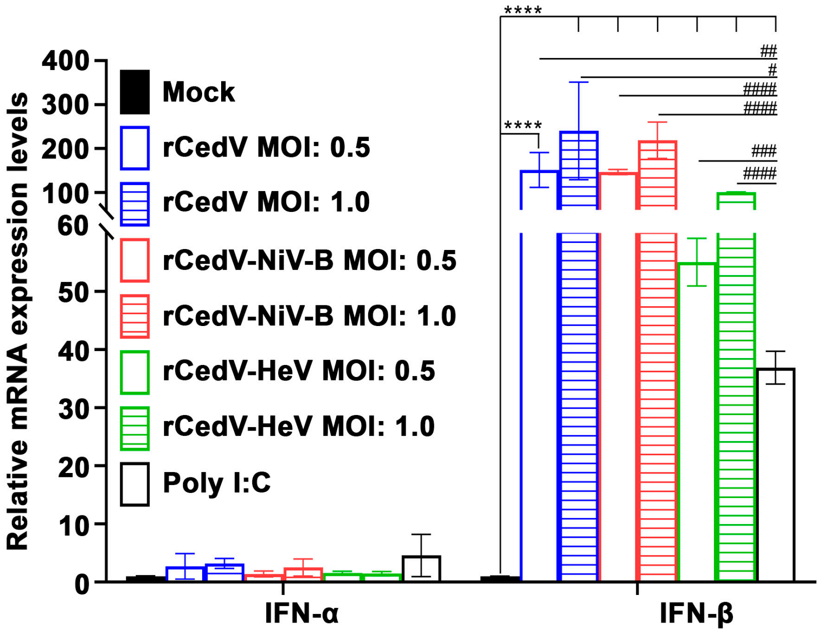

Figure 6. rCedV chimeras induce an IFN-β response. HeLa-CCL-2 cells were uninfected (Mock) or infected with rCedV-NiV-B, rCedV-HeV, or rCedV at a MOI of either 0.5 or 1.0 or transfected with Poly I:C (10 µg/mL) for 24 h. IFN-α and IFN-β mRNA expression were determined by qPCR. Fold changes were calculated relative to 18S ribosomal RNA and normalized to mock samples using the $2^{(-\Delta\Delta Ct)}$ method. These data represent mean ± standard deviation from two independent experiments, each performed in triplicate. Statistical analysis was performed with all samples in GraphPad Prism 9 by performing *t*-tests of each virus against Mock (asterisk *) or each virus against Poly I:C (hash #). **** $p < 0.0001$, # $p = 0.011$, ## $p = 0.012$, ### $p = 0.0001$ and #### $p < 0.0001$.

3.5. Plaque Reduction Neutralization Test (PRNT) of Chimeric Recombinant Cedar Viruses by Cross-Reactive NiV and HeV Specific Monoclonal Antibodies

To determine whether the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras could serve as suitable surrogate viruses for authentic NiV-B and HeV, respectively, the ability of the GFP expressing rCedV chimeras to be neutralized by a panel of well-characterized NiV/HeV cross-reactive neutralizing mAbs was conducted by PRNT. The antibody panel included the human mAb m102.4 specific to the G glycoprotein and the humanized mAb h5B3.1 and murine mAbs 12B2 and 1F5 specific to the F glycoprotein [44–46,71]. Figure 7 illustrates the dose-response neutralization profiles for each mAb against rCedV-NiV-B-GFP and rCedV-HeV-GFP performed at BSL-2 (Figure 7A). In addition, a set of parallel PRNTs using authentic NiV-B and HeV and both rCedV chimeras were also performed simultaneously in BSL-4 containment (Figure 7B,C). Each mAb tested neutralized the infectivity of rCedV-NiV-B-GFP and rCedV-HeV-GFP and NiV-B and HeV (Figure 7) with highly similar dose-response virus neutralization profiles. The mean 50% inhibitory concentrations (IC_{50}) for each of the mAbs against rCedV-NiV-B-GFP, rCedV-HeV-GFP, NiV-B, and HeV are summarized in Table 2. The most potent mAb was m102.4 with average IC_{50} values of ~20 ng/mL against rCedV-NiV-B-GFP and NiV-B, ~101 ng/mL against rCedV-HeV-GFP and ~50 ng/mL against HeV. The IC_{50} values for the mAbs tested here are within comparable ranges when compared to previous in vitro PRNT studies conducted with authentic NiV and HeV with the same set of cross-reactive neutralizing mAbs [43,44,46,66].

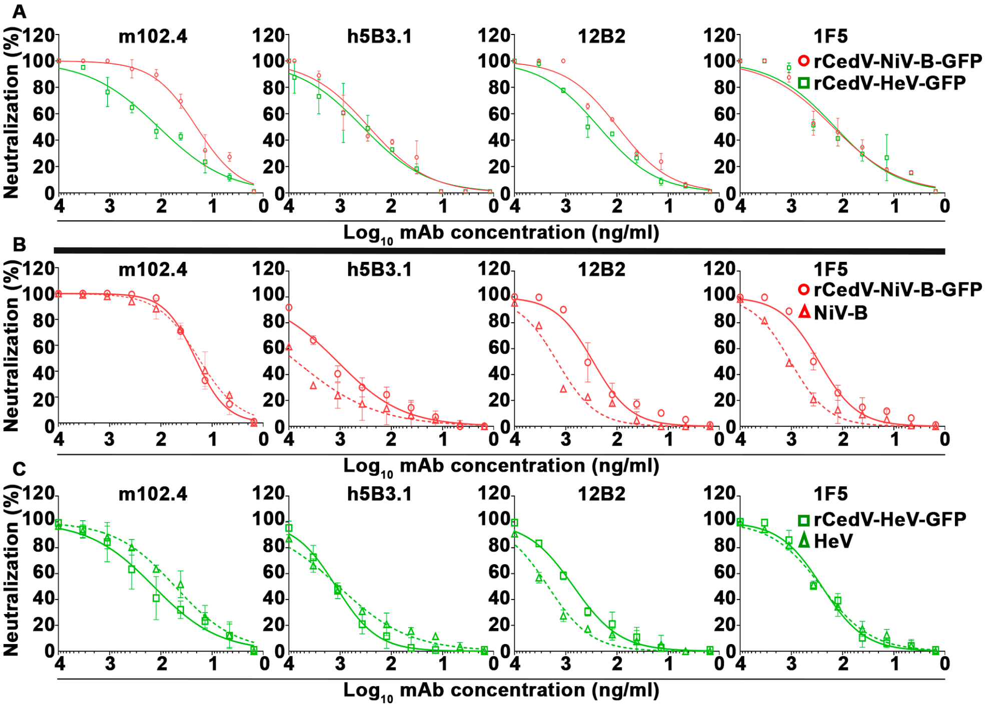

Figure 7. Neutralization of rCedV-NiV-B-GFP and rCedV-HeV-GFP by plaque reduction neutralization test (PRNT). Nine-point dose-response neutralization profiles for mAbs m102.4, h5B3.1, 12B2, and 1F5 against rCedV-NiV-B-GFP and rCedV-HeV-GFP at BSL-2 (**A**), at BSL-4 (**B**,**C**) and authentic NiV-B and HeV at BSL-4 (**B**,**C**). The diluted mAbs were incubated with an equal volume of either rCedV-NiV-B-GFP, rCedV-HeV-GFP, NiV-B, or HeV at an MOI of 0.0001 for 1 h at 37 °C, 5% CO_2. MOI was calculated for a tentative 10^6 cells/well and 0.4 mL virus and antibody mixture per well. Neutralization percent (%) was calculated based on PFU for each virus without mAb. These data represent mean ± standard deviation and are plotted as a non-linear regression curve fit with variable slope. BSL-2 studies are representative of two independent experiments, each performed in duplicate, and BSL-4 studies are from a single experiment performed in duplicate. The limit of detection for this assay was 50 PFU. Red circles and lines represent rCedV-NiV-B-GFP, red triangles and dashed lines represent NiV-B, green squares and lines represent rCedV-HeV-GFP, and green triangles and dashed lines represent HeV. The thick black line divides the BSL-2 PRNT from the BSL-4 PRNT.

Table 2. IC_{50} values of NiV and HeV cross-reactive monoclonal antibodies against henipavirus infection by PRNT.

Monoclonal Antibody (mAb)	IC_{50} (95% CI) (ng/mL)					
	BSL-2	BSL-4		BSL-2	BSL-4	
	rCedV-NiV-B-GFP	rCedV-NiV-B-GFP	NiV-B	rCedV-HeV-GFP	rCedV-HeV-GFP	HeV
m102.4	20.30 (16.58–24.99)	21.20 (18.80–23.89)	18.36 (15.21–22.17)	112.9 (82.82–154.1)	137.0 (89.09–208.8)	52.41 (39.32–70.07)
h5B3.1	274.8 (185.9–403.7)	1122 (813.6–1548)	7101 (4323–15,087)	363.5 (241.6–546.3)	1202 (975.2–1481)	1064 (827.4–1372)
12B2	130.0 (97.10–174.0)	291.9 (219.9–381.5)	1467 (1098–1925)	502.3 (377.4–658.3)	700.1 (570.0–857.0)	2202 (1692–2846)
1F5	153.8 (107.0–219.4)	289.4 (229.9–360.9)	1036 (812.3–1298)	140.6 (83.29–232.0)	253.2 (200.5–318.8)	259.8 (213.2–315.8)

Note: All IC_{50} values are calculated by a nonlinear fit model and are shown with 95% confidence intervals (95% CI). BSL-2 studies are representative of two independent experiments, each performed in duplicate, and BSL-4 studies are from a single experiment performed in duplicate.

3.6. Correlation Analysis of Plaque Reduction Neutralization Tests (PRNT) Using GFP Expressing Recombinant Cedar Virus Chimeras and Authentic NiV-B and HeV

To further evaluate if the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras are suitable surrogate virus platforms for authentic NiV-B and HeV antibody neutralization, a correlation analysis was performed. A Pearson correlation coefficient 'r' for each mAb was calculated by comparing the neutralization values derived from the rCedV chimeras BSL-2 PRNT with those of the NiV-B or HeV BSL-4 PRNT. The analysis indicated strong and statistically significant positive correlations between the two PRNTs (r values ranging from 0.86 to 0.99, *p* values from 0.005 to 0.0001) (Figure 8 and Table 3).

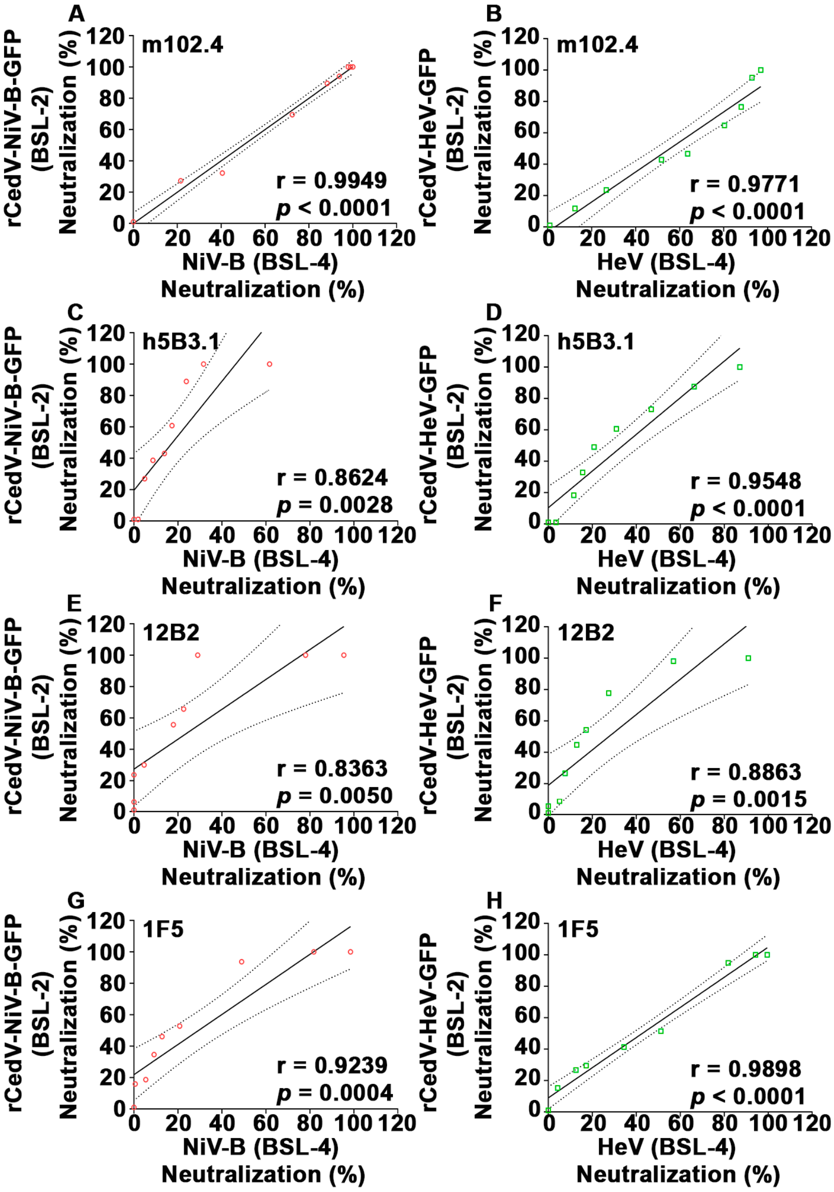

Figure 8. Correlation analysis of plaque reduction neutralization test (PRNT) neutralization values. Pearson correlation analysis of PRNT neutralization (%) values of rCedV-NiV-B-GFP versus NiV-B (**A**,**C**,**E**,**G**) and rCedV-HeV-GFP versus HeV (**B**,**D**,**F**,**H**) with mAbs m102.4, h5B3.1, 12B2 or 1F5. The Pearson correlation coefficient 'r,' *p*-value (two-tailed), linear regression line (solid lines), and 95% confidence intervals (dashed lines) are represented. Pearson's r $\geq$ 0.8 and *p*-value < 0.05 indicate a strong significant positive correlation.

Table 3. Correlation analysis of rCedV chimeric viruses in BSL-2 PRNT versus NiV-B and HeV BSL-4 PRNT assays.

Virus	Monoclonal Antibody (mAb)	Pearson's Correlation Coefficient (r)	Coefficient of Determination (R^2)	Significance (*p*)	95% Confidence Interval (CI)
rCedV-NiV-B-GFP vs. NiV-B	m102.4	0.9949	0.9898	<0.0001	0.9750–0.990
	h5B3.1	0.8624	0.7437	0.0028	0.4640–0.9706
	12B2	0.8363	0.6994	0.0050	0.3873–0.9647
	1F5	0.9239	0.8536	0.0004	0.6722–0.9842
rCedV-HeV-GFP vs. HeV	m102.4	0.9771	0.9547	<0.0001	0.8914–0.9953
	h5B3.1	0.9548	0.9117	<0.0001	0.7945–0.9907
	12B2	0.8863	0.7855	0.0015	0.5400–0.9760
	1F5	0.9898	0.9796	<0.0001	0.9503–0.9979

Note: Correlation analysis was performed with the neutralization values from Figure 7.

3.7. Establishment of a Fluorescence Reduction Neutralization Test (FRNT)

To further develop the utility of rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras as a surrogate platform for authentic NiV-B and HeV neutralization testing, we developed a high-throughput and quantitative assay based on the reduction in GFP fluorescent virus infection foci. Here, the virus neutralization efficacies of the same panel of mAbs used in the PRNT assays (Figure 7) were analyzed against rCedV-NiV-B-GFP and rCedV-HeV-GFP in a FRNT assay. As shown in Figure 9, the dose-response neutralization data were similar to those obtained by a PRNT (Figures 7A and 9). The IC_{50} values for each mAb against rCedV-NiV-B-GFP and rCedV-HeV-GFP are summarized in Table 4. The mAb m102.4 potently neutralized rCedV-NiV-B-GFP at an IC_{50} of 16.91 ng/mL, while rCedV-HeV-GFP was neutralized by m102.4 and 1F5 with similar potencies at IC_{50} values of 58.12 ng/mL and 50.16 ng/mL, respectively. These data reveal that rCedV-NiV-B-GFP and rCedV-HeV-GFP mAb neutralization values in a FRNT are comparable to those obtained in a PRNT (comparisons of Tables 2 and 4).

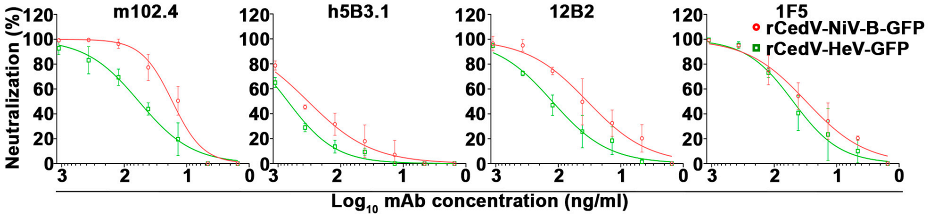

Figure 9. Neutralization profiles of NiV and HeV cross-reactive monoclonal antibodies by fluorescence reduction neutralization test (FRNT). Seven-point dose-response neutralization profiles for mAbs m102.4, h5B3.1, 12B2, and 1F5 against rCedV-NiV-B-GFP and rCedV-HeV-GFP. Neutralization percent (%) was calculated based on fluorescent foci for each virus without mAb. These data represent mean ± standard deviation from three independent experiments, each performed in triplicate. Data are plotted as non-linear regression curve fit with variable slope. The limit of detection for this assay was 50 fluorescent foci. Red circles and lines represent rCedV-NiV-B-GFP, and green squares and lines represent rCedV-HeV-GFP.

Table 4. IC_{50} values of NiV and HeV cross-reactive specific monoclonal antibodies against rCedV-NiV-B-GFP and rCedV-HeV-GFP infection by FRNT.

Monoclonal Antibody (mAb)	IC_{50} (95% CI) (ng/mL)	
	rCedV-NiV-B-GFP	rCedV-HeV-GFP
m102.4	16.91 (14.72–19.45)	58.12 (49.27–68.70)
h5B3.1	333.0 (255.5–439.9)	700.2 (620.0–798.8)
12B2	34.07 (24.88–46.48)	124.5 (98.17–157.2)
1F5	28.97 (22.86–36.65)	50.16 (40.95–61.07)

Note: All IC_{50} values are calculated by a nonlinear fit model from three independent experiments, each performed in triplicate, and are shown with 95% confidence intervals (95% CI).

3.8. Correlation Analysis of the Conventional PRNT and the FRNT Neutralization Assays

To further evaluate the FRNT assay as a suitable alternative virus neutralization assay to the standard PRNT, a correlation analysis was performed. A Pearson correlation analysis using the neutralization values obtained with each mAb against the rCedV-GFP chimeras by PRNT and FRNT assays was conducted (Figure 10 and Table 5), and a strong and significant positive correlation between the neutralization values obtained by PRNT versus the corresponding FRNT assay derived values was observed (r ≥ 0.9 and $p \leq 0.001$). Taken together, these data demonstrate that rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeric viruses are an ideal set of suitable surrogate viruses for authentic NiV-B and HeV for conducting a rapid FRNT-based assay for assessing NiV and HeV antibody neutralization.

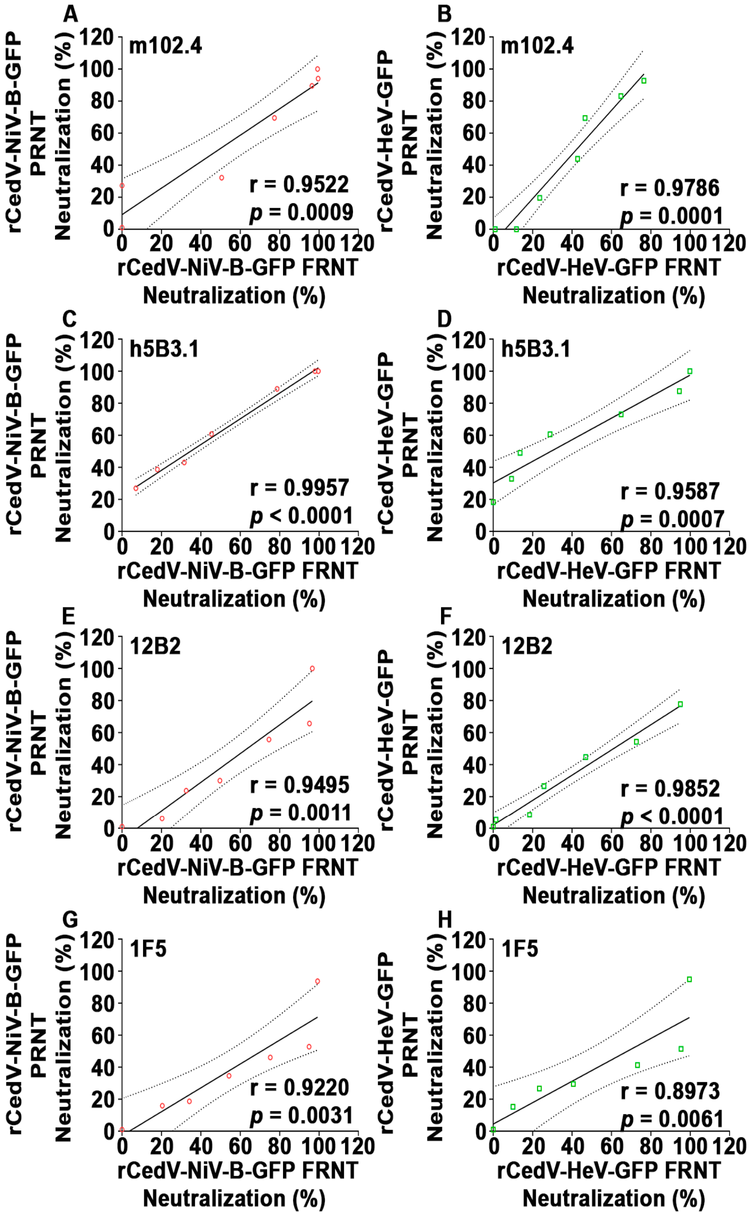

Figure 10. Correlation analysis of neutralization assays using the GFP expressing rCedV chimeric viruses. Pearson correlation analysis of neutralization (%) values from plaque reduction neutralization tests (PRNTs) (y-axes) and fluorescence reduction neutralization tests (FRNTs) (x-axes) performed with rCedV-NiV-B-GFP (**A**,**C**,**E**,**G**) and with rCedV-HeV-GFP (**B**,**D**,**F**,**H**) with mAbs m102.4, h5B3.1, 12B2 or 1F5. The Pearson correlation coefficient 'r,' p-value (two-tailed), linear regression line (solid lines), and 95% confidence intervals (dashed lines) are represented. Pearson's r ≥ 0.8 and p-value < 0.05 indicate a strong significant positive correlation.

Table 5. Correlation analysis of rCedV chimeric viruses in the PRNT versus FRNT assays.

Virus	Monoclonal Antibody (mAb)	Pearson's Correlation Coefficient (r)	Coefficient of Determination (R^2)	Significance (*p*)	95% Confidence Interval
rCedV-NiV-B-GFP	m102.4	0.9522	0.9067	0.0009	0.7038–0.9931
	h5B3.1	0.9957	0.9914	<0.0001	0.9698–0.9994
	12B2	0.9495	0.9016	0.0011	0.6894–0.9927
	1F5	0.9220	0.8501	0.0031	0.5527–0.9886
rCedV-HeV-GFP	m102.4	0.9786	0.9576	0.0001	0.8573–0.9970
	h5B3.1	0.9587	0.9191	0.0007	0.7397–0.9941
	12B2	0.9852	0.9707	<0.0001	0.8997–0.9979
	1F5	0.8973	0.8051	0.0061	0.4448–0.9849

Note: Correlation analysis was performed with the neutralization values from Figures 7A and 9.

3.9. Neutralization of rCedV-NiV-B-GFP and rCedV-HeV-GFP Using Henipavirus sG Immune Antisera

The utility of the rCedV-NiV-B-GFP and rCedV-HeV-GFP viruses in the FRNT assay for measuring immune serum neutralization was examined by testing sera from NiV-sG or HeV-sG immunized nonhuman primates (NHP) (rhesus macaques) and rabbits, respectively. NHP subjects were immunized with an equal mixture of recombinant NiV-M and NiV-B sG glycoproteins (see Section 2) [47]. Figure 11 shows the dose-response neutralization profiles for each NHP sera against the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras. The IC_{50} titers for each of the sera against rCedV-NiV-B-GFP and rCedV-HeV-GFP are summarized in Table 6. Sera collected on day 42 from subject 171269 had the highest neutralizing titer against rCedV-NiV-B-GFP (1:32,147) and rCedV-HeV-GFP (1:4157). In addition, we were also able to test sera collected on day 84 from 2 subjects, 171269 and 180227, and the IC_{50} titers are summarized in Table 6. Although still potently neutralizing, the 50% serum neutralization titers for animal 171269 declined 2-fold against rCedV-NiV-B-GFP to 1:16,101 and 4.8-fold against rCedV-HeV-GFP to 1:873.6. As expected, although the NHP sera were cross-neutralizing against rCedV-HeV-GFP the IC_{50} titers were much greater against the homologous immunized subjects (NiV).

Table 6. IC_{50} Anti-NiV G glycoprotein immunized rhesus macaque serum titers against rCedV-NiV-B-GFP and rCedV-HeV-GFP infection.

Animal ID	IC_{50} (95% CI) (Serum Titer)	
	rCedV-NiV-B-GFP	**rCedV-HeV-GFP**
171269 Day 42	1:32,147 (1:29,414–1:35,182)	1:4157 (1:3711–1:4658)
171269 Day 84	1:16,101 (1:12,288–1:21,024)	1:873.6 (1:809.4–1:941.3)
180274 Day 42	1:14,860 (1:14,018–1:15,761)	1:2704 (1:2375–1:3082)
180606 Day 42	1:19,480 (1:18,181–1:20,948)	1:3739 (1:3094–1:4542)
180227 Day 42	1:19,408 (1:17,817–1:21,158)	1:2048 (1:1668–1:2539)
180227 Day 84	1:7283 (1:6079–1:8739)	1:689.0 (1:499.2–1:904.4)

Note: All IC_{50} values are calculated by a nonlinear fit model from two independent experiments, each performed in triplicate, and are shown with 95% confidence intervals (95% CI).

We also analyzed sera from rabbits immunized with either HeV-sG or HeV-sG tetramer (HeV-sG_{tet}) (see Materials and Methods). The sG immune rabbit sera neutralized the infectivity of both rCedV-NiV-B-GFP and rCedV-HeV-GFP, as shown by the dose-response neutralization profiles in Figure 12. The IC_{50} titers for each of the rabbit sera against rCedV-NiV-B-GFP and rCedV-HeV-GFP are summarized in Table 7. The HeV-sG and HeV-sG_{tet} sera had similar and very high neutralizing titers of 1:65,820 and 1:59,457, respectively, against rCedV-HeV-GFP. Here, the HeV-sG-specific rabbit sera had higher cross-neutralizing heterologous titers against rCedV-NiV-B-GFP (HeV-sG: 1:6881 and HeV-sG_{tet}: 1:7367, respectively) with IC_{50} values again greater against the homologous immunized subjects (HeV), consistent with prior authentic NiV and HeV neutralization data derived from NiV-sG versus HeV-sG immunized cats [49].

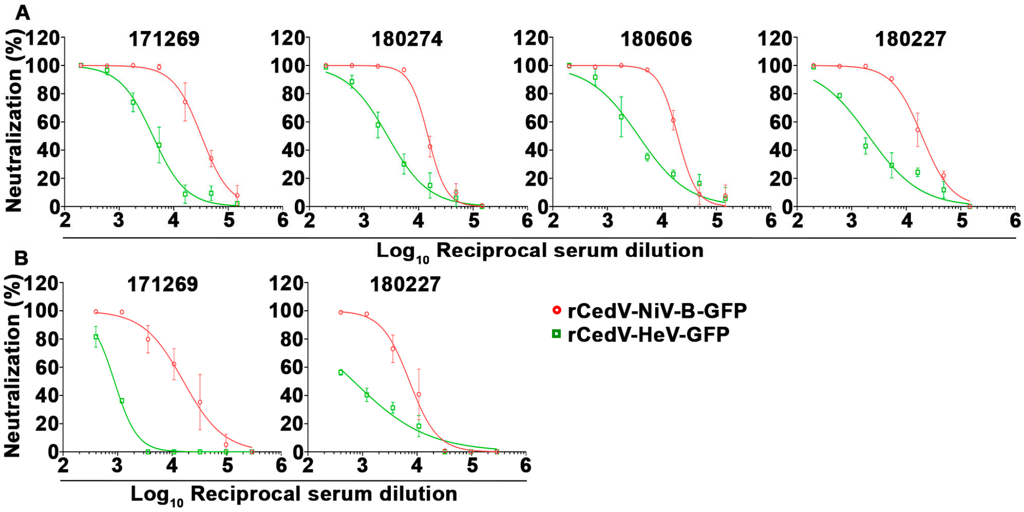

Figure 11. Neutralization profiles of NHP immunized sera against the GFP expressing rCedV chimeras by fluorescence reduction neutralization test (FRNT). Seven-point dose-response neutralization profiles of rhesus macaque sera collected on day 42 (**A**) and day 84 (**B**) post-immunization against rCedV-NiV-B-GFP and rCedV-HeV-GFP are shown. Neutralization percent (%) was calculated based on fluorescent foci for each virus without serum. These data represent mean ± standard deviation from two independent experiments, each performed in triplicate. Data are plotted as non-linear regression curve fit with variable slope. The limit of detection for this assay was 50 fluorescent foci. Animal ID numbers are 171269, 180274, 180606, and 180227. Red circles and lines represent rCedV-NiV-B-GFP, and green squares and lines represent rCedV-HeV-GFP.

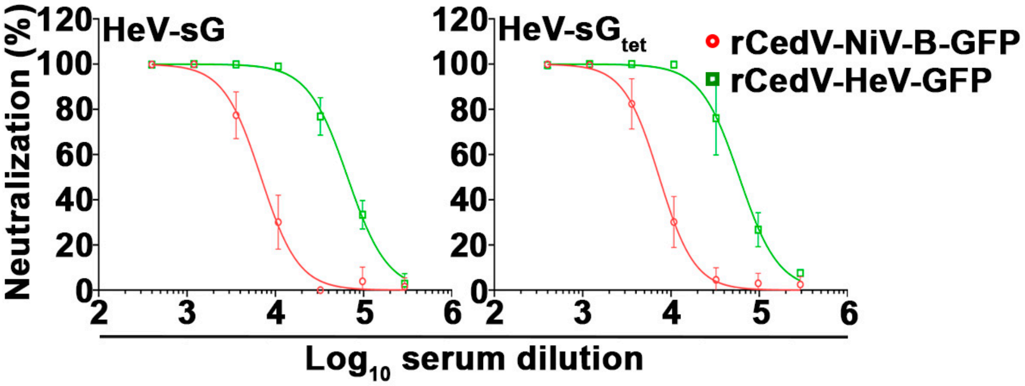

Figure 12. Neutralization profiles of rabbit immunized sera against rCedV chimeras expressing GFP. Seven-point dose-response neutralization profiles of HeV-sG (**left**) and HeV-sG$_{tet}$ (**right**) immunized sera against rCedV-NiV-B-GFP and rCedV-HeV-GFP are shown. Neutralization percent (%) was calculated based on fluorescent foci for each virus without serum. These data represent mean ± standard deviation from two independent experiments, each performed in triplicate. Data are plotted as non-linear regression curve fit with variable slope. The limit of detection for this assay was 50 fluorescent foci. Red circles and lines represent rCedV-NiV-B-GFP, and green squares and lines represent rCedV-HeV-GFP.

Table 7. IC_{50} rabbit serum titers against rCedV-NiV-B-GFP and rCedV-HeV-GFP infection.

Immunogen	**IC_{50} (95% CI) (Serum Titer)**	
	rCedV-NiV-B-GFP	**rCedV-HeV-GFP**
HeV-sG	1:6881 (1:6211–1:7616)	1:65,820 (1:61,407–1:70,409)
HeV-sG_{tet}	1:7367 (1:6642–1:8158)	1:59,457 (1:54,002–1:65,333)

Note: All IC_{50} values are calculated by a nonlinear fit model from two independent experiments, each performed in triplicate, and are shown with 95% confidence intervals (95% CI).

4. Discussion

There has been increased concern regarding respiratory pathogens such as NiV as a consequence of the COVID-19 pandemic [72]. The most recent significant outbreak of NiV-B, which occurred in Kerala, India, in 2018, had a case-fatality rate of 91% and revealed a high incidence of acute respiratory distress syndrome among those infected, correlating with nosocomial respiratory droplet-mediated human-to-human transmission by exposure to patient's coughing [73]. Experimentally, both NiV-M and NiV-B have been shown to cause lethal infection in NHPs when delivered as an aerosol [74,75], also the likely route of infection from deliberate release. Indeed, in 2020 the US Department of Health and Human Services (HHS) and Centers for Disease Control and Prevention (CDC) recommended that NiV be added to the list of Tier 1 Select Agents [76]. NiV-B has several characteristics enhancing its pandemic potential, including its respiratory tissue tropism; human susceptibility to infection; person-to-person transmission capability; and its potential to mutate, and the emergence of a human-adapted strain in South Asia could lead to the rapid spread of infection [77]. NiV and HeV have been important targets for vaccine development for more than 20 years, and these efforts have recently intensified [22,25].

In the present study, the rCedV reverse genetics platform [20,21] was modified by employing an optimized T7 promoter ($T7_{opt}$) and the self-cleaving HHRbzA in the pOLTV5-rCedV antigenome plasmid, which improved the rescue efficiency of rCedVs. A similar strategy was used to rescue a number of other single-stranded, negative-sense RNA viruses, including NiV [36,78–82]. We then expanded the utility of the rCedV platform by replacing the coding sequences of the CedV F and G glycoproteins with their NiV-B or HeV counterparts to generate a panel of non-reporter gene and reporter gene encoding versions of rCedV-NiV-B and rCedV-HeV chimeric viruses. Interestingly, all chimeras appeared more fusogenic than those observed in rCedV-infected cells, and a similar phenotype was observed by Yeo et al., where cell fusion levels of CedV were consistently lower than NiV in transfected HEK293T cells [83]. All rCedV chimeras expressed the heterologous envelope glycoproteins in infected cells, replicated similarly in comparison to rCedV, and infection tropism was specific for ephrin-B2 and ephrin-B3 as entry receptors.

Previous in vitro cell-based assays demonstrated that CedV and rCedV induced a robust IFN-β response [2,20], and CedV infection stimulated the expression of interferon response genes, such as *IFNA7*, *CCL5*, *STAT1*, and *STAT2* in primary hamster endothelial cells [11]. Here, the rCedV chimeric viruses also induced the expression of IFN-β mRNA in an infection dose-dependent manner to comparable levels observed with rCedV infection and Poly I:C treatment.

Several surrogate NiV neutralization assays using recombinant Vesicular Stomatitis Virus (VSV) as a replication-incompetent pseudovirus with a deletion of the VSV G glycoprotein gene have been developed as a tool to measure NiV neutralization at BSL-2 containment [84–86]. The VSV-based pseudotype virus particle system has also been utilized with the HeV and GhV envelope glycoproteins for measuring neutralization [87]. Preparation of VSV pseudoviruses involves the budding progeny virions from cells that are transiently expressing henipavirus F and G glycoproteins which can sometimes be technically challenging to produce large quantities of pseudovirus stocks with reproducible characteristics. We previously found similar challenges in developing a retrovirus-based pseudotyped virus assay system that also required significant optimization [88]. These replication-incompetent pseudovirus assays are sensitive and have a high correlation when samples are scored as either positive or negative for henipavirus neutralizing antibody. However, specific mAb neutralization potencies or the virus-neutralizing titers of sera against NiV using VSV pseudotypes, as examples, are often quite different in comparison to sera titers obtained using authentic NiV [84–86].

Here, we sought to develop an improved surrogate neutralization assay system for NiV and HeV using rCedV as replication-competent chimeric viruses. The rCedV chimeric viruses developed and characterized here can be readily produced and stored in large quantities and are an authentic replication-competent henipavirus platform that can be used to study NiV and HeV F and G glycoprotein-mediated infection and also as surrogate viruses for authentic NiV and HeV in neutralization assays without the requirement for BSL-4 containment. Indeed, similar dose-response neutralization data and comparable IC_{50} concentrations of well-characterized NiV and HeV cross-reactive mAbs were observed between PRNTs using rCedV-NiV-B-GFP and NiV-B or rCedV-HeV-GFP and HeV. A strong and significant correlation between the overall neutralization values of the BSL-2 and BSL-4 PRNTs (Figure 8 and Table 3) validated the utility of the rCedV chimeric platform as suitable surrogate viruses for authentic NiV and HeV by PRNT.

We also expanded the utility of these novel reporter genes encoding rCedV chimeric viruses by developing a rapid and high-throughput fluorescence-based neutralization assay, FRNT. In contrast to the PRNT, which is the current gold standard for determining the presence of neutralizing antibodies and measuring the neutralizing titer in henipavirus-specific antisera, the FRNT (i) is high-throughput and allows for more samples to be assayed with more replicates in a 96-well plate format, (ii) requires smaller sample volumes, (iii) is less time consuming taking less than 36 h from infection to assay completion, and (iv) reduces the requirement for other reagents such as luciferase substrate. The rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeric viruses were used to assess the neutralization potencies of mAbs by FRNT assay, and the neutralization values at each mAb concentration obtained by FRNT were found to be highly correlated with those values obtained by PRNT (Table 5). We further evaluated the utility of the rCedV-NiV-B-GFP and rCedV-HeV-GFP FRNT by measuring the neutralization activities of henipavirus sG immunized NHP sera and rabbit sera. The NiV-sG immunized NHP sera, and the HeV-sG immunized rabbit sera were both cross-neutralizing to rCedV-NiV-B-GFP and rCedV-HeV-GFP, with higher homotypic serum neutralization titers as expected. The rabbit HeV-sG immune sera exhibited greater heterologous neutralization titers in comparison to the NHP NiV-sG immune sera, which was also consistent with neutralization data derived from NiV-sG versus HeV-sG immunized cats against authentic NiV-M and HeV [49].

In summary, a surrogate henipavirus-based system for NiV and HeV using the rCedV platform suitable for use at BSL-2 containment has been developed and well-characterized. These rCedV chimeras can serve as useful tools to study NiV and HeV entry, membrane fusion mechanisms, and F and G glycoprotein interactions and aid in the discovery and development of henipavirus countermeasures. More importantly, the specificity and utility of the rCedV-NiV-B-GFP and rCedV-HeV-GFP viruses as a surrogate neutralization assay for authentic NiV and HeV to evaluate the neutralization potential of mAbs and NiV/HeV specific antisera has also been demonstrated. The rCedV chimeras will reduce the cost and technical challenges of the high-containment environment, particularly when large numbers of serum samples derived from NiV or HeV vaccine development programs will require testing and quantitation.

5. Patents

C.C.B. and M.A. are United States federal employees and co-inventors on US and foreign patents pertaining to Recombinant Cedar Virus Chimeras, whose assignees are the United States as represented by the Henry M Jackson Foundation for the Advancement of Military Medicine, Inc. (Bethesda, MD, USA).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/v15051077/s1, Table S1: Mutations identified by genome sequencing of recombinant Cedar virus chimeras.

Author Contributions: Conceptualization, M.A. and C.C.B.; Methodology, M.A., C.C.B., R.Y., L.Y., V.B., B.N.A., A.B., F.M., R.Z.C. and K.A.B.-L.; Formal Analysis, M.A., A.S.D., R.W.C., K.A.B.-L., B.N.A., A.B., F.M., R.Z.C., T.W.G. and C.C.B.; Writing—Original Draft Preparation, M.A., A.S.D. and C.C.B.; Writing—Review and Editing, M.A., A.S.D., K.A.B.-L. and C.C.B.; Funding Acquisition, T.W.G. and C.C.B. C.C.B. revised and prepared the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: Research support was provided by National Institutes of Health grant U19AI142764 to C.C.B. and CEPI (Coalition for Epidemic Preparedness Innovations (CEPI)) to C.C.B. Operations support of the Galveston National Laboratory was funded by NIAID/NIH grant UC7AI094660 and support by Naval Medical Research Center was funded by Navy WUN A1417.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The views expressed in the manuscript are solely those of the authors, and they do not represent official views or opinions of the Department of Defense, Department of the Navy, the U.S. Government, the Uniformed Services University, nor the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. Several of the authors are U.S. Government employees. This work was prepared as part of their official duties. Title 17 U.S.C. § 105 provides that 'Copyright protection under this title is not available for any work of the United States Government.' Title 17 U.S.C. §101 defines U.S. Government work as work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. We thank the CSIRO Australian Animal Health Laboratory for providing the polyclonal CedV-N sera. The authors thank Auro Vaccines, LLC, for providing the rhesus macaque NiV-M and NiV-B sG immune sera.

Conflicts of Interest: C.C.B is a United States federal employee and co-inventor on United States and foreign patents pertaining to soluble forms of Nipah virus and Hendra virus G glycoproteins, and Cedar Virus and Methods of Use, whose assignees are the United States as represented by the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (Bethesda, MD, USA). All other authors declare no conflict of interest.

References

1. Wang, L.-F.; Mackenzie, J.S.; Broder, C.C. Henipaviruses. In *Fields Virology*; Knipe, D.M., Howley, P.M., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2013; Volume 1, pp. 1070–1085.
2. Marsh, G.A.; de Jong, C.; Barr, J.A.; Tachedjian, M.; Smith, C.; Middleton, D.; Yu, M.; Todd, S.; Foord, A.J.; Haring, V.; et al. Cedar virus: A novel Henipavirus isolated from Australian bats. *PLoS Pathog.* **2012**, *8*, e1002836. [CrossRef] [PubMed]
3. Lee, S.H.; Kim, K.; Kim, J.; No, J.S.; Park, K.; Budhathoki, S.; Lee, S.H.; Lee, J.; Cho, S.H.; Cho, S.; et al. Discovery and Genetic Characterization of Novel Paramyxoviruses Related to the Genus Henipavirus in Crocidura Species in the Republic of Korea. *Viruses* **2021**, *13*, 2020. [CrossRef] [PubMed]
4. Zhang, X.A.; Li, H.; Jiang, F.C.; Zhu, F.; Zhang, Y.F.; Chen, J.J.; Tan, C.W.; Anderson, D.E.; Fan, H.; Dong, L.Y.; et al. A Zoonotic Henipavirus in Febrile Patients in China. *N. Engl. J. Med.* **2022**, *387*, 470–472. [CrossRef]
5. Drexler, J.F.; Corman, V.M.; Muller, M.A.; Maganga, G.D.; Vallo, P.; Binger, T.; Gloza-Rausch, F.; Rasche, A.; Yordanov, S.; Seebens, A.; et al. Bats host major mammalian paramyxoviruses. *Nat. Commun.* **2012**, *3*, 796. [CrossRef] [PubMed]
6. Wu, Z.; Yang, L.; Yang, F.; Ren, X.; Jiang, J.; Dong, J.; Sun, L.; Zhu, Y.; Zhou, H.; Jin, Q. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg. Infect. Dis.* **2014**, *20*, 1064–1066. [CrossRef]
7. Vanmechelen, B.; Meurs, S.; Horemans, M.; Loosen, A.; Joly Maes, T.; Laenen, L.; Vergote, V.; Koundouno, F.R.; Magassouba, N.; Konde, M.K.; et al. The characterization of multiple novel paramyxoviruses highlights the diverse nature of the subfamily Orthoparamyxovirinae. *Virus Evol.* **2022**, *8*, veac061. [CrossRef]
8. Madera, S.; Kistler, A.; Ranaivoson, H.C.; Ahyong, V.; Andrianiaina, A.; Andry, S.; Raharinosy, V.; Randriambolamanantsoa, T.H.; Ravelomanantsoa, N.A.F.; Tato, C.M.; et al. Discovery and Genomic Characterization of a Novel Henipavirus, Angavokely Virus, from Fruit Bats in Madagascar. *J. Virol.* **2022**, *96*, e0092122. [CrossRef]
9. Eaton, B.T.; Broder, C.C.; Middleton, D.; Wang, L.F. Hendra and Nipah viruses: Different and dangerous. *Nat. Rev. Microbiol.* **2006**, *4*, 23–35. [CrossRef]

10. Geisbert, T.W.; Feldmann, H.; Broder, C.C. Animal Challenge Models of Henipavirus Infection and Pathogenesis. In *Current Topics in Microbiology and Immunology*, 1st ed.; Lee, B., Rota, P., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; Volume 359, pp. 153–177.
11. Schountz, T.; Campbell, C.; Wagner, K.; Rovnak, J.; Martellaro, C.; DeBuysscher, B.L.; Feldmann, H.; Prescott, J. Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **2019**, *11*, 291. [CrossRef]
12. Basler, C.F. Nipah and hendra virus interactions with the innate immune system. In *Current Topics in Microbiology and Immunology*, 1st ed.; Lee, B., Rota, P., Eds.; Springer: Berlin/Heidelberg, Germany, 2012; Volume 359, pp. 123–152.
13. Marsh, G.A.; Wang, L.F. Hendra and Nipah viruses: Why are they so deadly? *Curr. Opin. Virol.* **2012**, *2*, 242–247. [CrossRef]
14. Lieu, K.G.; Marsh, G.A.; Wang, L.F.; Netter, H.J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antivir. Res.* **2015**, *124*, 69–76. [CrossRef] [PubMed]
15. Satterfield, B.A.; Borisevich, V.; Foster, S.L.; Rodriguez, S.E.; Cross, R.W.; Fenton, K.A.; Agans, K.N.; Basler, C.F.; Geisbert, T.W.; Mire, C.E. Antagonism of STAT1 by Nipah virus P gene products modulates disease course but not lethal outcome in the ferret model. *Sci. Rep.* **2019**, *9*, 16710. [CrossRef] [PubMed]
16. Satterfield, B.A.; Cross, R.W.; Fenton, K.A.; Agans, K.N.; Basler, C.F.; Geisbert, T.W.; Mire, C.E. The immunomodulating V and W proteins of Nipah virus determine disease course. *Nat. Commun.* **2015**, *6*, 7483. [CrossRef]
17. Satterfield, B.A.; Cross, R.W.; Fenton, K.A.; Borisevich, V.; Agans, K.N.; Deer, D.J.; Graber, J.; Basler, C.F.; Geisbert, T.W.; Mire, C.E. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J. Virol.* **2016**, *90*, 6326–6343. [CrossRef]
18. Yoneda, M.; Guillaume, V.; Sato, H.; Fujita, K.; Georges-Courbot, M.C.; Ikeda, F.; Omi, M.; Muto-Terao, Y.; Wild, T.F.; Kai, C. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS ONE* **2010**, *5*, e12709. [CrossRef] [PubMed]
19. *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th ed.; HHS Publication No. (CDC) 300859; US Government Printing Office: Washington, DC, USA, 2020; pp. 248–250.
20. Laing, E.D.; Amaya, M.; Navaratnarajah, C.K.; Feng, Y.R.; Cattaneo, R.; Wang, L.F.; Broder, C.C. Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol. J.* **2018**, *15*, 56. [CrossRef]
21. Laing, E.D.; Navaratnarajah, C.K.; Cheliout Da Silva, S.; Petzing, S.R.; Xu, Y.; Sterling, S.L.; Marsh, G.A.; Wang, L.F.; Amaya, M.; Nikolov, D.B.; et al. Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 20707–20715. [CrossRef]
22. Amaya, M.; Broder, C.C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu. Rev. Virol.* **2020**, *7*, 447–473. [CrossRef]
23. Mehand, M.S.; Al-Shorbaji, F.; Millett, P.; Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antivir. Res.* **2018**, *159*, 63–67.
24. Priority Diseases. 2021. Available online: https://cepi.net/research_dev/priority-diseases/ (accessed on 15 December 2021).
25. Brende, B.; Farrar, J.; Gashumba, D.; Moedas, C.; Mundel, T.; Shiozaki, Y.; Vardhan, H.; Wanka, J.; Rottingen, J.A. CEPI-a new global R&D organisation for epidemic preparedness and response. *Lancet* **2017**, *389*, 233–235. [CrossRef]
26. Middleton, D.; Pallister, J.; Klein, R.; Feng, Y.R.; Haining, J.; Arkinstall, R.; Frazer, L.; Huang, J.A.; Edwards, N.; Wareing, M.; et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg. Infect. Dis.* **2014**, *20*, 372–379. [CrossRef] [PubMed]
27. Geisbert, T.W.; Bobb, K.; Borisevich, V.; Geisbert, J.B.; Agans, K.N.; Cross, R.W.; Prasad, A.N.; Fenton, K.A.; Yu, H.; Fouts, T.R.; et al. A single dose investigational subunit vaccine for human use against Nipah virus and Hendra virus. *NPJ Vaccines* **2021**, *6*, 23. [CrossRef] [PubMed]
28. Playford, E.G.; Munro, T.; Mahler, S.M.; Elliott, S.; Gerometta, M.; Hoger, K.L.; Jones, M.L.; Griffin, P.; Lynch, K.D.; Carroll, H.; et al. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: A first-in-human, randomised, controlled, phase 1 study. *Lancet Infect. Dis.* **2020**, *20*, 445–454. [CrossRef]
29. Bossart, K.N.; Fusco, D.L.; Broder, C.C. Paramyxovirus entry. *Adv. Exp. Med. Biol.* **2013**, *790*, 95–127. [CrossRef] [PubMed]
30. Bishop, K.A.; Stantchev, T.S.; Hickey, A.C.; Khetawat, D.; Bossart, K.N.; Krasnoperov, V.; Gill, P.; Feng, Y.R.; Wang, L.; Eaton, B.T.; et al. Identification of hendra virus G glycoprotein residues that are critical for receptor binding. *J. Virol.* **2007**, *81*, 5893–5901. [CrossRef]
31. Bonaparte, M.I.; Dimitrov, A.S.; Bossart, K.N.; Crameri, G.; Mungall, B.A.; Bishop, K.A.; Choudhry, V.; Dimitrov, D.S.; Wang, L.F.; Eaton, B.T.; et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 10652–10657. [CrossRef]
32. Negrete, O.A.; Levroney, E.L.; Aguilar, H.C.; Bertolotti-Ciarlet, A.; Nazarian, R.; Tajyar, S.; Lee, B. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* **2005**, *436*, 401–405. [CrossRef]
33. Negrete, O.A.; Wolf, M.C.; Aguilar, H.C.; Enterlein, S.; Wang, W.; Muhlberger, E.; Su, S.V.; Bertolotti-Ciarlet, A.; Flick, R.; Lee, B. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog.* **2006**, *2*, e7. [CrossRef]
34. Pryce, R.; Azarm, K.; Rissanen, I.; Harlos, K.; Bowden, T.A.; Lee, B. A key region of molecular specificity orchestrates unique ephrin-B1 utilization by Cedar virus. *Life Sci. Alliance* **2020**, *3*, e201900578. [CrossRef]
35. Navaratnarajah, C.K.; Generous, A.R.; Yousaf, I.; Cattaneo, R. Receptor-mediated cell entry of Paramyxoviruses: Mechanisms, and consequences for tropism and pathogenesis. *J. Biol. Chem.* **2020**, *295*, 2771–2786. [CrossRef]

36. Yun, T.; Park, A.; Hill, T.E.; Pernet, O.; Beaty, S.M.; Juelich, T.L.; Smith, J.K.; Zhang, L.; Wang, Y.E.; Vigant, F.; et al. Efficient reverse genetics reveals genetic determinants of budding and fusogenic differences between Nipah and Hendra viruses and enables real-time monitoring of viral spread in small animal models of henipavirus infection. *J. Virol.* **2015**, *89*, 1242–1253. [CrossRef] [PubMed]
37. Amaya, M.; Cheng, H.; Borisevich, V.; Navaratnarajah, C.K.; Cattaneo, R.; Cooper, L.; Moore, T.W.; Gaisina, I.N.; Geisbert, T.W.; Rong, L.; et al. A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antivir. Res.* **2021**, *193*, 105084. [CrossRef] [PubMed]
38. Buchholz, U.J.; Finke, S.; Conzelmann, K.K. Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *J. Virol.* **1999**, *73*, 251–259. [CrossRef] [PubMed]
39. Bossart, K.N.; Geisbert, T.W.; Feldmann, H.; Zhu, Z.; Feldmann, F.; Geisbert, J.B.; Yan, L.; Feng, Y.R.; Brining, D.; Scott, D.; et al. A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Sci. Transl. Med.* **2011**, *3*, 105ra103. [CrossRef]
40. Bossart, K.N.; Zhu, Z.; Middleton, D.; Klippel, J.; Crameri, G.; Bingham, J.; McEachern, J.A.; Green, D.; Hancock, T.J.; Chan, Y.P.; et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS Pathog.* **2009**, *5*, e1000642. [CrossRef]
41. Di Rubbo, A.; McNabb, L.; Klein, R.; White, J.R.; Colling, A.; Dimitrov, D.S.; Broder, C.C.; Middleton, D.; Lunt, R.A. Optimization and diagnostic evaluation of monoclonal antibody-based blocking ELISA formats for detection of neutralizing antibodies to Hendra virus in mammalian sera. *J. Virol. Methods* **2019**, *274*, 113731. [CrossRef]
42. Geisbert, T.W.; Mire, C.E.; Geisbert, J.B.; Chan, Y.P.; Agans, K.N.; Feldmann, F.; Fenton, K.A.; Zhu, Z.; Dimitrov, D.S.; Scott, D.P.; et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci. Transl. Med.* **2014**, *6*, 242ra282. [CrossRef]
43. Zhu, Z.; Bossart, K.N.; Bishop, K.A.; Crameri, G.; Dimitrov, A.S.; McEachern, J.A.; Feng, Y.; Middleton, D.; Wang, L.F.; Broder, C.C.; et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J. Infect. Dis.* **2008**, *197*, 846–853. [CrossRef]
44. Dang, H.V.; Chan, Y.P.; Park, Y.J.; Snijder, J.; Da Silva, S.C.; Vu, B.; Yan, L.; Feng, Y.R.; Rockx, B.; Geisbert, T.W.; et al. An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat. Struct. Mol. Biol.* **2019**, *26*, 980–987. [CrossRef]
45. Mire, C.E.; Chan, Y.P.; Borisevich, V.; Cross, R.W.; Yan, L.; Agans, K.N.; Dang, H.V.; Veesler, D.; Fenton, K.A.; Geisbert, T.W.; et al. A Cross-Reactive Humanized Monoclonal Antibody Targeting Fusion Glycoprotein Function Protects Ferrets against Lethal Nipah Virus and Hendra Virus Infection. *J. Infect. Dis.* **2020**, *221* (Suppl. S4), S471–S479. [CrossRef]
46. Dang, H.V.; Cross, R.W.; Borisevich, V.; Bornholdt, Z.A.; West, B.R.; Chan, Y.P.; Mire, C.E.; Da Silva, S.C.; Dimitrov, A.S.; Yan, L.; et al. Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat. Struct. Mol. Biol.* **2021**, *28*, 426–434. [CrossRef] [PubMed]
47. Wang, Z.; Amaya, M.; Addetia, A.; Dang, H.V.; Reggiano, G.; Yan, L.; Hickey, A.C.; DiMaio, F.; Broder, C.C.; Veesler, D. Architecture and antigenicity of the Nipah virus attachment glycoprotein. *Science* **2022**, *375*, 1373–1378. [CrossRef] [PubMed]
48. Bossart, K.N.; Crameri, G.; Dimitrov, A.S.; Mungall, B.A.; Feng, Y.R.; Patch, J.R.; Choudhary, A.; Wang, L.F.; Eaton, B.T.; Broder, C.C. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J. Virol.* **2005**, *79*, 6690–6702. [CrossRef] [PubMed]
49. Mungall, B.A.; Middleton, D.; Crameri, G.; Bingham, J.; Halpin, K.; Russell, G.; Green, D.; McEachern, J.; Pritchard, L.I.; Eaton, B.T.; et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J. Virol.* **2006**, *80*, 12293–12302. [CrossRef]
50. McEachern, J.A.; Bingham, J.; Crameri, G.; Green, D.J.; Hancock, T.J.; Middleton, D.; Feng, Y.R.; Broder, C.C.; Wang, L.F.; Bossart, K.N. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* **2008**, *26*, 3842–3852. [CrossRef] [PubMed]
51. Pallister, J.; Middleton, D.; Wang, L.F.; Klein, R.; Haining, J.; Robinson, R.; Yamada, M.; White, J.; Payne, J.; Feng, Y.R.; et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine* **2011**, *29*, 5623–5630. [CrossRef]
52. Bossart, K.N.; Rockx, B.; Feldmann, F.; Brining, D.; Scott, D.; Lacasse, R.; Geisbert, J.B.; Feng, Y.R.; Chan, Y.P.; Hickey, A.C.; et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci. Transl. Med.* **2012**, *4*, 146ra107. [CrossRef] [PubMed]
53. Pallister, J.A.; Klein, R.; Arkinstall, R.; Haining, J.; Long, F.; White, J.R.; Payne, J.; Feng, Y.R.; Wang, L.F.; Broder, C.C.; et al. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol. J.* **2013**, *10*, 237. [CrossRef]
54. Mire, C.E.; Geisbert, J.B.; Agans, K.N.; Feng, Y.R.; Fenton, K.A.; Bossart, K.N.; Yan, L.; Chan, Y.P.; Broder, C.C.; Geisbert, T.W. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J. Virol.* **2014**, *88*, 4624–4631. [CrossRef]
55. Yan, L.; Sterling, S.L.; Fusco, D.L.; Chan, Y.P.; Xu, K.; Laing, E.D.; Broder, C.C. Recombinant Soluble Henipavirus Glycoprotein Preparation. *Methods Mol. Biol.* 2021, *in press*.

56. Cheliout Da Silva, S.; Yan, L.; Dang, H.V.; Xu, K.; Epstein, J.H.; Veesler, D.; Broder, C.C. Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **2021**, *13*, 517. [CrossRef]
57. Li, P.E.; Lo, C.C.; Anderson, J.J.; Davenport, K.W.; Bishop-Lilly, K.A.; Xu, Y.; Ahmed, S.; Feng, S.; Mokashi, V.P.; Chain, P.S. Enabling the democratization of the genomics revolution with a fully integrated web-based bioinformatics platform. *Nucleic Acids Res* **2017**, *45*, 67–80. [CrossRef] [PubMed]
58. Andrews, S. FastQC: A Quality Control Tool for High throughput Sequence Data. Available online: http://www.bioinformatics.babraham.ac.uk/projects/fastqc (accessed on 1 June 2020).
59. Bushnell, B. BBMap: A Fast, Accurate, Splice-Aware Aligner. United States. Available online: https://www.osti.gov/servlets/purl/1241166 (accessed on 1 February 2020).
60. Bankevich, A.; Nurk, S.; Antipov, D.; Gurevich, A.A.; Dvorkin, M.; Kulikov, A.S.; Lesin, V.M.; Nikolenko, S.I.; Pham, S.; Prjibelski, A.D.; et al. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **2012**, *19*, 455–477. [CrossRef] [PubMed]
61. Buchfink, B.; Xie, C.; Huson, D.H. Fast and sensitive protein alignment using DIAMOND. *Nat. Methods* **2015**, *12*, 59–60. [CrossRef] [PubMed]
62. Grubaugh, N.D.; Gangavarapu, K.; Quick, J.; Matteson, N.L.; De Jesus, J.G.; Main, B.J.; Tan, A.L.; Paul, L.M.; Brackney, D.E.; Grewal, S.; et al. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *Genome Biol.* **2019**, *20*, 8. [CrossRef]
63. Weingartl, H.M.; Berhane, Y.; Caswell, J.L.; Loosmore, S.; Audonnet, J.C.; Roth, J.A.; Czub, M. Recombinant nipah virus vaccines protect pigs against challenge. *J. Virol.* **2006**, *80*, 7929–7938. [CrossRef]
64. Doyle, M.P.; Kose, N.; Borisevich, V.; Binshtein, E.; Amaya, M.; Nagel, M.; Annand, E.J.; Armstrong, E.; Bombardi, R.; Dong, J.; et al. Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep.* **2021**, *36*, 109628. [CrossRef]
65. Wang, Z.; Dang, H.V.; Amaya, M.; Xu, Y.; Yin, R.; Yan, L.; Hickey, A.C.; Annand, E.J.; Horsburgh, B.A.; Reid, P.A.; et al. Potent monoclonal antibody-mediated neutralization of a divergent Hendra virus variant. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2122769119. [CrossRef]
66. Mire, C.E.; Satterfield, B.A.; Geisbert, J.B.; Agans, K.N.; Borisevich, V.; Yan, L.; Chan, Y.P.; Cross, R.W.; Fenton, K.A.; Broder, C.C.; et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci. Rep.* **2016**, *6*, 30916. [CrossRef]
67. Chan, Y.P.; Yan, L.; Feng, Y.R.; Broder, C.C. Preparation of recombinant viral glycoproteins for novel and therapeutic antibody discovery. *Methods Mol. Biol.* **2009**, *525*, 31–58. [CrossRef]
68. Tsutsumi, H.; Takeuchi, R.; Ohsaki, M.; Seki, K.; Chiba, S. Respiratory syncytial virus infection of human respiratory epithelial cells enhances inducible nitric oxide synthase gene expression. *J. Leukoc. Biol.* **1999**, *66*, 99–104. [CrossRef]
69. Shaw, M.L.; Cardenas, W.B.; Zamarin, D.; Palese, P.; Basler, C.F. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J. Virol.* **2005**, *79*, 6078–6088. [CrossRef] [PubMed]
70. Nicot, N.; Hausman, J.F.; Hoffmann, L.; Evers, D. Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *J. Exp. Bot.* **2005**, *56*, 2907–2914. [CrossRef] [PubMed]
71. Xu, K.; Rockx, B.; Xie, Y.; Debuysscher, B.L.; Fusco, D.L.; Zhu, Z.; Chan, Y.P.; Xu, Y.; Luu, T.; Cer, R.Z.; et al. Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog.* **2013**, *9*, e1003684. [CrossRef] [PubMed]
72. Thiagarajan, K. Why the World Should Be More Than a Bit Worried about India's Nipah Virus Outbreak. Available online: https://www.npr.org/sections/goatsandsoda/2021/09/12/1035571714/why-the-world-should-be-more-than-a-bit-worried-about-indias-nipah-virus-outbrea (accessed on 5 October 2021).
73. Arunkumar, G.; Chandni, R.; Mourya, D.T.; Singh, S.K.; Sadanandan, R.; Sudan, P.; Bhargava, B. Outbreak investigation of Nipah Virus Disease in Kerala, India, 2018. *J. Infect. Dis.* **2019**, *219*, 1867–1878. [CrossRef]
74. Cong, Y.; Lentz, M.R.; Lara, A.; Alexander, I.; Bartos, C.; Bohannon, J.K.; Hammoud, D.; Huzella, L.; Jahrling, P.B.; Janosko, K.; et al. Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS Negl. Trop. Dis.* **2017**, *11*, e0005532. [CrossRef]
75. Prasad, A.N.; Agans, K.N.; Sivasubramani, S.K.; Geisbert, J.B.; Borisevich, V.; Mire, C.E.; Lawrence, W.S.; Fenton, K.A.; Geisbert, T.W. A Lethal Aerosol Exposure Model of Nipah Virus Strain Bangladesh in African Green Monkeys. *J. Infect. Dis.* **2020**, *221* (Suppl. S4), S431–S435. [CrossRef]
76. *Possession, Use, and Transfer of Select Agents and Toxins*; Biennial Review; Department of Health and Human Services (Ed.) United States Government, Federal Register: Washington, DC, USA, 2020; Volume 85, FR 15087.
77. Luby, S.P. The pandemic potential of Nipah virus. *Antivir. Res.* **2013**, *100*, 38–43. [CrossRef]
78. Chumbe, A.; Izquierdo-Lara, R.; Calderon, K.; Fernandez-Diaz, M.; Vakharia, V.N. Development of a novel Newcastle disease virus (NDV) neutralization test based on recombinant NDV expressing enhanced green fluorescent protein. *Virol. J.* **2017**, *14*, 232. [CrossRef]
79. Ghanem, A.; Kern, A.; Conzelmann, K.K. Significantly improved rescue of rabies virus from cDNA plasmids. *Eur. J. Cell Biol.* **2012**, *91*, 10–16. [CrossRef]

80. Griffin, B.D.; Leung, A.; Chan, M.; Warner, B.M.; Ranadheera, C.; Tierney, K.; Audet, J.; Frost, K.L.; Safronetz, D.; Embury-Hyatt, C.; et al. Establishment of an RNA polymerase II-driven reverse genetics system for Nipah virus strains from Malaysia and Bangladesh. *Sci. Rep.* **2019**, *9*, 11171. [CrossRef]
81. Kwilas, A.R.; Yednak, M.A.; Zhang, L.; Liesman, R.; Collins, P.L.; Pickles, R.J.; Peeples, M.E. Respiratory syncytial virus engineered to express the cystic fibrosis transmembrane conductance regulator corrects the bioelectric phenotype of human cystic fibrosis airway epithelium in vitro. *J. Virol.* **2010**, *84*, 7770–7781. [CrossRef] [PubMed]
82. Munoz-Alia, M.A.; Russell, S.J. Probing Morbillivirus Antisera Neutralization Using Functional Chimerism between Measles Virus and Canine Distemper Virus Envelope Glycoproteins. *Viruses* **2019**, *11*, 688. [CrossRef] [PubMed]
83. Yeo, Y.Y.; Buchholz, D.W.; Gamble, A.; Jager, M.; Aguilar, H.C. Headless Henipaviral Receptor Binding Glycoproteins Reveal Fusion Modulation by the Head/Stalk Interface and Post-receptor Binding Contributions of the Head Domain. *J. Virol.* **2021**, *95*, e0066621. [CrossRef] [PubMed]
84. Kaku, Y.; Noguchi, A.; Marsh, G.A.; Barr, J.A.; Okutani, A.; Hotta, K.; Bazartseren, B.; Fukushi, S.; Broder, C.C.; Yamada, A.; et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: Sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. *J. Virol. Methods* **2012**, *179*, 226–232. [CrossRef]
85. Kaku, Y.; Noguchi, A.; Marsh, G.A.; McEachern, J.A.; Okutani, A.; Hotta, K.; Bazartseren, B.; Fukushi, S.; Broder, C.C.; Yamada, A.; et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. *J. Virol. Methods* **2009**, *160*, 7–13. [CrossRef]
86. Tamin, A.; Harcourt, B.H.; Lo, M.K.; Roth, J.A.; Wolf, M.C.; Lee, B.; Weingartl, H.; Audonnet, J.C.; Bellini, W.J.; Rota, P.A. Development of a neutralization assay for Nipah virus using pseudotype particles. *J. Virol. Methods* **2009**, *160*, 1–6. [CrossRef]
87. Pernet, O.; Schneider, B.S.; Beaty, S.M.; LeBreton, M.; Yun, T.E.; Park, A.; Zachariah, T.T.; Bowden, T.A.; Hitchens, P.; Ramirez, C.M.; et al. Evidence for henipavirus spillover into human populations in Africa. *Nat. Commun.* **2014**, *5*, 5342. [CrossRef]
88. Khetawat, D.; Broder, C.C. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol. J.* **2010**, *7*, 312. [CrossRef]

ANNUAL REVIEWS

Annual Review of Virology

Vaccines to Emerging Viruses: Nipah and Hendra

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Annu. Rev. Virol. 2020. 7:447–73

The *Annual Review of Virology* is online at virology.annualreviews.org

https://doi.org/10.1146/annurev-virology-021920-113833

Keywords

henipavirus, Hendra virus, Nipah virus, vaccine, subunit vaccine, henipavirus countermeasures

Abstract

Hendra virus (HeV) and Nipah virus (NiV) are bat-borne zoonotic paramyxoviruses identified in the mid- to late 1990s in outbreaks of severe disease in livestock and people in Australia and Malaysia, respectively. HeV repeatedly re-emerges in Australia while NiV continues to cause outbreaks in South Asia (Bangladesh and India), and these viruses have remained transboundary threats. In people and several mammalian species, HeV and NiV infections present as a severe systemic and often fatal neurologic and/or respiratory disease. NiV stands out as a potential pandemic threat because of its associated high case-fatality rates and capacity for human-to-human transmission. The development of effective vaccines, suitable for people and livestock, against HeV and NiV has been a research focus. Here, we review the progress made in NiV and HeV vaccine development, with an emphasis on those approaches that have been tested in established animal challenge models of NiV and HeV infection and disease.

INTRODUCTION

Nipah virus (NiV) and Hendra virus (HeV) are bat-borne viral zoonoses that were discovered in the mid- to late 1990s in outbreaks of severe disease in livestock and people in Australia (HeV) and Malaysia [NiV-Malaysia (NiV-M)] (1). They are the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae* (2). NiV outbreaks have also been recorded in Bangladesh and India by a closely related strain, NiV-Bangladesh (NiV-B) (3). Three other henipaviruses are also recognized: Cedar virus (CedV) as an isolate and Ghana virus (GhV) and Mojiang virus (MojV) known only from sequence data (4–7). Both NiV and HeV are highly pathogenic in a broad range of mammalian hosts that are capable of infecting and causing severe disease in humans, monkeys, pigs, horses, cats, dogs, ferrets, hamsters, and guinea pigs and that span six mammalian orders including bats, although bats do not exhibit disease when infected (8–21). In contrast, CedV is nonpathogenic in well-characterized models of HeV and NiV disease including ferrets and hamsters (4, 22). The pathogenic potential of GhV and MojV is unknown.

Several species of *Pteropus* fruit bats are the natural reservoir hosts of NiV, HeV, and CedV (4, 23–27). NiV- or HeV-mediated disease has not been reported in wild or experimentally infected bats (13, 28–30). NiV and HeV infections in people and many animals manifest as severe systemic and often fatal neurologic and/or respiratory diseases (31–33). Both NiV and HeV are regarded as transboundary biological threats to both human and animal health and are classified as biosafety level 4 (BSL-4) select agents (34, 35). NiV and henipaviral diseases are included in the World Health Organization (WHO) R&D Blueprint list of priority pathogens with epidemic potential that need research attention (36). This review summarizes the important characteristics of the NiV and HeV pathogens, the modes of virus transmission, and the immunization strategies being developed against them.

Emergence and Outbreaks of Hendra and Nipah Viruses

In 1994 in the Brisbane suburb of Hendra, Australia, an outbreak of severe respiratory disease resulted in the deaths of 14 horses and their trainer, along with the nonfatal infection of 7 other horses and 1 other person. This led to the discovery of a novel paramyxovirus initially termed equine morbillivirus, now known as HeV (37–39). The first known cases of HeV in horses and a human actually occurred a few months prior, where one person became ill after assisting in the necropsies of two horses later shown to have died from HeV (40, 41). This individual experienced a relapsed fatal encephalitis caused by HeV 13 months later (42). HeV has since re-emerged in Australia 62 times with a total of 104 horse deaths (fatal or euthanized), along with 4 human fatalities of 7 cases (43). Every recorded occurrence of HeV in Australia has involved horses, all resulting in a severe or fatal disease, and all cases of human infection were acquired from virus-shedding horses (31, 44).

In 1998, an outbreak of encephalitis among pig farmers in Peninsular Malaysia occurred and a virus was isolated from samples of cerebrospinal fluid (CSF) of two patients who had died; cells infected with this virus cross-reacted with antibodies against HeV (45). Genetic studies revealed a new paramyxovirus that was closely related to HeV, and it was named Nipah after the village in Malaysia where one of the patients had lived (45). There were 265 cases of human infection with 105 fatalities in Malaysia and 11 cases and 1 fatality among abattoir workers in Singapore (46, 47). This outbreak was controlled through the culling of more than 1 million pigs, resulting in significant economic impacts to the region (48, 49).

A genetically similar but distinct strain of NiV was identified as the causative agent of fatal encephalitis in people in Bangladesh (NiV-B) (3, 50). Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and there have been three outbreaks in

India (51–54). The recent 2018 NiV outbreak in Kerala, India, was significant, having occurred in a new geographic region far from locations in Bangladesh and India where all prior outbreaks had occurred and with a case fatality rate of 91% (51). In 2014, an outbreak of NiV-M encephalitis occurred in the Philippines with 9 fatalities of 11 human cases of acute encephalitis and influenza-like illness or meningitis in another 6 individuals (55). Altogether, there have been over 650 cases of human NiV infection (combined ~60% fatality rate) in South Asia and Southeast Asia in five countries (54, 56).

Transmission of Hendra and Nipah Viruses

The routes of transmission of virus infection to humans from animals are different for HeV and NiV, with horses the only spillover host of HeV in Australia, while for NiV it was pigs in Malaysia and horses in the Philippines (**Figure 1**). However, human NiV infections in Bangladesh, India, and the Philippines also include bat-to-human and human-to-human transmission (57–60). Transmission routes of HeV and NiV to animals are likely urine from infected bats contaminating pastures or pigsties and/or virus-contaminated fruit spat from bats that is ingested (61, 62) (**Figure 1**). Recoverable virus is shed in the urine of experimentally infected bats and can also be detected in throat and rectal swabs (13, 28–30). Pooled urine samples from flying foxes are also routinely used to detect and isolate henipaviruses (4, 13, 23, 27, 63–65).

It was previously suggested that infected horses could transmit HeV to people during the feeding of ill animals (38). Also, the majority of all HeV-infected horse cases have involved a single animal, suggesting that HeV is not readily transmitted between horses, and multiple horse outbreaks are likely via contamination of fomites (43, 66). The transmission risk of HeV from infected horses to humans appears to be virus-contaminated fluids or tissues during examination procedures and/or the necropsy of horses (31, 67) (**Figure 1**). Indeed, all cases of human HeV infection have been associated with postmortem examination of horses or close contact with ill horses (31, 38, 42, 68). In Malaysia, it was contact with infected pigs or fresh infected pig products that was required for transmission of virus to humans (45, 69, 70) (**Figure 1**). NiV shedding in respiratory fluids of infected pigs suggested that it probably spread among farmed animals by aerosol droplets or direct contact (16, 71, 72). In Bangladesh, the transmission of NiV from bats to people has been linked to the consumption of virus-contaminated fresh date palm sap, and bats will consume sap during its collection (57, 73, 74). Domestic animals have also been linked to NiV infection in people in Bangladesh from unwell animals (cows and goats) and pigs (50, 59). Human-to-human transmission of NiV has been well documented in Bangladesh and India (52, 58–60, 75) (**Figure 1**). A study of human NiV-B cases in Bangladesh spanning 14 years reported that of 248 cases studied, one-third were caused by human-to-human transmission (56). Human-to-human transmission of NiV-M was not apparent in Malaysia (76, 77), whereas in the Philippines' NiV-M outbreak, human cases were linked to horse slaughtering and horse meat consumption or exposure to other human patients, indicating both horse-to-human and human-to-human transmission (55) (**Figure 1**). The NiV-B outbreak in Kerala had a very high rate of human-to-human transmission (22 of 23 cases) at three different hospital locations (51).

Naturally acquired NiV infections were also recorded in cats, dogs, and horses in the initial Malaysian outbreak (**Figure 1**), and serological evidence of natural NiV infection in dogs was linked to outbreak farms (11, 61, 78). In the Philippines, both dogs and cats were linked to NiV-M infection, with cats dying after eating horse meat and dogs having NiV-neutralizing antibodies (55) (**Figure 1**). In Australia, a dog was found to be seropositive for HeV and later euthanized but showed no signs of disease, and a second HeV-positive dog was identified in 2013 following exposure to blood from an infected horse (79) (**Figure 1**). Dogs are susceptible to experimental HeV infection and shed virus but show little evidence of clinical illness (80).

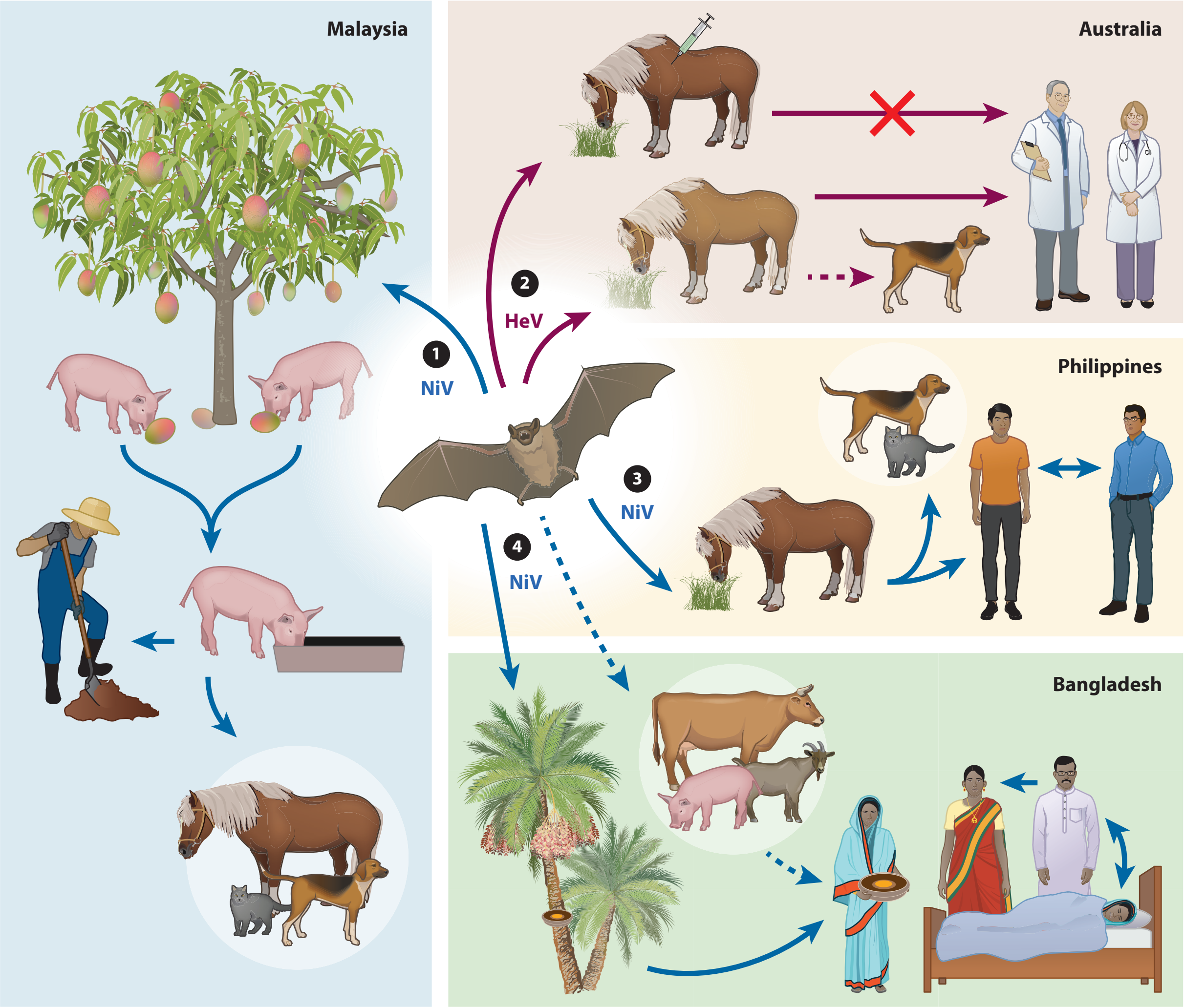

Figure 1

Nipah virus (NiV) and Hendra virus (HeV) modes of transmission in different countries. The transmission routes of NiV in Malaysia (*left*), Philippines (*middle right*), Bangladesh (*bottom right*), and HeV (*top right*) are depicted. Solid lines represent transmission that has been observed and documented, and dashed lines represent suspected transmission in natural conditions. Fruit bats are the natural reservoirs of NiV and HeV. (①) Pigs are infected by consuming partially eaten or contaminated fruit from infected bats (urine, saliva) and transmit NiV to other pigs, pig farmers, or other animals (dogs, cats, and horses) through close or direct contact. (②) Horses can be infected from grazing in contaminated pastures and transmit HeV to humans and on occasion domestic dogs through close contact. A One Health vaccine approach was developed for vaccination of horses in Australia with the dual purpose of saving horses from lethal HeV infection and preventing HeV transmission from horses to humans. (③) NiV is transmitted to humans through close contact with infected horses. NiV transmission to humans, cats, and dogs appears to have occurred following close contact with or consumption of infected horse meat. Human-to-human NiV transmission can occur through close contact. (④) Bat-to-human NiV transmission occurs through consumption of contaminated date palm sap. Human-to-human transmission can occur through close contact with infected patients. Humans may also become infected through contact with infected animals. Figure adapted with permission from Reference 171.

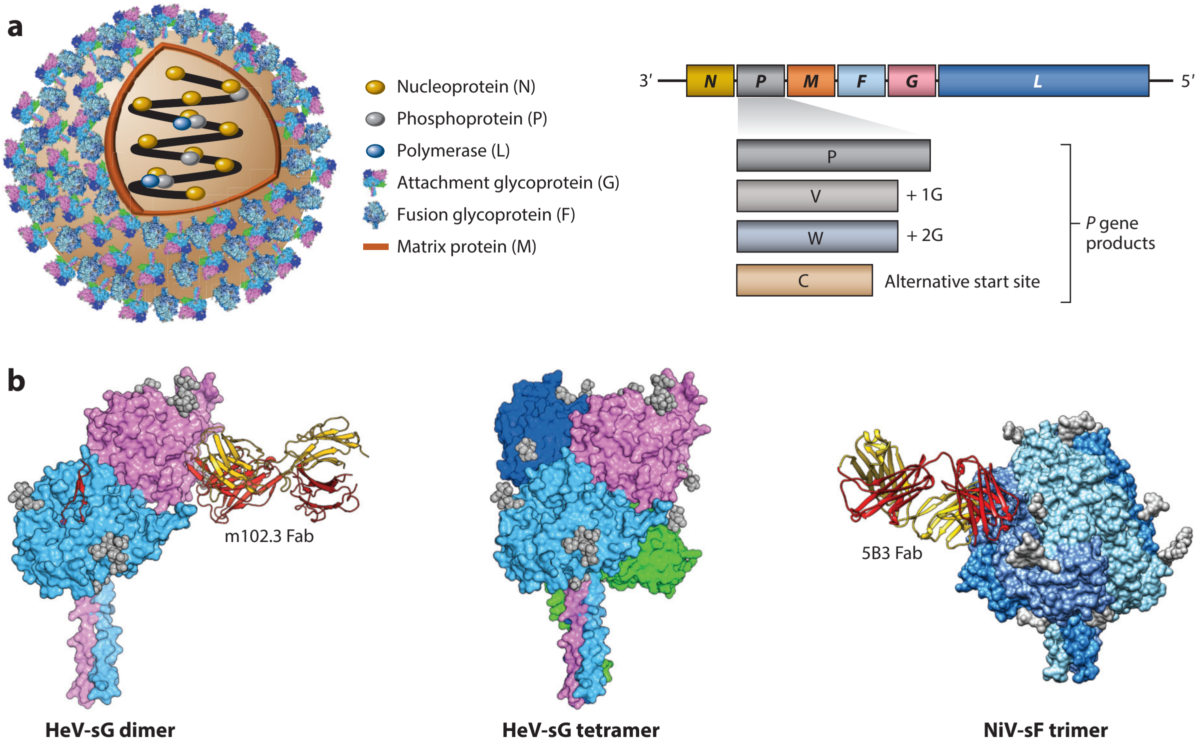

Figure 2

Henipavirus structure and genome organization and models of the G and F glycoprotein soluble ectodomains, Hendra virus (HeV-sG) and Nipah virus (NiV-sF), respectively, and their complexes with respective NiV and HeV cross-reactive neutralizing monoclonal antibodies m102.3 (anti-G) and 5B3 (anti-F). (*a*) Schematic representation of a henipavirus particle with the structural proteins depicted in different colors (*left*) and the henipavirus genome (*right*). HeV and NiV *P* genes encode 3 nonstructural proteins: The C protein is expressed from an alternative start site, and the V and W proteins are expressed following the addition of one or two G residues at the messenger RNA editing site, respectively (*right*). (*b*, *left*) HeV-sG shown as a dimer solvent-accessible surface view with one monomer (*cyan*) overlaid with the monoclonal antibody m102.3 CDR-H3 loop (*red*) at the receptor binding site, and the other monomer (*magenta*) in complex with m102.3 Fab, which has an identical heavy chain and a similar light chain, that was used in place of the m102.4 monoclonal antibody (mAb) in the structural solution of the complex (109). The HeV-sG consists of amino acids 76–604, and the structures of the two globular head domains of HeV-sG are derived from the crystal structure (103, 172). The stalk regions of each G monomer (residues 77–136) are modeled (173). The light chain of m102.3 Fab is colored in yellow, and the heavy chain is colored in red. (*b*, *middle*) The HeV-sG tetramer surface view is modeled with one dimer (*cyan* and *magenta*) in front and the other dimer (*blue* and *green*) in back. *N*-linked glycans are gray spheres. (*b*, *right*) Structural model of the NiV-sF trimer in complex with the 5B3 Fab derived from the cryo–electron microscopy structure (110). The NiV-sF consists of amino acid residues 1–494 with a FLAG tag (DYKDDDK) introduced between residues L104-V105 and a C-terminal GCN4 motif. Each monomer of NiV-sF is in a different shade of blue, 5B3 heavy chain is in red, and light chain is in gold. *N*-linked glycans are illustrated in gray.

Entry and Tropism of Nipah and Hendra Viruses

NiV and HeV are enveloped viruses containing an unsegmented, single-stranded, negative-sense RNA genome (2). **Figure 2*a*** is an illustration of the viral particle and the associated viral proteins. The genomes of HeV and NiV, and also CedV, GhV, and MojV, are considerably longer than the genomes of other paramyxoviruses, at greater than 18 kb. Henipavirus genomes encode 6 structural proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion glycoprotein (F), attachment glycoprotein (G), and the polymerase protein (L) (**Figure 2*a***). The N, P, and L proteins comprise the replication complex. The *P* gene undergoes RNA editing to produce 2 additional nonstructural proteins, V and W, that are interferon (IFN) antagonists (81–84). The C protein is transcribed from a second open reading frame in the *P* gene (**Figure 2*a***). NiV has been central to understanding the V, W, P, and C protein roles in antagonizing the innate immune responses via a diverse set of mechanisms (85, 86). Recent in vivo studies with recombinant NiV variants have further defined the varying importance of these nonstructural proteins in pathogenesis, but only a lack of the V protein results in a nonlethal infection (87–89).

The henipavirus virion bears surface projections composed of the F and G glycoproteins that are anchored in the viral membrane and together mediate infection of host cells, and they are the major antigens of vaccine strategies (1) (**Figure 2*a***). The F glycoprotein facilitates membrane fusion between the virus and host cell. The G glycoprotein consists of a characteristic stalk with a globular head that engages entry receptors on host cells, leading to the fusion activation of F and virus infection. The native structure of G is a tetramer while F is a trimer, and together they are the key determinants of infection and tropism (90–92). Models of the soluble ectodomain of the HeV G (HeV-sG) as a dimer and tetramer and the soluble ectodomain of the NiV F (NiV-sF) as a trimer are shown in **Figure 2*b***. NiV and HeV utilize the host cell proteins ephrin-B2 and ephrin-B3 for entry (93–96). Ephrin-B2 and ephrin-B3 are members of a large family of ligands that bind to Eph receptors and are highly sequence conserved among mammals (97, 98). Ephrin-B2 expression is prominent in the vasculature of multiple organs, whereas ephrin-B3 is found predominantly in the nervous system (99–101). The ability of HeV and NiV to use these ephrins as receptors provided explanations of their broad host and tissue tropism (32, 33, 102). The NiV and HeV G head domain structures alone and in complex with ephrin-B2 and ephrin-B3 receptors have been determined (103–106). The structures of both the NiV and HeV F in their prefusion conformation have also been determined (107, 108). These studies have provided insights into understanding the virus entry receptors and host tropism features of the viruses on the molecular level and also facilitated further structural studies of henipavirus G and F glycoproteins in complexes with specific virus-neutralizing antibodies, providing valuable information that has aided vaccine design and choice (109, 110).

Nipah Virus and Hendra Virus Infection in Humans and Animals

Human NiV and HeV infections are generally accepted to occur via the oronasal route, and the incubation periods for both have been estimated to be 1 to 2 weeks (31, 51, 111). Acute infection in people is a systemic infection likely via hematogenous spread of the virus from the respiratory system (112). In general, HeV and NiV disease onset is characterized by fever, myalgia, shortness of breath, and cough (38, 111). Human HeV infections have resulted in both fatal respiratory or encephalitic disease and also recovery from infection (31, 38, 42, 68). The predominant clinical feature in the NiV-M outbreak in Malaysia was encephalitis, but respiratory symptoms were also common with fever, cough, and headache (47, 111, 112). The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease. In the 2018 NiV-B outbreak in Kerala, 83% of cases presented with acute respiratory distress syndrome (ARDS) (51, 113). Central findings of human NiV and HeV infection are a widespread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected (45, 112, 114). Human NiV and HeV infections can also take a protracted course following apparent recovery, and some patients can experience late-onset encephalitis or relapsed encephalitis can occur in patients who previously recovered (42, 115). Relapsed encephalitis caused by NiV appears to result from a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later (116–118). Recrudescence of virus has important implications for vaccine development.

The development of animal models of NiV and HeV infection and pathogenesis has been a major focus since the late 1990s and an essential component of vaccine development and testing. Also, the approval process of countermeasures for NiV and HeV would fall under the animal rule requirement set forth by the US Food and Drug Administration (FDA) in 2002 as an alternative licensing pathway for countermeasures against highly pathogenic agents when human efficacy studies are not feasible or ethical (119, 120). Several animal models of NiV and HeV infection have emerged that well reflect the pathogenesis seen in infected people, which includes a systemic vasculitis with both respiratory and neurological diseases. Detailed reviews of NiV and HeV infections of a variety of mammalian species have recently been published (33, 121–123). It is generally accepted that the pathogenic processes of NiV and HeV infection in the hamster, ferret, and African green monkey (AGM) best reflect the pathogenesis observed in humans, whereas the most appropriate models for livestock are the horse and pig themselves.

VACCINATION

The attachment and fusion glycoproteins of paramyxoviruses such as measles, mumps, and parainfluenza viruses are the viral antigens to which virtually all neutralizing antibodies are directed (124–126). Likewise, immunization strategies for NiV and HeV have largely targeted their G and F glycoproteins.

Passive Immunization Strategies

Early passive immunization studies in the hamster model demonstrated that polyclonal antiserums or mouse monoclonal antibodies (mAbs) to NiV F or G could provide complete protection against NiV-M or HeV when administered before and immediately after virus infection (10, 127, 128). These studies demonstrated a major role of a viral glycoprotein-specific antibody in protection.

Recombinant human antibody technology was used to generate a potent cross-neutralizing mAb against NiV and HeV (m102.4) (129, 130). The m102.4 mAb epitope maps to the ephrin receptor binding site of G and blocks virus infection (see the left side of **Figure 2*b***), and it can neutralize NiV-M, NiV-B, and HeV (8, 109). The m102.4 mAb provided complete protection from NiV-M-mediated disease in ferrets as a single 50 mg dose administered 10 h post-challenge (8). In the AGM model, m102.4 administered as two 20 mg/kg doses, intravenously, at 10 h and again on day 3, on days 1 and 3 (days 1/3), or on days 3/5, after HeV challenge [4×10^5 50% tissue culture infectious dose ($TCID_{50}$)] by intratracheal (i.t.) administration, protected 100% of treated subjects (131). All treated subjects seroconverted against HeV F glycoprotein with a rise in antibody titer over time, indicating all animals had become infected with HeV and recovered, whereas untreated control subjects succumbed to HeV disease and failed to mount a protective immune response. No clinical signs were evident at any time in the early treatment groups; although neurological symptoms were observed in subjects in the late treatment group (days 3/5), all later recovered from infection. There was no HeV antigen or virus-specific histopathology detected in the lung or brain at the conclusion of the study in any treated subject, and infectious virus could not be recovered from any tissue. A similar study evaluated m102.4 against NiV-M disease in the AGM model at several time points following virus challenge (5×10^5 PFU), including a late cohort where treatment was initiated at the onset of clinical illness (day 5) (132). All subjects became infected after challenge, and all subjects that received m102.4 survived infection and all controls succumbed to disease. Subjects in the late day 5/7 treatment group exhibited disease, but all recovered. A comparative study in AGMs using NiV-M and NiV-B [5×10^5 PFU divided by i.t. and intranasal (i.n.) administration] revealed that NiV-B caused a more aggressive disease, with a shortened time to death and higher virus loads in tissues and fluids (133). When m102.4 was tested in this model, all subjects in the days 1/3 and days 3/5 post-infection treatment groups survived NiV-B challenge, but subjects in the days 5/7 treatment group succumbed, indicating a shorter therapeutic window in treating NiV-B infection (133). Another well-characterized, humanized mouse mAb, 5B3 (h5B3.1), that is cross-reactive to the F glycoprotein of NiV and HeV and binds a prefusion conformation epitope on F, preventing membrane fusion, was recently tested (110, 134) (**Figure 2*b***). The h5B3.1 mAb was given to ferrets in 20 mg/kg doses by intraperitoneal (i.p.) injection, at 1 to several days post-challenge, with either NiV or HeV ($\sim 5 \times 10^3$ PFU) delivered i.n. (135). All subjects that received h5B3.1 after infection were protected from disease and had increasing neutralizing antibody titers, whereas all controls died. No pathology was observed, and no infectious virus could be isolated at the study endpoint. Altogether, these studies demonstrate that passive immunization with mAbs can provide therapeutic benefit and allow the infected host an extended period to mount a protective immune response. The findings from these experiments were also important because they suggest that vaccine approaches designed to induce adequate neutralizing antibody responses to NiV and HeV should be effective.

The m102.4 mAb producing cell line was provided to the Queensland Government, Australia, to produce the mAb for compassionate use in future cases of high-risk human HeV infection. To date, 14 individuals exposed to either HeV in Australia ($n = 13$) or NiV in the United States ($n = 1$) have been given high-dose m102.4 therapy (15–20 mg/kg) by emergency use protocols, and all have remained well. In Australia, m102.4 was used in a randomized, controlled phase I study in healthy adults (136). The study included four single and one repeat dosing groups, and the m102.4 mAb was found to be safe and well tolerated, with a half-life ranging between ~16.5 and 27 days, and no observed immunogenicity was reported. Two doses of 20 mg/kg (days 1/3) were as well tolerated as a single dose. This study's findings will aid in the design of future dosing regimens of mAbs for evaluating their ability to prevent and/or treat HeV and NiV human infections.

Active Immunization Strategies

A variety of immunization strategies have been developed to prevent NiV and HeV infection including several live-recombinant virus vectors, protein subunit, and virus-like particle (VLP) approaches, and all target the virus attachment and entry steps of infection by employing the G and/or F glycoprotein antigens. Here we summarize these various vaccination countermeasure approaches to NiV and HeV infection (**Tables 1** and **2**).

Poxvirus vectored. Poxviruses have a long history as a platform for the expression of heterologous genes to study protein function and serve as vaccine candidates as a live-attenuated viral vaccine platform capable of inducing both cell-mediated and humoral immune responses (137). The F and G glycoproteins of NiV and HeV were functionally characterized using recombinant vaccinia viruses in the early 2000s (138, 139). The first NiV vaccine tested used a highly attenuated vaccinia virus strain (NYVAC) encoding either the F or G glycoproteins from NiV-M (127). Hamsters were vaccinated by subcutaneous (s.c.) injection in a prime-boost strategy with NYVAC-NiV-F or NYVAC-NiV-G, individually and in combination, and then 3 months later challenged i.p. with NiV-M. Vaccination yielded complete protection from NiV-M with no detection of viral RNA, and control subjects succumbed 7–10 days after challenge (127) (**Table 1**). Another poxvirus-based approach was examined as a vaccine for pigs using canarypox (ALVAC) vaccine vectors encoding either NiV-M F or G glycoprotein (140). A prime-boost strategy with ALVAC-NiV-F or ALVAC-NiV-G vectors was tested alone or in combination in pigs. The animals were then challenged 28 days later with NiV-M via i.n. administration. All vaccinated animals survived NiV-M challenge

Table 1 Virus vectored vaccine strategies for NiV and HeV

Approach	Name(s)	Animal model	Vaccination dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Correlate(s) of immunity/ protection	Reference
Poxvirus	NYVAC-NiV-F and/or -G	Hamster	2 doses at 1×10^7 PFU, s.c., 1 month apart	None	NiV-M	1×10^3 PFU, i.p., 3 months later	100%	NAb response, viral RNA	127
	ALVAC-NiV-F and/or -G	Pigs	2 doses at 1×10^8 PFU, i.m., 2 weeks apart	None	NiV-M	2.5×10^5 PFU, i.n., 28 days later	100%	NAb response, viral RNA, infectious virus, viral shedding, cytokine production	140
	ALVAC-HeV-F and/or -G	Hamster	2 doses at 7.4 or 5.4 log10 $CCID_{50}$, s.c., 3 weeks apart	None	HeV	1×10^3 LD_{50}, i.p., 21 days later	89% and 63%	NAb response, viral RNA, viral antigen, viral shedding	141
		Ponies	2 doses at 6 log10 $CCID_{50}$, i.m., 3 weeks apart	None	NT	NA	NA	High NAb titers	
	MVA-NiV-sG and/or MVA-NiV-G	IFNAR−/− mice	1 or 2 doses at 1×10^8 PFU, i.m., 3 weeks apart	None	NT	NA	NA	High serum IgG titers, NiV-G-specific CD8 and CD4 T cells	142

(*Continued*)

Table 1 (*Continued*)

Approach	Name(s)	Animal model	Vaccination dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Correlate(s) of immunity/ protection	Reference
VSV	VSV-NiV-F and/or -G	Mice	5 × 10^3 PFU, i.n. or i.m.	None	NT	NA	NA	High NAb titers	144
		Hamster	1 × 10^6 infectious particles, i.m.		NiV-M	1 × 10^5 $TCID_{50}$, i.p., 32 days later	100%	NAb response, viral RNA, viral antigen	145
	VSV-NiV-B F and/or G	Ferret	1 × 10^7 PFU, i.m.	None	NiV-M	5 × 10^3 PFU, i.n., 28 days later	100%	Serum IgG response, viral RNA, viral antigen	146
		AGM			NiV-B	5 × 10^5 PFU, i.t. and i.n., 28 days later		NAb response, viral RNA, viral antigen	147
	VSV-ZEBOV-GP-NiV F, G, or N	Hamster	1 × 10^5 PFU, i.p.	None	NiV-M	1 × 10^3 LD_{50}, i.p., 28 days later	100%	NAb response, viral RNA, infectious virus	148
		AGM	1 × 10^7 PFU, i.m.			1 × 10^5 $TCID_{50}$, i.t., 29 days later		NAb response, viral RNA, infectious virus, viral shedding	149
	VSV-HeV-G	Mice	1 × 10^5 PFU, i.m.	None	NT	NA	NA	Serum IgG, NAb response	150
AAV	AAV8 NiV.G	Mice	2 × 10^{10} genome particles, i.m. or 1 × 10^{10} genome particles, i.d.	None	NT	NA	NA	Serum IgG, NAb response	151
		Hamster	6 × 10^{11} genome particles, i.m.		NiV-M	1 × 10^4 PFU, i.p., 5 weeks later	100%	Serum IgG, NAb response, viral RNA, viral antigen	
					HeV		50%		

(*Continued*)

Table 1 (*Continued*)

Approach	Name(s)	Animal model	Vaccination dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Correlate(s) of immunity/ protection	Reference
Adenovirus	ChAdOx1 NiV-B G	Hamster	2 doses at 1×10^8 IU, i.m., 28 days apart	None	NiV-B	5.3×10^5 $TCID_{50}$, i.p., 28 days later	100%	NAb response, viral RNA, infectious virus, virus shedding	153
			1×10^8 IU, i.m.		NiV-M	6.8×10^4 $TCID_{50}$, i.p., 28 days later	100%		
					HeV	6×10^3 $TCID_{50}$, i.p., 28 days later	33%		
Measles virus	rMV-Ed-G or rMV-HL-G	Hamster	2 doses at 2×10^4 $TCID_{50}$, i.p., 3 weeks apart	None	NiV-M	1×10^3 $TCID_{50}$, i.p., 1 week later	100%	Serum IgG response	NA
	rMV-Ed-G	AGM	2 doses at 1×10^5 $TCID_{50}$, s.c., 4 weeks apart			1×10^5 $TCID_{50}$, i.p., 1 week later		Serum IgG response, viral RNA	154
Inactivated RABV	RABV-HeV-G	Mice	3 doses at 10 μg, i.m., 2 weeks apart	None	NT	NA	NA	High NAb titers, serum IgG response	150
	RABV-NiV-B G		2 doses at 10 μg, i.m., 4 weeks apart						155
RABV	RABV-NiV-F and/or -G	Mice	$1 \times 10^{6.5}$ FFU, oral	None	NT	NA	NA	Serum IgG, NAb response	156

All NiV glycoprotein vaccines employ the NiV-M strain unless otherwise indicated.

Abbreviations: AAV, adeno-associated virus; AGM, African green monkey; $CCID_{50}$, 50% cell culture infectious dose; ChAdOx1, chimpanzee adenovirus Oxford 1; F, fusion glycoprotein; FFU, focus forming units; G, attachment glycoprotein; HeV, Hendra virus; i.d., intradermal; i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; IFNAR, interferon receptor; IgG, immunoglobulin G; IU, infectious unit; LD_{50}, 50% lethal dose; MVA, modified vaccinia virus Ankara; NA, not applicable; NAb, neutralizing antibody; NiV, Nipah virus; NiV-B, Nipah virus Bangladesh; NiV-M, Nipah virus Malaysia; NT, not tested; PFU, plaque forming unit; RABV, rabies virus; rMV-Ed, recombinant measles virus Edmonston; rMV-HL, recombinant measles virus HL; s.c., subcutaneous; sF, F glycoprotein soluble ectodomain; sG, G glycoprotein soluble ectodomain; $TCID_{50}$, 50% tissue culture infectious dose; VSV, vesicular stomatitis virus; ZEBOV-GP, *Zaire ebolavirus* glycoprotein.

Table 2 VLP, subunit, and nucleic acid–based vaccine strategies for NiV and HeV

Approach	Name	Animal model	Immunization dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Immune correlate(s) of survival	Reference
VLPs	VLPs-NiV M/F/G	Mice	2 doses at 1.75, 3.5, 7, or 14 µg and 6 µg, s.c., 2 weeks apart	None	NT	NA	NA	High NAb titers	159
		Hamster	1 dose or 3 doses at 30 µg, i.m., 3 weeks apart	Alhydrogel/MPLA or Alhydrogel/CpG	NiV-M	1.6×10^4 PFU (3-dose trial) or 3.3×10^4 PFU (1-dose trial), i.p., 28 days later	100%	NAb response, viral RNA	160
Subunit vaccines	NiV-sG	Cat	3 doses at 100 µg, s.c., 2 weeks apart	CSIRO triple adjuvant	NiV-M	5×10^2 $TCID_{50}$, s.c., 2 months later	100%	NAb response, viral antigen, viral genome	14
	HeV-sG	Cat	3 doses at 100 µg, s.c., 2 weeks apart	CSIRO triple adjuvant	NiV-M	5×10^2 $TCID_{50}$, s.c., 2 months later	100%	NAb response, viral antigen, viral genome	14
			2 doses at 50, 25, or 5 µg, i.m., 3 weeks apart	CpG/Alhydrogel		5×10^4 $TCID_{50}$, o.n., 2 weeks later	100%	Serum IgG, NAb response, viral RNA, viral shedding, infectious virus	162
		Ferret	2 doses at 100, 20, or 4 µg, i.m., 20 days apart	CpG	HeV	5×10^3 $TCID_{50}$, o.n., 3 weeks later	100%	NAb response, viral RNA, infectious virus	163
				CpG/Alhydrogel	NiV-B	5×10^4 $TCID_{50}$, 20 days later or 12 months later	100%	viral RNA, viral antigen, infectious virus	164

(*Continued*)

Table 2 ***(Continued)***

Approach	Name	Animal model	Immunization dose, route, schedule	Adjuvant	Challenge	Challenge dose, route, schedule	Survival	Immune correlate(s) of survival	Reference
		AGM	2 doses at 100, 50, or 10 μg, i.m., 3 weeks apart	CpG/Alhydrogel	NiV-M	1×10^5 TCID$_{50}$, i.t., 3 weeks later	100%	Serum IgG, NAb response, viral RNA, viral antigen, infectious virus	165
			2 doses at 100 μg, i.m., 3 weeks apart	Alhydrogel or CpG/Alhydrogel	HeV	5×10^5 PFU, i.t., 21 days later	100%	NAb response, viral RNA	166
		Horse	2 doses at 100 or 50 μg, i.m., 3 weeks apart	Zoetis	HeV	2×10^6 TCID$_{50}$, o.n., 28 or 194 days later	100%	NAb response, viral RNA, viral antigen, infectious virus	167
		Pig	2 doses of 2 mL preformulation, i.m., 3 weeks apart	Zoetis	HeV	5×10^5 PFU, i.n., 35 days later	Partial	NAb response, viral RNA, infectious virus, viral shedding	168
					NiV-M		0%		
Nucleic acid–based vaccine	HeV-sG mRNA LNP	Hamster	10 or 30 μg, i.m.	None	NiV-M	1×10^5 TCID$_{50}$, i.p., 30 days later	30% or 70%	Serum IgG, NAb response	169

All NiV glycoprotein vaccines employ the NiV-M strain.

Abbreviations: AGM, African green monkey; CSIRO, Commonwealth Scientific and Industrial Research Organisation; F, fusion glycoprotein; G, attachment glycoprotein; HeV, Hendra virus; i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; i.t., intratracheal; IgG, immunoglobulin G; LNP, lipid nanoparticle; M, matrix protein; MPLA, monophosphoryl lipid A; mRNA, messenger RNA; NA, not applicable; NAb, neutralizing antibody; NiV, Nipah virus; NiV-B, Nipah virus Bangladesh; NiV-M, Nipah virus Malaysia; NT, not tested; o.n., oronasal; PFU, plaque forming unit; s.c., subcutaneous; sG, G glycoprotein soluble ectodomain; TCID$_{50}$, 50% tissue culture infectious dose; VLP, virus-like particle.

as determined by the lack of NiV RNA and infectious virus from nasal washes, pharyngeal swabs, and a variety of sampled organs (140).

ALVAC-vectored vaccines encoding HeV F or G glycoprotein for potential use in horses were also examined (141). ALVAC-HeV-F or ALVAC-HeV-G vectors were combined and first used to vaccinate hamsters at a high or low dose of each vector, by s.c. injection, and then challenged with HeV by i.p. administration. Vaccination did not result in complete protection, with 8 out of 9 subjects in the high-dose group and 5 out of 8 subjects in the low-dose group surviving challenge. No signs of disease were noted, and viral antigen or viral RNA could not be detected in survivors. Nine ponies vaccinated using the same prime-boost regimen were able to develop high cross-neutralizing antibody titers to HeV and NiV-M at day 28 after vaccination. Although ponies were not challenged, most animals yielded titers of at least 1:32 and were considered likely protective (141).

More recently, a modified vaccinia virus Ankara (MVA) vector encoding NiV-M G glycoprotein and a soluble version of G (NiV-sG) were examined in interferon receptor α and β (IFNAR−/−) knockout mice (142) (**Table 1**). IFNAR−/− mice were immunized once with MVA-NiV-G or MVA-NiV-sG or prime-boosted. IFNAR−/− mice developed high serum immunoglobulin G (IgG) titers to NiV-G and also generated NiV-G-specific CD8 and CD4 T cells following vaccination. MVA-NiV-sG vaccination induced rapid and significantly higher amounts of NiV-G epitope-specific CD8 T cells compared with the MVA-NiV-G candidate vaccine, suggesting superior immunogenicity. Together, these immunization studies with poxvirus vectors highlight that both T cell and B cell responses play a role in an adaptive immune response to NiV and HeV. However, detailed studies on the adaptive immune responses in animal experiments with henipaviruses have been limited. Future studies evaluating the role of NiV-specific T cells will be important because two human survivors of NiV-B infection in the 2018 outbreak in Kerala showed marked elevation of activated $CD8^+$ T cells, which coincided with virus clearance (143).

Vesicular stomatitis virus vectored. Recombinant vesicular stomatitis virus (rVSV) vectors as a vaccine platform suitable for single immunization strategies to potentially meet emergency use requirements have been tested by several groups (**Table 1**). A method of using two defective VSVΔG vectors each expressing only the NiV G or F glycoprotein was devised using VSV G glycoprotein complementation that can generate replication-defective VSV vectors that could elicit NiV-neutralizing antibodies (144). Using this technique, researchers tested rVSV vaccines expressing either NiV-M F or G glycoproteins (VSV-ΔG-NiVG, VSV-ΔG-NiVF) in hamsters by intramuscular (i.m.) vaccination (145). Hamsters were then challenged 32 days later with NiV-M by i.p. administration. All vaccinated animals survived lethal infection with no clinical signs of disease. No viral RNA or viral antigen could be detected in the sampled tissues when compared with controls, and there was a lack of an anamnestic immune response in vaccinated subjects following challenge, suggesting the induction of sterilizing immunity.

Another study used rVSV-ΔG vectors expressing NiV-B F or G glycoprotein and also tested them as single-injection vaccinations in NiV-M-challenged ferrets (146). Ferrets were vaccinated i.m. with rVSV-NiV-B F or rVSV-NiV-B G complemented with VSV G or a mix of both vectors, rVSV-NiV-B F/G, that was generated as a complementing pair in the absence of VSV G and then challenged at 28 days with NiV-M by i.n. administration. All vaccinated ferrets were completely protected against NiV-M challenge. Although viral RNA was detected in blood at day 6 post-challenge in 2 of 5 animals in each group, those levels were 100 times lower than in the unvaccinated controls, and by day 21 no viral RNA was detected (146). In a second study, rVSV-NiV-B F and rVSV-NiV-B G were assessed separately and in combination in AGMs (147). Cohorts were vaccinated with the rVSVs by i.m. injection and challenged 28 days later with NiV-B divided between the i.t. and i.n. routes (147). Complete protection was recorded from NiV-B disease with no gross pathology and no detectable NiV antigen in lung or spleen tissues. Viral RNA was detected in nasal and oral swabs of the vaccinated groups, but no viral RNA could be detected in blood samples.

Replication-competent rVSV-NiV-M F or G vectors, generated by the retention of the envelope glycoprotein from *Zaire ebolavirus* (ZEBOV-GP), which allowed virus stocks to be propagated (rVSV-ZEBOV-GP-NiVF, rVSV-ZEBOV-GP-NiVG, and rVSV-ZEBOV-GP-NiVN), have also been tested (148). These rVSVs were used to immunize hamsters by i.p. administration and were challenged 28 days later with NiV-M. All subjects vaccinated with either the NiV F or G glycoprotein encoding rVSV vectors were completely protected with no clinical disease or pathology, whereas those vaccinated with the NiV N protein were only partially protected (2 of 6 animals) with no clinical signs of disease and the other subjects succumbed to infection. The protective efficacy of the rVSV-ZEBOV-GP-NiVG was also tested in AGMs, where vaccinated subjects were challenged with NiV-M by i.t. administration 29 days later (149). All vaccinated subjects were protected from lethal challenge and showed no signs of clinical disease, no viral RNA was detected in the blood or oral and nasal swabs, and no infectious virus could be recovered. Another study using a rVSV vector expressing a codon-optimized HeV *G* gene together with an inactivated counterpart was evaluated in mice for humoral immune responses only as a comparator to a recombinant rabies virus vaccine encoding HeV G as a HeV vaccine candidate (150). Here, the live rVSV vectors induced greater levels of HeV G-specific antibodies and higher levels of HeV-neutralizing antibodies than did the recombinant rabies virus vectors (see the section titled Rabies Virus Vectored).

Adeno-associated virus and adenovirus vectored. Adeno-associated virus (AAV) vectors as a vaccine platform against infectious diseases, particularly viral pathogens, have been explored. AAV is a small, single-stranded DNA virus in the family *Parvoviridae*. Immunization of hamsters with an AAV vector expressing NiV-M G glycoprotein (AAV8 NiV.G) by i.m. injection demonstrated complete protection from a challenge of NiV-M by i.p. administration, and no signs of clinical disease were recorded (151) (**Table 1**). Neutralizing antibodies to NiV were induced, no viral RNA or viral antigen was detected in any of the sampled tissues, and there was only a moderate anamnestic response observed in a single subject, suggestive of potential sterilizing immunity. However, in a cross-protection study, AAV8 NiV.G protected only 50% of hamsters challenged with HeV.

Chimpanzee adenoviral (ChAd) vectors circumvent issues of the preexisting immunity observed with human adenovirus vectors (152). Adenoviruses are double-stranded DNA viruses in the family *Adenoviridae*. An engineered replication-deficient ChAd vector, Oxford 1 (ChAdOx1), was tested as a NiV/HeV vaccine (153). Here, ChAdOx1 encoding NiV-B (ChAdOx1 NiV-B) G glycoprotein was used to vaccinate hamsters by i.m. injection, either as a single dose or as a prime-boost protocol. Hamsters were challenged by i.p. administration with NiV-B 42 days following the booster or the single vaccination. Neutralizing antibodies were detectable, and all vaccinated hamsters were protected against lethal disease with no lung pathology, suggesting that a single dose of ChAdOx1 NiV-B was sufficient to completely protect against NiV-B. No viral RNA in the lung tissue and no viral shedding in oropharyngeal swabs could be detected, and no infectious virus could be isolated. A second cohort using a single dose of ChAdOx1 NiV-B to vaccinate hamsters was trialed, and these animals were challenged 28 days later with NiV-M or HeV. All vaccinated animals were protected from lethal NiV-M challenge, but 4 out of 6 hamsters succumbed to HeV disease between days 5 and 7 post-challenge. Neither virus shedding in oropharyngeal swabs nor infectious virus was detected in the lung or brain tissues of NiV-M-challenged vaccinated hamsters. In contrast, infectious virus was detected in the lung tissues of 75% of the HeV-challenged vaccinated animals. The lower cross-protection observation using NiV G vaccination followed by HeV challenge was not unexpected, as it was previously shown that when the G glycoprotein (as a recombinant soluble subunit immunogen) of either HeV or NiV was used to vaccinate cats, both could completely protect against lethal NiV-M challenge, and that the HeV-sG elicited greater heterologous neutralizing antibody responses in comparison to NiV-sG (14).

Measles virus vectored. Recombinant measles virus vectors based on the HL (rMV-HL) and Edmonston (rMV-Ed) measles virus strains have also been explored in which they encoded the NiV-M G glycoprotein (rMV-HL-G and rMV-Ed-G) (154) (**Table 1**). Hamsters were immunized twice by i.p. administration of rMV-HL-G or rMV-Ed-G. All vaccinated animals produced NiV G-specific antibody titers after the booster immunization. Animals were challenged 1 week after the second immunization with NiV-M by i.p. administration. All immunized hamsters exhibited no clinical symptoms and survived challenge. The study was extended to non-human primates (NHPs), where 2 AGMs were immunized twice by s.c. injection with rMV-Ed-G. Subjects were challenged 2 weeks after the second immunization with NiV-M by i.p. administration. Here, immunization completely protected the AGMs with no observed clinical disease and no detectable pathological changes, and no viral RNA could be detected in sampled tissues. Although this was a small study, the safety profile and success of the live-attenuated measles virus vaccine suggests that a recombinant platform encoding the NiV G glycoprotein as a NiV vaccine candidate is promising and should induce a balanced and long-lasting immune response against NiV.

Rabies virus vectored. A rabies virus (RABV) SAD B19 vaccine strain, BNSP333, expressing HeV or NiV G glycoproteins has been evaluated (150, 155). Recombinant BNSP333 encoding either the wild-type or a codon-optimized HeV *G* gene, together with their inactivated counterparts, was used in mice (150) (**Table 1**). Mice were immunized by i.m. injection with a single dose of the RABV-based vectors or with 3 doses of their inactivated versions. The inactivated RABV-based vectors induced higher and more rapid HeV G-specific antibody responses and higher neutralizing antibody titers than their live counterparts. The inactivated RABV-coHeV-G induced cross-neutralizing antibodies against NiV. A similar study used the BNSP333 vector expressing NiV-B G glycoprotein (RABV-NiV-BG) (**Table 1**) and elicited NiV G-specific neutralizing antibodies (155).

Recently, the recombinant RABV Evelyn-Rokitnicki-Abelseth (ERA) strain ($\text{rERAG}_{333\text{E}}$) expressing either NiV-M F or G glycoproteins was evaluated in mice and pigs (156) (**Table 1**). This vector, $\text{rERAG}_{333\text{E}}$, serves as an oral vaccine in dogs. Here, mice were orally immunized with RABV-NiV-F or RABV-NiV-G either individually or in combination. Pigs were also immunized in a similar manner but with 2 doses of each vector either alone or in combination. RABV-NiV-F and/or RABV-NiV-G immunization induced NiV F- and G-specific IgG antibody responses and neutralizing antibodies in both mice and pigs with the combination vaccine inducing higher titers. Although not suitable for human use, the live-attenuated $\text{rERAG}_{333\text{E}}$ vector is of interest as a potential veterinary vaccine for NiV because it is already approved for use in some animals and could be adapted for emergency use to protect against NiV infection in livestock, particularly swine.

Many of these virus-vectored vaccines for NiV are promising candidates because of their established safety profiles and ease of genetic modification. Several of these virus-vectored vaccines also require no adjuvants, and some are clearly efficacious as a single immunization strategy, suitable features for emergency use circumstances. In addition, several of these platforms are able to induce both cell-mediated and humoral immune responses, which may also be desirable but as yet are not fully explored in the development of vaccines for NiV and HeV. Although animals immunized with viral vectors encoding the NiV G glycoprotein and challenged with the homologous virus were completely protected, cross-protection studies with some of these vaccines against a HeV challenge were less effective. For example, only 50% of AAV8 NiV.G-immunized hamsters or 33% of ChAdOx1 NiV-B G glycoprotein–immunized hamsters were protected from a lethal HeV challenge (151, 153). In addition, the ALVAC-HeV-F and ALVAC-HeV-G vaccination studies showed that these vectors did not provide 100% protection in hamsters challenged with HeV, perhaps due to either a suboptimal immunization dose or the immunization route (141).

Virus-like particles. VLPs have been explored as a vaccine platform because of the resemblance of their surface structure, dimensions, and compositions to authentic virus yet are of high safety because of the lack of viral genetic material. Earlier studies revealed that the M protein of NiV was capable of orchestrating the formation and budding of NiV VLPs when expressed in cells that appeared structurally similar to authentic NiV virions, and these VLPs could also incorporate other viral proteins such as the F and G glycoproteins (157, 158). VLPs composed of NiV M, F, and G were used to vaccinate mice s.c. at weeks 0, 2, and 4 and demonstrated they could induce high neutralizing antibody titers by day 35 (159) (**Table 2**). NiV VLPs were later used in NiV-M challenge studies either alone or in combination with adjuvant, monophosphoryl lipid A (MPLA) and Alhydrogel^{TM} (15 μg/50 μg) or CpG and Alhydrogel (40 μg/50 μg) (160). Hamsters were vaccinated i.m. either as a single dose or as a 3-dose regimen and then challenged via i.p. administration of NiV-M at 28 days or 58 days, respectively. In all cohorts, 100% of the vaccinated animals survived with no clinical signs of disease and no detection of viral RNA in any of the sampled tissues, regardless of the presence of adjuvant. VLPs are thus an alternative means, with inherent safety, of producing an inactivated whole virus vaccine from an otherwise highly pathogenic virus.

Subunit vaccine. The HeV-sG subunit vaccine has been extensively evaluated in several studies. Here, a brief summary of earlier reports is made, but the focus is on studies in NHPs and livestock. Recombinant HeV-sG and NiV-sG can elicit a potent neutralizing antibody response and were first tested as vaccine immunogens in the feline model (14, 161) (**Table 2**). Both HeV-sG and NiV-sG vaccination of cats completely protected against lethal NiV-M challenge, and HeV-sG elicited greater heterologous neutralizing titers than did NiV-sG, demonstrating that a single subunit vaccine may be effective against both NiV and HeV (14). Other studies using lower doses of HeV-sG (**Table 2**) demonstrated that a pre-challenge neutralizing titer of 1:32 could protect against NiV-M (162). Additional studies in ferrets showed that low doses of HeV-sG could protect against HeV and NiV-B (163, 164) (**Table 2**). Also, a longevity study showed that vaccinated ferrets challenged with NiV-B at 14 months post-immunization, with pre-challenge neutralizing titers of 1:16 to 1:128, were also protected (164).

The HeV-sG vaccine has been extensively evaluated in AGMs (**Table 2**). In a cross-protection study, 100 μg, 50 μg, or 10 μg doses of HeV-sG in combination with Alhydrogel and CpG were administered i.m. as a prime-boost, on days 0 and 21. Pre-challenge 50% neutralization titers ranged from 1:28 to 1:379. All subjects were challenged with NiV-M by i.t. administration on day 42. All vaccinated subjects were completely protected, displaying no clinical signs of disease, and no viral RNA could be detected in blood and tissues and no infectious virus was isolated (165). Similarly, HeV-sG vaccination HeV challenge in AGMs has also been performed. Using a prime-boost regimen, AGMs were vaccinated twice, 3 weeks apart, by i.m. injection with 100 μg HeV-sG with Alhydrogel or HeV-sG with Alhydrogel and CpG, and then challenged 3 weeks later with HeV by i.t. administration (166). All vaccinated animals were completely protected from clinical disease, and no HeV RNA or viral antigen could be detected in swabs, blood, or tissues, and notably HeV-sG formulated in only Alhydrogel protected (166).

The efficacy and inherent safety of the HeV-sG subunit led to its development as an equine vaccine to prevent HeV infection of horses and also reduce the risk of HeV transmission to people, as a One Health concept (**Figure 1**). HeV-sG, formulated in an approved equine adjuvant (Zoetis, Inc.), was evaluated in two efficacy studies; the first tested 50 μg and 100 μg doses of the same HeV-sG used in prior animal studies to vaccinate horses, and the second used 100 μg doses of HeV-sG produced in Chinese hamster ovary cells (Zoetis, Inc.). Two vaccinations were given by i.m. administration 3 weeks apart. All horses in these efficacy studies were challenged by oronasal inoculation with HeV (**Table 2**). Seven horses were challenged at 28 days and 3 horses were challenged at 194 days after the second immunization. All vaccinated horses remained clinically healthy following challenge; pre-challenge neutralization titers ranged from 1:128 to more than 4,096 in horses challenged 21 days after vaccination and only from 1:16 to 1:32 in horses challenged at 6 months. There was no gross or histologic evidence of infection in any of the vaccinated horses at study completion, and all tissues examined were negative for viral antigen, with no viral genome detected in any tissue. In 9 of 10 vaccinated horses, HeV nucleic acid was not detected in daily nasal, oral, or rectal swab samples or from blood, urine, or fecal samples collected before euthanasia, no recoverable virus was present, and no rise in antibody titer was detected in any vaccinated horse following challenge (167). The HeV-sG horse vaccine (Equivac® HeV) was launched by Zoetis, Inc., in November 2012 on a minor use permit by the regulatory authority, the Australian Pesticides and Veterinary Medicines Authority (APVMA), and is the first commercially developed and deployed vaccine against a BSL-4 agent. All vaccinated horses are microchipped, and a database is maintained. Equivac HeV received full registration by the APVMA in 2015. To date, more than 765,000 doses of Equivac HeV vaccine have been administered to more than 179,000 unique horses, and laboratory-confirmed HeV infections in horses have since occurred only in unvaccinated animals.

Studies showed HeV-sG as a NiV vaccine in the pig model (which is a non-lethal challenge model) was much less effective in comparison to results observed in the cat, ferret, NHP, and horse, and HeV-sG was only partially protective against HeV challenge and unprotective against NiV-M in the pig (168). These experiments also indicated that both humoral and cellular immune responses were required for protection of swine against NiV and HeV. Here, pigs were immunized with HeV-sG in a proprietary adjuvant (Zoetis, Inc.), and subjects were challenged with HeV or NiV via i.n. administration (**Table 2**). HeV-sG-vaccinated pigs developed neutralizing titers ranging from 1:160 to 1:320 to HeV, but only partial protection was achieved with reduced viral RNA in tissues and no recoverable virus, and there was no reduction of viral shedding in nasal washes. These HeV-sG-vaccinated pigs did not develop neutralizing antibodies to NiV-M that were considered protective (low), nor did they have measurable activation of cellular immune memory. Only a comparative group of pigs that were first orally infected (vaccinated) with NiV (and recovered) were subsequently protected against an i.n. rechallenge with NiV. This group of pigs developed protective antibody levels and cell-mediated immune memory responses (168).

Single-dose lipid nanoparticle mRNA, HeV-sG vaccine. More recently, messenger RNA (mRNA)-based vaccines have emerged as an attractive vaccine strategy because of safety, efficacy, and rapid implementation features. In a recent study, the efficacy of an mRNA vaccine approach was assessed in a NiV-M animal challenge model (169). mRNA transcripts encoding HeV-sG were complexed with lipid nanoparticles (LNPs) to generate HeV-sG mRNA LNP. Two groups of 10 hamsters were vaccinated with a single dose of HeV-sG mRNA LNP at either 10 μg or 30 μg by i.m. injection. Subjects were challenged with NiV-M by i.p. administration 30 days post-vaccination (**Table 2**). The HeV-sG mRNA LNP was only partially protective, with 3 hamsters from the low-dose group and 7 hamsters from the high-dose group surviving challenge. Of the surviving animals, signs of clinical disease were observed in 2 low-dose group and 6 high-dose group hamsters; however, disease symptoms were gone by study termination. NiV *N* gene RNA levels in the blood and a variety of tissues in surviving hamsters were lower compared with nonsurvivors, but NiV RNA copy levels were not different compared with controls. No anti-NiV IgG or virus-neutralizing activity was detected in vaccinated animals prior to challenge; however, all post-challenge survivors were positive for anti-NiV IgG antibodies, and all survivors (in both groups) had similar neutralizing titers ranging from 1:160 to 1:640. Euthanized animals had little to undetectable neutralizing activity, highlighting the correlation of this immune response to protection. Although promising, the partial efficacy of HeV-sG mRNA LNP observed in this study suggests that further optimization of vaccination route, addition of an adjuvant, and/or a prime-boost regimen is needed.

SUMMARY AND FUTURE PERSPECTIVES

The frequency of henipavirus outbreaks and human infections is a significant global health concern. A promising passive immunization strategy has been developed using a human mAb, m102.4, shown effective in the NHP challenge model, which has also been administered numerous times to people by compassionate use protocol and has successfully completed a phase I safety trial in Australia. In addition, the Equivac HeV vaccine is available, targeting the protection of horses and also people by breaking the chain of HeV transmission to people, and is an example of a One Health approach to counter an infectious disease threat. Over the past 15 years, nearly a dozen NiV and HeV vaccine approaches have been trialed in animal challenge models, and many show promise as effective human-use vaccines. Recently, the formation of the Coalition for Epidemic Preparedness Innovations (CEPI), a global partnership between public and private organizations, was undertaken with the goals of developing vaccines against emerging infectious diseases and offering equitable access to those vaccines (170). Indeed, without the support of CEPI, the prospects of having a NiV or HeV vaccine suitable for use in people, at a deployable stage in the event of a significant outbreak, would have remained academic. Research teams can now capitalize on the large body of basic and preclinical vaccine development data on a half-dozen important emerging viral threats including NiV and, with the support of CEPI, can develop vaccine candidates for clinical use and future licensure. Several of the NiV human vaccine candidates described in this review are now supported by CEPI.

DISCLOSURE STATEMENT

C.C.B. is a US federal employee and co-inventor on US and foreign patents pertaining to soluble forms of HeV and NiV G and F glycoproteins and monoclonal antibodies against HeV and NiV whose assignee is the United States as represented by the Henry M. Jackson Foundation for the Advancement of Military Medicine (Bethesda, Maryland). The soluble forms of the HeV and NiV G glycoproteins are licensed to Zoetis, Inc., and Aurobindo Pharma USA Inc. M.A. declares no competing interests.

ACKNOWLEDGMENTS

C.C.B. is supported by National Institutes of Health (NIH) grant AI142764 and Coalition for Epidemic Preparedness Innovations grant to Aurobindo Pharma USA Inc. The authors thank

Dr. Kai Xu, NIH, Bethesda, Maryland, and Ha V. Dang and Dr. David Veesler, University of Washington, Seattle, Washington, for providing the glycoprotein models in **Figure 2**.

LITERATURE CITED

1. Eaton BT, Broder CC, Middleton D, Wang LF. 2006. Hendra and Nipah viruses: different and dangerous. *Nat. Rev. Microbiol.* 4:23–35
2. Wang L-F, Mackenzie JS, Broder CC. 2013. Henipaviruses. In *Fields Virology*, ed. DM Knipe, PM Howley, pp. 1070–85. Philadelphia: Lippincott Williams & Wilkins
3. Harcourt BH, Lowe L, Tamin A, Liu X, Bankamp B, et al. 2005. Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg. Infect. Dis.* 11:1594–97
4. Marsh GA, de Jong C, Barr JA, Tachedjian M, Smith C, et al. 2012. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLOS Pathog.* 8:e1002836
5. Drexler JF, Corman VM, Gloza-Rausch F, Seebens A, Annan A, et al. 2009. Henipavirus RNA in African bats. *PLOS ONE* 4:e6367
6. Wu Z, Yang L, Yang F, Ren X, Jiang J, et al. 2014. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg. Infect. Dis.* 20:1064–66
7. Maes P, Amarasinghe GK, Ayllon MA, Basler CF, Bavari S, et al. 2019. Taxonomy of the order *Mononegavirales*: second update 2018. *Arch. Virol.* 164:1233–44
8. Bossart KN, Zhu Z, Middleton D, Klippel J, Crameri G, et al. 2009. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLOS Pathog.* 5:e1000642
9. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, Smith MA, Chan YP, et al. 2010. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLOS ONE* 5:e10690
10. Guillaume V, Wong KT, Looi RY, Georges-Courbot MC, Barrot L, et al. 2009. Acute Hendra virus infection: analysis of the pathogenesis and passive antibody protection in the hamster model. *Virology* 387:459–65
11. Hooper P, Zaki S, Daniels P, Middleton D. 2001. Comparative pathology of the diseases caused by Hendra and Nipah viruses. *Microbes Infect.* 3:315–22
12. Hooper PT, Westbury HA, Russell GM. 1997. The lesions of experimental equine morbillivirus disease in cats and guinea pigs. *Vet. Pathol.* 34:323–29
13. Middleton DJ, Morrissy CJ, van der Heide BM, Russell GM, Braun MA, et al. 2007. Experimental Nipah virus infection in pteropid bats (*Pteropus poliocephalus*). *J. Comp. Pathol.* 136:266–72
14. Mungall BA, Middleton D, Crameri G, Bingham J, Halpin K, et al. 2006. Feline model of acute Nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J. Virol.* 80:12293–302
15. Rockx B, Bossart KN, Feldmann F, Geisbert JB, Hickey AC, et al. 2010. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. *J. Virol.* 84:9831–39
16. Weingartl H, Czub S, Copps J, Berhane Y, Middleton D, et al. 2005. Invasion of the central nervous system in a porcine host by Nipah virus. *J. Virol.* 79:7528–34
17. Westbury HA, Hooper PT, Brouwer SL, Selleck PW. 1996. Susceptibility of cats to equine morbillivirus. *Aust. Vet. J.* 74:132–34
18. Westbury HA, Hooper PT, Selleck PW, Murray PK. 1995. Equine morbillivirus pneumonia: susceptibility of laboratory animals to the virus. *Aust. Vet. J.* 72:278–79
19. Wong KT, Grosjean I, Brisson C, Blanquier B, Fevre-Montange M, et al. 2003. A golden hamster model for human acute Nipah virus infection. *Am. J. Pathol.* 163:2127–37
20. Li M, Embury-Hyatt C, Weingartl HM. 2010. Experimental inoculation study indicates swine as a potential host for Hendra virus. *Vet. Res.* 41:33
21. Marianneau P, Guillaume V, Wong T, Badmanathan M, Looi RY, et al. 2010. Experimental infection of squirrel monkeys with Nipah virus. *Emerg. Infect. Dis.* 16:507–10
22. Schountz T, Campbell C, Wagner K, Rovnak J, Martellaro C, et al. 2019. Differential innate immune responses elicited by Nipah virus and Cedar virus correlate with disparate in vivo pathogenesis in hamsters. *Viruses* 11:291

23. Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, et al. 2002. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect.* 4:145–51
24. Halpin K, Young PL, Field HE, Mackenzie JS. 2000. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J. Gen. Virol.* 81:1927–32
25. Rahman SA, Hassan SS, Olival KJ, Mohamed M, Chang LY, et al. 2010. Characterization of Nipah virus from naturally infected *Pteropus vampyrus* bats, Malaysia. *Emerg. Infect. Dis.* 16:1990–93
26. Reynes JM, Counor D, Ong S, Faure C, Seng V, et al. 2005. Nipah virus in Lyle's flying foxes, Cambodia. *Emerg. Infect. Dis.* 11:1042–47
27. Anderson DE, Islam A, Crameri G, Todd S, Islam A, et al. 2019. Isolation and full-genome characterization of Nipah viruses from bats, Bangladesh. *Emerg. Infect. Dis.* 25:166–70
28. Halpin K, Hyatt AD, Fogarty R, Middleton D, Bingham J, et al. 2011. Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission. *Am. J. Trop. Med. Hyg.* 85:946–51
29. Williamson MM, Hooper PT, Selleck PW, Gleeson LJ, Daniels PW, et al. 1998. Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. *Aust. Vet. J.* 76:813–18
30. Williamson MM, Hooper PT, Selleck PW, Westbury HA, Slocombe RF. 2000. Experimental Hendra virus infection in pregnant guinea-pigs and fruit bats (*Pteropus poliocephalus*). *J. Comp. Pathol.* 122:201–7
31. Playford EG, McCall B, Smith G, Slinko V, Allen G, et al. 2010. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg. Infect. Dis.* 16:219–23
32. Wong KT, Ong KC. 2011. Pathology of acute henipavirus infection in humans and animals. *Pathol. Res. Int.* 2011:567248
33. Geisbert TW, Feldmann H, Broder CC. 2012. Animal challenge models of henipavirus infection and pathogenesis. *Curr. Top. Microbiol. Immunol.* 359:153–77
34. CDC (Cent. Dis. Control Prev.). 2009. *Biosafety in Microbiological and Biomedical Laboratories*, ed. LC Chosewood, DE Wilson. Washington, DC: Natl. Inst. Health. 5th ed.
35. ARS (Agricult. Res. Serv.). 2018. *Henipavirus Gap Analysis*. Washington, DC: US Dep. Agricult.
36. Sweileh WM. 2017. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health* 13:9
37. Murray K, Rogers R, Selvey L, Selleck P, Hyatt A, et al. 1995. A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg. Infect. Dis.* 1:31–33
38. Selvey LA, Wells RM, McCormack JG, Ansford AJ, Murray K, et al. 1995. Infection of humans and horses by a newly described morbillivirus. *Med. J. Aust.* 162:642–45
39. Murray K, Selleck P, Hooper P, Hyatt A, Gould A, et al. 1995. A morbillivirus that caused fatal disease in horses and humans. *Science* 268:94–97
40. Hooper PT, Gould AR, Russell GM, Kattenbelt JA, Mitchell G. 1996. The retrospective diagnosis of a second outbreak of equine morbillivirus infection. *Aust. Vet. J.* 74:244–45
41. Rogers RJ, Douglas IC, Baldock FC, Glanville RJ, Seppanen KT, et al. 1996. Investigation of a second focus of equine morbillivirus infection in coastal Queensland. *Aust. Vet. J.* 74:243–44
42. O'Sullivan JD, Allworth AM, Paterson DL, Snow TM, Boots R, et al. 1997. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* 349:93–95
43. Queensland Gov. 2019. Summary of Hendra virus incidents in horses. *Queensland Government*. **https://www.business.qld.gov.au/industries/service-industries-professionals/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary**
44. Barclay AJ, Paton DJ. 2000. Hendra (equine morbillivirus). *Vet. J.* 160:169–76
45. Chua KB, Goh KJ, Wong KT, Kamarulzaman A, Tan PS, et al. 1999. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* 354:1257–59
46. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, et al. 2000. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 288:1432–35
47. Paton NI, Leo YS, Zaki SR, Auchus AP, Lee KE, et al. 1999. Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* 354:1253–56
48. Ahmad K. 2000. Malaysia culls pigs as Nipah virus strikes again. *Lancet* 356:230
49. Lam SK, Chua KB. 2002. Nipah virus encephalitis outbreak in Malaysia. *Clin. Infect. Dis.* 34:S48–51

50. Hsu VP, Hossain MJ, Parashar UD, Ali MM, Ksiazek TG, et al. 2004. Nipah virus encephalitis reemergence, Bangladesh. *Emerg. Infect. Dis.* 10:2082–87
51. Arunkumar G, Chandni R, Mourya DT, Singh SK, Sadanandan R, et al. 2019. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. *J. Infect. Dis.* 219:1867–78
52. Chadha MS, Comer JA, Lowe L, Rota PA, Rollin PE, et al. 2006. Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg. Infect. Dis.* 12:235–40
53. Harit AK, Ichhpujani RL, Gupta S, Gill KS, Lal S, et al. 2006. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. *Indian J. Med. Res.* 123:553–60
54. Luby SP, Hossain MJ, Gurley ES, Ahmed BN, Banu S, et al. 2009. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. *Emerg. Infect. Dis.* 15:1229–35
55. Ching PK, de los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, et al. 2015. Outbreak of henipavirus infection, Philippines, 2014. *Emerg. Infect. Dis.* 21:328–31
56. Nikolay B, Salje H, Hossain MJ, Khan A, Sazzad HMS, et al. 2019. Transmission of Nipah virus—14 years of investigations in Bangladesh. *N. Engl. J. Med.* 380:1804–14
57. Luby SP, Rahman M, Hossain MJ, Blum LS, Husain MM, et al. 2006. Foodborne transmission of Nipah virus, Bangladesh. *Emerg. Infect. Dis.* 12:1888–94
58. Gurley ES, Montgomery JM, Hossain MJ, Bell M, Azad AK, et al. 2007. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerg. Infect. Dis.* 13:1031–37
59. Luby SP, Gurley ES, Hossain MJ. 2009. Transmission of human infection with Nipah virus. *Clin. Infect. Dis.* 49:1743–48
60. Homaira N, Rahman M, Hossain MJ, Epstein JH, Sultana R, et al. 2010. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol. Infect.* 138:1630–36
61. Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J. 2001. The natural history of Hendra and Nipah viruses. *Microbes Infect.* 3:307–14
62. Field HE. 2016. Hendra virus ecology and transmission. *Curr. Opin. Virol.* 16:120–25
63. Edson D, Field H, McMichael L, Vidgen M, Goldspink L, et al. 2015. Routes of Hendra virus excretion in naturally-infected flying-foxes: implications for viral transmission and spillover risk. *PLOS ONE* 10:e0140670
64. Peel AJ, Wells K, Giles J, Boyd V, Burroughs A, et al. 2019. Synchronous shedding of multiple bat paramyxoviruses coincides with peak periods of Hendra virus spillover. *Emerg. Microbes Infect.* 8:1314–23
65. Barr J, Smith C, Smith I, de Jong C, Todd S, et al. 2015. Isolation of multiple novel paramyxoviruses from pteropid bat urine. *J. Gen. Virol.* 96:24–29
66. Middleton D. 2014. Hendra virus. *Vet. Clin. North Am. Equine Pract.* 30:579–89
67. Mahalingam S, Herrero LJ, Playford EG, Spann K, Herring B, et al. 2012. Hendra virus: an emerging paramyxovirus in Australia. *Lancet Infect. Dis.* 12:799–807
68. Hanna JN, McBride WJ, Brookes DL, Shield J, Taylor CT, et al. 2006. Hendra virus infection in a veterinarian. *Med. J. Aust.* 185:562–64
69. Parashar UD, Sunn LM, Ong F, Mounts AW, Arif MT, et al. 2000. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998–1999 outbreak of severe encephalitis in Malaysia. *J. Infect. Dis.* 181:1755–59
70. Amal NM, Lye MS, Ksiazek TG, Kitsutani PD, Hanjeet KS, et al. 2000. Risk factors for Nipah virus transmission, Port Dickson, Negeri Sembilan, Malaysia: results from a hospital-based case-control study. *Southeast Asian J. Trop. Med. Public Health* 31:301–6
71. Mohd Nor MN, Gan CH, Ong BL. 2000. Nipah virus infection of pigs in peninsular Malaysia. *Rev. Sci. Tech.* 19:160–65
72. Middleton DJ, Westbury HA, Morrissy CJ, van der Heide BM, Russell GM, et al. 2002. Experimental Nipah virus infection in pigs and cats. *J. Comp. Pathol.* 126:124–36
73. Khan MS, Hossain J, Gurley ES, Nahar N, Sultana R, Luby SP. 2010. Use of infrared camera to understand bats' access to date palm sap: implications for preventing Nipah virus transmission. *Ecohealth* 7:517–25

74. Rahman MA, Hossain MJ, Sultana S, Homaira N, Khan SU, et al. 2012. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector-Borne Zoonotic Dis.* 12:65–72
75. Sazzad HM, Hossain MJ, Gurley ES, Ameen KM, Parveen S, et al. 2013. Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. *Emerg. Infect. Dis.* 19:210–17
76. Tan CT, Tan KS. 2001. Nosocomial transmissibility of Nipah virus. *J. Infect. Dis.* 184:1367
77. Mounts AW, Kaur H, Parashar UD, Ksiazek TG, Cannon D, et al. 2001. A cohort study of health care workers to assess nosocomial transmissibility of Nipah virus, Malaysia, 1999. *J. Infect. Dis.* 183:810–13
78. Mills JN, Alim AN, Bunning ML, Lee OB, Wagoner KD, et al. 2009. Nipah virus infection in dogs, Malaysia, 1999. *Emerg. Infect. Dis.* 15:950–52
79. Kirkland PD, Gabor M, Poe I, Neale K, Chaffey K, et al. 2015. Hendra virus infection in dog, Australia, 2013. *Emerg. Infect. Dis.* 21:2182–85
80. Middleton DJ, Riddell S, Klein R, Arkinstall R, Haining J, et al. 2017. Experimental Hendra virus infection of dogs: virus replication, shedding and potential for transmission. *Aust. Vet. J.* 95:10–18
81. Ciancanelli MJ, Volchkova VA, Shaw ML, Volchkov VE, Basler CF. 2009. Nipah virus sequesters inactive STAT1 in the nucleus via a P gene-encoded mechanism. *J. Virol.* 83:7828–41
82. Rodriguez JJ, Horvath CM. 2004. Host evasion by emerging paramyxoviruses: Hendra virus and Nipah virus V proteins inhibit interferon signaling. *Viral. Immunol.* 17:210–19
83. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. 2005. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J. Virol.* 79:6078–88
84. Shaw ML, Garcia-Sastre A, Palese P, Basler CF. 2004. Nipah virus V and W proteins have a common STAT1-binding domain yet inhibit STAT1 activation from the cytoplasmic and nuclear compartments, respectively. *J. Virol.* 78:5633–41
85. Basler CF. 2012. Nipah and Hendra virus interactions with the innate immune system. *Curr. Top. Microbiol. Immunol.* 359:123–52
86. Pelissier R, Iampietro M, Horvat B. 2019. Recent advances in the understanding of Nipah virus immunopathogenesis and anti-viral approaches. *F1000Research* 8:1763
87. Satterfield BA, Borisevich V, Foster SL, Rodriguez SE, Cross RW, et al. 2019. Antagonism of STAT1 by Nipah virus P gene products modulates disease course but not lethal outcome in the ferret model. *Sci. Rep.* 9:16710
88. Satterfield BA, Cross RW, Fenton KA, Agans KN, Basler CF, et al. 2015. The immunomodulating V and W proteins of Nipah virus determine disease course. *Nat. Commun.* 6:7483
89. Satterfield BA, Cross RW, Fenton KA, Borisevich V, Agans KN, et al. 2016. Nipah virus C and W proteins contribute to respiratory disease in ferrets. *J. Virol.* 90:6326–43
90. Chang A, Dutch RE. 2012. Paramyxovirus fusion and entry: multiple paths to a common end. *Viruses* 4:613–36
91. Aguilar HC, Iorio RM. 2012. Henipavirus membrane fusion and viral entry. *Curr. Top. Microbiol. Immunol.* 359:79–94
92. Navaratnarajah CK, Generous AR, Yousaf I, Cattaneo R. 2020. Receptor-mediated cell entry of paramyxoviruses: mechanisms, and consequences for tropism and pathogenesis. *J. Biol. Chem.* 295:2771–86
93. Bishop KA, Stantchev TS, Hickey AC, Khetawat D, Bossart KN, et al. 2007. Identification of Hendra virus G glycoprotein residues that are critical for receptor binding. *J. Virol.* 81:5893–901
94. Bonaparte MI, Dimitrov AS, Bossart KN, Crameri G, Mungall BA, et al. 2005. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *PNAS* 102:10652–57
95. Negrete OA, Levroney EL, Aguilar HC, Bertolotti-Ciarlet A, Nazarian R, et al. 2005. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* 436:401–5
96. Negrete OA, Wolf MC, Aguilar HC, Enterlein S, Wang W, et al. 2006. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLOS Pathog.* 2:e7
97. Drescher U. 2002. Eph family functions from an evolutionary perspective. *Curr. Opin. Genet. Dev.* 12:397–402
98. Bossart KN, Tachedjian M, McEachern JA, Crameri G, Zhu Z, et al. 2008. Functional studies of host-specific ephrin-B ligands as Henipavirus receptors. *Virology* 372:357–71

99. Gale NW, Baluk P, Pan L, Kwan M, Holash J, et al. 2001. Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. *Dev. Biol.* 230:151–60

100. Poliakov A, Cotrina M, Wilkinson DG. 2004. Diverse roles of eph receptors and ephrins in the regulation of cell migration and tissue assembly. *Dev. Cell* 7:465–80

101. Pasquale EB. 2008. Eph-ephrin bidirectional signaling in physiology and disease. *Cell* 133:38–52

102. Wong KT, Tan CT. 2012. Clinical and pathological manifestations of human henipavirus infection. *Curr. Top. Microbiol. Immunol.* 359:95–104

103. Xu K, Chan YP, Rajashankar KR, Khetawat D, Yan L, et al. 2012. New insights into the Hendra virus attachment and entry process from structures of the virus G glycoprotein and its complex with ephrin-B2. *PLOS ONE* 7:e48742

104. Xu K, Rajashankar KR, Chan YP, Himanen JP, Broder CC, Nikolov DB. 2008. Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. *PNAS* 105:9953–58

105. Bowden TA, Aricescu AR, Gilbert RJ, Grimes JM, Jones EY, Stuart DI. 2008. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. *Nat. Struct. Mol. Biol.* 15:567–72

106. Bowden TA, Crispin M, Harvey DJ, Jones EY, Stuart DI. 2010. Dimeric architecture of the Hendra virus attachment glycoprotein: evidence for a conserved mode of assembly. *J. Virol.* 84:6208–17

107. Wong JJ, Paterson RG, Lamb RA, Jardetzky TS. 2016. Structure and stabilization of the Hendra virus F glycoprotein in its prefusion form. *PNAS* 113:1056–61

108. Xu K, Chan YP, Bradel-Tretheway B, Akyol-Ataman Z, Zhu Y, et al. 2015. Crystal structure of the pre-fusion Nipah virus fusion glycoprotein reveals a novel hexamer-of-trimers assembly. *PLOS Pathog.* 11:e1005322

109. Xu K, Rockx B, Xie Y, Debuysscher BL, Fusco DL, et al. 2013. Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLOS Pathog.* 9:e1003684

110. Dang HV, Chan YP, Park YJ, Snijder J, Da Silva SC, et al. 2019. An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat. Struct. Mol. Biol.* 26:980–87

111. Goh KJ, Tan CT, Chew NK, Tan PS, Kamarulzaman A, et al. 2000. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N. Engl. J. Med.* 342:1229–35

112. Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, et al. 2002. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am. J. Pathol.* 161:2153–67

113. Hossain MJ, Gurley ES, Montgomery JM, Bell M, Carroll DS, et al. 2008. Clinical presentation of Nipah virus infection in Bangladesh. *Clin. Infect. Dis.* 46:977–84

114. Wong KT, Robertson T, Ong BB, Chong JW, Yaiw KC, et al. 2009. Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathol. Appl. Neurobiol.* 35:296–305

115. Tan CT, Goh KJ, Wong KT, Sarji SA, Chua KB, et al. 2002. Relapsed and late-onset Nipah encephalitis. *Ann. Neurol.* 51:703–8

116. Wong SC, Ooi MH, Wong MN, Tio PH, Solomon T, Cardosa MJ. 2001. Late presentation of Nipah virus encephalitis and kinetics of the humoral immune response. *J. Neurol. Neurosurg. Psychiatry* 71:552–54

117. Chong HT, Tan CT. 2003. Relapsed and late-onset Nipah encephalitis, a report of three cases. *Neurol. J. Southeast Asia* 8:109–12

118. Abdullah S, Chang LY, Rahmat K, Goh KT, Tan CT. 2012. Late-onset Nipah virus encephalitis 11 years after the initial outbreak: a case report. *Neurol. Asia* 17:71–74

119. Aebersold P. 2012. FDA experience with medical countermeasures under the animal rule. *Adv. Prev. Med.* 2012:507571

120. Crawford LM. 2002. New drug and biological drug products; evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible. *Fed. Reg.* 67:37988–98

121. de Wit E, Munster VJ. 2015. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *J. Pathol.* 235:196–205

122. Dhondt KP, Horvat B. 2013. Henipavirus infections: lessons from animal models. *Pathogens* 2:264–87

123. Rockx B. 2014. Recent developments in experimental animal models of Henipavirus infection. *Pathog. Dis.* 71:199–206
124. Rubin SA, Sauder CJ, Carbone KM. 2013. Mumps virus. In *Fields Virology*, ed. DM Knipe, PM Howley, pp. 1024–41. Philadelphia: Lippincott Williams & Wilkins
125. Griffin DE. 2013. Measles virus. In *Fields Virology*, ed. DM Knipe, PM Howley, pp. 1042–69. Philadelphia: Lippincott Williams & Wilkins
126. Karron RAC, Collins PL. 2013. Parainfluenza viruses. In *Fields Virology*, ed. DM Knipe, PM Howley, pp. 996–1023. Philadelphia: Lippincott Williams & Wilkins
127. Guillaume V, Contamin H, Loth P, Georges-Courbot MC, Lefeuvre A, et al. 2004. Nipah virus: vaccination and passive protection studies in a hamster model. *J. Virol.* 78:834–40
128. Guillaume V, Contamin H, Loth P, Grosjean I, Courbot MC, et al. 2006. Antibody prophylaxis and therapy against Nipah virus infection in hamsters. *J. Virol.* 80:1972–78
129. Zhu Z, Dimitrov AS, Bossart KN, Crameri G, Bishop KA, et al. 2006. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J. Virol.* 80:891–99
130. Zhu Z, Bossart KN, Bishop KA, Crameri G, Dimitrov AS, et al. 2008. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J. Infect. Dis.* 197:846–53
131. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, et al. 2011. A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Sci. Transl. Med.* 3:105ra03
132. Geisbert TW, Mire CE, Geisbert JB, Chan YP, Agans KN, et al. 2014. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci. Transl. Med.* 6:242ra82
133. Mire CE, Satterfield BA, Geisbert JB, Agans KN, Borisevich V, et al. 2016. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. *Sci. Rep.* 6:30916
134. Chan YP, Lu M, Dutta S, Yan L, Barr J, et al. 2012. Biochemical, conformational, and immunogenic analysis of soluble trimeric forms of henipavirus fusion glycoproteins. *J. Virol.* 86:11457–71
135. Mire CE, Chan YP, Borisevich V, Cross RW, Yan L, et al. 2019. A cross-reactive humanized monoclonal antibody targeting fusion glycoprotein function protects ferrets against lethal Nipah virus and Hendra virus infection. *J. Infect. Dis.* 2019:jiz515
136. Playford EG, Munro T, Mahler SM, Elliott S, Gerometta M, et al. 2020. Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect. Dis.* 20:445–54
137. Prow NA, Jimenez Martinez R, Hayball JD, Howley PM, Suhrbier A. 2018. Poxvirus-based vector systems and the potential for multi-valent and multi-pathogen vaccines. *Expert Rev. Vaccines* 17:925–34
138. Bossart KN, Wang LF, Eaton BT, Broder CC. 2001. Functional expression and membrane fusion tropism of the envelope glycoproteins of Hendra virus. *Virology* 290:121–35
139. Bossart KN, Wang LF, Flora MN, Chua KB, Lam SK, et al. 2002. Membrane fusion tropism and heterotypic functional activities of the Nipah virus and Hendra virus envelope glycoproteins. *J. Virol.* 76:11186–98
140. Weingartl HM, Berhane Y, Caswell JL, Loosmore S, Audonnet JC, et al. 2006. Recombinant Nipah virus vaccines protect pigs against challenge. *J. Virol.* 80:7929–38
141. Guillaume-Vasselin V, Lemaitre L, Dhondt KP, Tedeschi L, Poulard A, et al. 2016. Protection from Hendra virus infection with Canarypox recombinant vaccine. *NPJ Vaccines* 1:16003
142. Kalodimou G, Veit S, Jany S, Kalinke U, Broder CC, et al. 2019. A soluble version of Nipah virus glycoprotein G delivered by vaccinia virus MVA activates specific CD8 and CD4 T cells in mice. *Viruses* 12:26
143. Arunkumar G, Devadiga S, McElroy AK, Prabhu S, Sheik S, et al. 2019. Adaptive immune responses in humans during Nipah virus acute and convalescent phases of infection. *Clin. Infect. Dis.* 69:1752–56
144. Chattopadhyay A, Rose JK. 2011. Complementing defective viruses that express separate paramyxovirus glycoproteins provide a new vaccine vector approach. *J. Virol.* 85:2004–11

145. Lo MK, Bird BH, Chattopadhyay A, Drew CP, Martin BE, et al. 2014. Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters. *Antivir. Res.* 101:26–29

146. Mire CE, Versteeg KM, Cross RW, Agans KN, Fenton KA, et al. 2013. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol. J.* 10:353

147. Mire CE, Geisbert JB, Agans KN, Versteeg KM, Deer DJ, et al. 2019. Use of single-injection recombinant vesicular stomatitis virus vaccine to protect nonhuman primates against lethal Nipah virus disease. *Emerg. Infect. Dis.* 25:1144–52

148. DeBuysscher BL, Scott D, Marzi A, Prescott J, Feldmann H. 2014. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. *Vaccine* 32:2637–44

149. Prescott J, DeBuysscher BL, Feldmann F, Gardner DJ, Haddock E, et al. 2015. Single-dose live-attenuated vesicular stomatitis virus-based vaccine protects African green monkeys from Nipah virus disease. *Vaccine* 33:2823–29

150. Kurup D, Wirblich C, Feldmann H, Marzi A, Schnell MJ. 2015. Rhabdovirus-based vaccine platforms against henipaviruses. *J. Virol.* 89:144–54

151. Ploquin A, Szecsi J, Mathieu C, Guillaume V, Barateau V, et al. 2013. Protection against henipavirus infection by use of recombinant adeno-associated virus–vector vaccines. *J. Infect. Dis.* 207:469–78

152. Ewer K, Sebastian S, Spencer AJ, Gilbert S, Hill AVS, Lambe T. 2017. Chimpanzee adenoviral vectors as vaccines for outbreak pathogens. *Hum. Vaccines Immunother.* 13:3020–32

153. van Doremalen N, Lambe T, Sebastian S, Bushmaker T, Fischer R, et al. 2019. A single-dose ChAdOx1-vectored vaccine provides complete protection against Nipah Bangladesh and Malaysia in Syrian golden hamsters. *PLOS Negl. Trop. Dis.* 13:e0007462

154. Yoneda M, Georges-Courbot MC, Ikeda F, Ishii M, Nagata N, et al. 2013. Recombinant measles virus vaccine expressing the Nipah virus glycoprotein protects against lethal Nipah virus challenge. *PLOS ONE* 8:e58414

155. Keshwara R, Shiels T, Postnikova E, Kurup D, Wirblich C, et al. 2019. Rabies-based vaccine induces potent immune responses against Nipah virus. *NPJ Vaccines* 4:15

156. Shuai L, Ge J, Wen Z, Wang J, Wang X, Bu Z. 2020. Immune responses in mice and pigs after oral vaccination with rabies virus vectored Nipah disease vaccines. *Vet. Microbiol.* 241:108549

157. Ciancanelli MJ, Basler CF. 2006. Mutation of YMYL in the Nipah virus matrix protein abrogates budding and alters subcellular localization. *J. Virol.* 80:12070–78

158. Patch JR, Crameri G, Wang LF, Eaton BT, Broder CC. 2007. Quantitative analysis of *Nipah virus* proteins released as virus-like particles reveals central role for the matrix protein. *Virol. J.* 4:1

159. Walpita P, Barr J, Sherman M, Basler CF, Wang L. 2011. Vaccine potential of Nipah virus-like particles. *PLOS ONE* 6:e18437

160. Walpita P, Cong Y, Jahrling PB, Rojas O, Postnikova E, et al. 2017. A VLP-based vaccine provides complete protection against Nipah virus challenge following multiple-dose or single-dose vaccination schedules in a hamster model. *NPJ Vaccines* 2:21

161. Bossart KN, Crameri G, Dimitrov AS, Mungall BA, Feng YR, et al. 2005. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J. Virol.* 79:6690–702

162. McEachern JA, Bingham J, Crameri G, Green DJ, Hancock TJ, et al. 2008. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine* 26:3842–52

163. Pallister J, Middleton D, Wang LF, Klein R, Haining J, et al. 2011. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine* 29:5623–30

164. Pallister JA, Klein R, Arkinstall R, Haining J, Long F, et al. 2013. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol. J.* 10:237

165. Bossart KN, Rockx B, Feldmann F, Brining D, Scott D, et al. 2012. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci. Transl. Med.* 4:146ra07

166. Mire CE, Geisbert JB, Agans KN, Feng YR, Fenton KA, et al. 2014. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J. Virol.* 88:4624–31

167. Middleton D, Pallister J, Klein R, Feng YR, Haining J, et al. 2014. Hendra virus vaccine, a One Health approach to protecting horse, human, and environmental health. *Emerg. Infect. Dis.* 20:372–79

168. Pickering BS, Hardham JM, Smith G, Weingartl ET, Dominowski PJ, et al. 2016. Protection against henipaviruses in swine requires both, cell-mediated and humoral immune response. *Vaccine* 34:4777–86

169. Lo MK, Spengler JR, Welch SR, Harmon JR, Coleman-McCray JD, et al. 2019. Evaluation of a single-dose nucleoside-modified messenger RNA vaccine encoding Hendra virus-soluble glycoprotein against lethal Nipah virus challenge in Syrian hamsters. *J. Infect. Dis.* 2019:jiz553

170. Gouglas D, Christodoulou M, Plotkin SA, Hatchett R. 2019. CEPI: driving progress towards epidemic preparedness and response. *Epidemiol. Rev.* 41:28–33

171. Clayton BA. 2017. Nipah virus: transmission of a zoonotic paramyxovirus. *Curr. Opin. Virol.* 22:97–104

172. Colgrave ML, Snelling HJ, Shiell BJ, Feng YR, Chan YP, et al. 2011. Site occupancy and glycan compositional analysis of two soluble recombinant forms of the attachment glycoprotein of Hendra virus. *Glycobiology* 22:572–84

173. Kelley LA, Sternberg MJ. 2009. Protein structure prediction on the Web: a case study using the Phyre server. *Nat. Protoc.* 4:363–71

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of Virology

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Errata

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Date: Tuesday, May 2, 2023 1:28:20 PM
Attachments: MAmaya-Published-Viruses-15-01077.pdf
AA-Amaya-Published-annurev-virology-021920-113833.pdf

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hi Chris,

great to meet you today.

attached is Cedar chimera paper.
also a recent Nipah/Hendra review

A good plan for Nipah/Hendra as the prototypes in **VSVdeltaG**
Could be
NiV G(bangladesh)
NiV sG(bangladesh)
HeV sG(redlands)

We could also consider a comparison of these vaccines to recombinant Cedar virus encoding HeV sG ?

AAV and adenovirus platforms with NiV G did not work well against Hendra challenge

Mouse sera could be sent to us for testing. against NiV/HeV
and any prechallenge sera (if animals are not yet moved to bsl4)

Or we could simply collaborate on the project and MTA/Supply
virus stocks to your Cores
v/r
Chris

--
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https://medschool.usuhs.edu/mic
https://medschool.usuhs.edu/mic/research/broder-lab

TEL: 301-295-3401
FAX: 301-295-3773

(b) (6)
[redacted]
fax - 301-295-3773

OMB No. 0925-0001 and 0925-0002 (Rev. 12/2020 Approved Through 02/28/2024)

Name of Individual: **Broder, Christopher**
Commons ID: (b) (6)

Other Support – Project/Proposal

ACTIVE

Title: Collaborative Development and Evaluation of a Human Subunit Vaccine (HeV-sG) to Protect Against Nipah and Hendra Viruses

Major Goals: This project will develop, characterize and test chimeric, luciferase encoding henipaviruses using CedV along with the fusion and attachment glycoproteins of Nipah virus and Hendra virus

Overlap: None. This Project supported the development of the chimeric recombinant CedV serum neutralization test platform to be supplied to AuroVaccines LLC.

Status of Support: Active
Project Number: N/A
Name of PD/PI: Hamm, Stefan.
Source of Support: Subward from Profectus (Now AuroVaccines LLC) / CEPI CRADA
Primary Place of Performance: USUHS, Bethesda, MD
Project/Proposal Start and End Date: (MM/YYYY): 01/30/2019 – 01/29/2024
Total Award Amount (including Indirect Costs): $796,308
Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
4, 5. 2023	1.2 calendar
5. 2024	0.1 calendar

Title: Serological Biosurveillance for Spillover of Henipaviruses and Filoviruses at Agricultural and Hunting Human-Animal Interfaces in Peninsular Malaysia

Major Goals: The overarching goal is to characterize the distribution and detect the spillover of henipaviruses and filoviruses among indigenous farming and hunting communities in Peninsular Malaysia.

Status of Support: Active
Project Number: 07-103-7096
Name of PD/PI: Daszak, P.
Source of Support: Subward from EcoHealth Alliance / DTRA
Primary Place of Performance: USUHS, Bethesda, MD
Project/Proposal Start and End Date: (MM/YYYY) 05/01/2017-04/30/2023
Total Award Amount (including Indirect Costs): $1,339,017
Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
7. 2023	0.3 calendar

Name of Individual: **Broder, Christopher**
Commons ID: (b) (6)

Title: Advancement of Vaccines and Therapies for Henipaviruses

Major Goals: The primary objective of the Center is to perform pivotal studies that will facilitate the development of products used for the prevention and treatment of Nipah and Hendra infections. All three RPs, and Center Cores: Administrative; human monoclonal antibody; and Biosafety Level (BSL)-4 work together to provide broadly effective countermeasures.

Overlap: None. This program is focused on vaccine and antiviral monoclonal antibody countermeasure development. It uses recombinant CedV and recombinant CedV chimeras to measure virus neutralizing potencies of antibodies and to develop novel vaccine platforms and small animal models. Not antiviral drug discovery.

Status of Support: Active

Project Number: U19AI142764

Name of PD/PI: Broder, C.

Source of Support: NIAID/NIH (CETR)

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 03/01/19 – 02/28/24

Total Award Amount (including Indirect Costs): $24,587,556

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
4, 5. 2023	1.2 calendar – Project 0.6 calendar – Core Admin
5. 2024	1.2 calendar – Project 0.6 calendar – Core Admin

Title: Biosurveillance for Spillover of Henipaviruses and Filoviruses in Rural Communities in India

Major Goals: The major goals of this project are to enhance early detection and surveillance capacity in India.

Status of Support: Active

Project Number: HDTRA12010026

Name of PD/PI: Epstein, Jonathan

Source of Support: Subaward from EcoHealth Alliance / DTRA

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 10/01/2020-9/30/2023

Total Award Amount (including Indirect Costs): $175,000

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2023	0.6 calendar

Name of Individual: **Broder, Christopher**
Commons ID: (b) (6)

Title: Understanding Risk of Zoonotic Virus Emergence in EID Hotspots of Southeast Asia

Major Goals: The major goals of this project is to develop multidisciplinary teams of investigators in the program will conduct pathogen/host surveillance, study pathogen transmission, pathogenesis and immunologic responses in the host, and will develop reagents and diagnostic assays for improved detection for important emerging pathogens and their vectors.

Status of Support: Active

Project Number: U01AI151797 NIH/NIAID/CREID 07-049-7012-52338

Name of PD/PI: Daszak, P.

Source of Support: Subaward from EcoHealth Alliance / CREID, NIAID, NIH

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 06/17/2020-05/31/2024

Total Award Amount (including Indirect Costs): $136,903

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2023, 2024	0.6 calendar
4. 2024	0.6 calendar

Title: Furopyrimidines as novel inhibitors of henipaviruses

Major Goals: The major goals of this project are to discover, refine and optimize antiviral molecules from lead chemical hits, to improve antiviral activities against henipaviruses using CedV.

Status of Support: Active
Project Number: R41AI157095

Name of PD/PI: Rong, Lijun

Source of Support: Subaward from Chicago BioSolutions, Inc. / NIAID, NIH

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 01/01/2021-12/31/2023

Total Award Amount (including Indirect Costs): $45,750

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2023	0.5 calendar

Name of Individual: **Broder, Christopher**
Commons ID: (b) (6) [redacted]

Title: Biosurveillance for Viral Zoonoses and Bat-Livestock-Human interfaces in South Africa

Major Goals: This project will focus on building capacity and enhancing surveillance, including early detection programs for henipa-, filo- and zoonotic coronaviruses in Southern Africa.

Status of Support: Active

Project Number: HDTRA12010025

Name of PD/PI: Markotter, W.

Source of Support: Subaward from University of Pretoria / DTRA

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 07/31/2020-07/30/2025

Total Award Amount (including Indirect Costs): $1,120,965

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2023	0.5 calendar
4. 2024	0.5 calendar
5. 2025	0.5 calendar

Title: Study of Nipah virus dynamics and genetics in its bat reservoir and of human exposure of NiV across Bangladesh to understand patterns of human outbreaks

Major Goals: This project will use serological and genetic surveillance approaches to detect the presence of Nipah virus in serological samples by anti-Nipah virus antibody detection assays in human and wildlife samples from different locals and seasonal periods to improve knowledge of virus location and emergence dynamics.

Status of Support: Active

Project Number: U01AI153420

Name of PD/PI: Epstein, Jonathan

Source of Support: subaward from EcoHealth Alliance / NIAID, NIH

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 11/15/2020-06/30/2025

Total Award Amount (including Indirect Costs): $45,939

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
4. 2023, 2024	0.5 calendar
5. 2024, 2025	0.5 calendar

Name of Individual: **Broder, Christopher**
Commons ID: (b) (6)

Title: Recombinant HeV-sG Vaccine Formulation and Delivery via Microneedle Patch

Major Goals: This project will use recombinant soluble Hendra virus G antigen protein and test the feasibility of a novel immunization and vaccine platform to elicit anti-Hendra/Nipah virus antibodies in animal models.

Status of Support: Active

Project Number: 011882

Name of PD/PI: Broder, C.

Source of Support: Uniformed Services University

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 11/30/2021-11/29/2024 (pending NCE)

Total Award Amount (including Indirect Costs): $2,575,800

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2023	1.2 calendar
3. 2024	1.2 calendar

Title: Efficacy testing of a novel human monoclonal antibody therapy for late-stage rabies/lyssavirus infection

Major Goals: The major goals of this project is to test the efficacy of mAb therapy at times beyond day seven post-infection, to more completely define the degree to which mAb therapy can confer protection from death post-onset of clinically evident severe neurological disease.

Status of Support: Active

Project Number: HU00011920118

Name of PD/PI: Broder, C.

Source of Support: CGHE / USUHS

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 09/30/2019-09/29/2024 (pending NCE)

Total Award Amount (including Indirect Costs): $518,000

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2022, 2023	0.6 calendar
4. 2023,2024	0.6 calendar

Name of Individual: **Broder, Christopher**
Commons ID: (b) (6)

Title: UTMB-Novartis Alliance for Pandemic Preparedness (UNAPP). (An NIH AViDD Center)

Sub-project 3: Henipaviruses.

Major Goals: This project will use recombinant Cedar virus-based reporters to screen chemical libraries for antiviral activities; then tool compound generation, target identification, and lead compound optimization; then evaluate lead compounds in animal models and select Development Candidate (DC).

Status of Support: Active

Project Number: U19 AI171413

Name of PD/PI: Diagana, Thierry

Shi, Pei-Yong

Source of Support: NIAID-NIH; subaward from University of Texas Med. Br. Galveston, TX

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 05/01/2022-04/30/2027

Total Award Amount (including Indirect Costs): $2,885,380

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1, 2. 2023	0.6 calendar
2, 3. 2024	0.6 calendar
3, 4. 2025	0.6 calendar
4,5. 2026	0.6 calendar
5. 2027	0.6 calendar

Title: Informing biosurveillance: contribution of pteropdid fruit bats to virus spillover in the Philippines

Major Goals: This project aims to characterize viruses shedding pulses and polymicrobial infection dynamics; investigate seasonal and temporal variation of host immune status; and conduct risk assessment of bat-borne viruses spillover to incidental hosts.

Status of Support: Pending

Project Number: N/A

Name of PD/PI: Laing, E.

Source of Support: DTRA / Duke NUS

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 05/01/2022-04/30/2027

Total Award Amount (including Indirect Costs): $1,213,743

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	1 calendar
1, 2. 2023	1 calendar
2, 3. 2024	1 calendar
3, 4. 2025	1 calendar
4,5. 2026	1 calendar
5. 2027	1 calendar

Name of Individual: **Broder, Christopher**
Commons ID: (b) (6)

Title: Establishment of a Bat Resource for Infectious Disease Research

Major Goals: The major goals of this proposal is to contribute significant insight into the viral determinants that confer henipaviral pathogenesis. The construction and generation of recombinant henipavirus chimeric viruses will be the primary means for determining how virus receptor usage and the expression of virulence factors act individually or in conjunction to cause limited or severe disease. The recombinant constructs generated from the proposed studies will also be used to determine host determinants of immunity in Jamaican fruit bats and animal models.

Status of Support: Pending

Project Number: R24AI165424

Name of PD/PI: Schountz, T.

Source of Support: Colorado State University (NIH / NIAID flow through)

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY): 01/01/2023-12/30/2027

Total Award Amount (including Indirect Costs): $695,937

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	0.5 calendar
2. 2024	0.5 calendar
3. 2025	0.5 calendar
4. 2026	0.5 calendar
5. 2027	0.25 calendar

IN-KIND

NONE

OVERLAP

There is no overlap with the pending awards with any of the projects listed above. A statement addressing overlap is made above under each active and pending item of support.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

*Signature: (b) (6)

ALL PERSONNEL REPORT

Place this form at the end of the signed original copy of the application. Do not duplicate.

GRANT NUMBER
U19AI171413

Always list the PD/PI(s). In addition, list all other personnel who participated in the project during the current budget period for at least one person month or more, regardless of the source of compensation (a person month equals approximately 160 hours or 8.3% of annualized effort). Use the following abbreviated categories for describing Role on Project:

- **PD/PI**
- **Co-Investigator**
- **Faculty**
- **Postdoctoral (scholar, fellow, or other postdoctoral position)**
- **Technician**
- **Staff Scientist (doctoral level)**
- **Statistician**
- **Graduate Student (research assistant)**
- **Non-student Research Assistant**
- **Undergraduate Student**
- **High School Student**
- **Consultant**
- **Other (please specify)**

If personnel are supported by a Reentry or Diversity Supplement please indicate such after the Role on Project, using the following abbreviations: RS - Reentry Supplement; DS - Diversity Supplement.

Use Cal (calendar), Acad, or Summer to enter months devoted to project.

Commons ID	Name	Degree(s)	SSN (last 4 digits)	Role on Project	DoB (MM /YY)	Cal	Acad	Summer
(b) (6)	Christopher Broder	Ph.D.		Site Principal Investigator		.6		
(b) (6)	Moushimi Amaya	Ph.D.		Co-Investigator		2		
N/A	Lianying Yan			Lead, Manager Recombinant Prot		.20		
N/A	Madison Thammavong			Research Assistant I		.38		
N/A	Alka Jays			Research Associate I		.18		
(b) (6)	Antony Dimitrov	Ph.D.		Senior Scientist		.10		
N/A	Julia Paoli			Research Assistant I		.10		
N/A	Randy Yin			Research Assistant I		.35		
(b) (6)	Kimberly Boxley			Program Manager III		.10		

Name of Individual: Amaya, Moushimi
Commons ID: (b) (6) [redacted]

Other Support – Project/Proposal

ACTIVE

Title: Advancement of Vaccines and Therapies for Henipaviruses

Major Goals: The primary objective of the Center is to perform pivotal studies that will facilitate the development of products used for the prevention and treatment of Nipah and Hendra infections. All three RPs, and Center Cores: Administrative; human monoclonal antibody; and Biosafety Level (BSL)-4 work together to provide broadly effective countermeasures.

Overlap: None. This program is focused on vaccine and antiviral monoclonal antibody countermeasure development. It uses recombinant CedV and recombinant CedV chimeras to measure virus neutralizing potencies of antibodies and to develop novel vaccine platforms and small animal models. Not antiviral drug discovery.

Status of Support: Active

Project Number: U19AI142764

Name of PD/PI: Broder, C.

Source of Support: NIAID/NIH (CETR)

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY) (if available): 03/01/19 – 02/28/24

Total Award Amount (including Indirect Costs): $24,587,556

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
5. 2023	3.0 calendar – Project
6. 2024	3.0 calendar – Project

Title: Furopyrimidines as novel inhibitors of henipaviruses

Major Goals: The major goals of this project are to discover, refine and optimize small molecules from lead chemical hits to improve antiviral activities against henipaviruses using recombinant Cedar virus.

Overlap: None. There is no relation to the pending award with UTMB/Novartis which will be using recombinant CedV to screen new and proprietary drug libraries from Novartis.

Status of Support: Active

Project Number: HR41AI157095

Name of PD/PI: Broder, C.

Source of Support: Sub-award from Chicago BioSolutions, Inc. / NIAID, NIH

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/01/2021-12/31/2023

Total Award Amount (including Indirect Costs): $45,750

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2023	0.5 calendar

Title: UTMB-Novartis Alliance for Pandemic Preparedness (UNAPP). (An NIH AViDD Center)

Sub-project 3: Henipaviruses.

Major Goals: This project will use recombinant Cedar virus-based reporters to screen chemical libraries for antiviral activities; then tool compound generation, target identification, and lead compound optimization; then evaluate lead compounds in animal models and select Development Candidate (DC).

Status of Support: Pending

Project Number: N/A

Name of PD/PI: Broder, C.

Source of Support: NIAID-NIH; Sub-award from University of Texas Med. Br. Galveston, TX

Primary Place of Performance: USUHS, Bethesda, MD

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/01/2022-04/30/2027

Total Award Amount (including Indirect Costs): $2,885,380

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2023	6 calendar
3. 2024	3 calendar
4. 2025	3 calendar
5. 2026	3 calendar
6. 2027	3 calendar

IN-KIND

NONE

OVERLAP

There is no overlap with the pending awards with any of the projects listed above. A statement addressing overlap is made above under each active and pending item of support.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

* SIGNATURE: (b) (6)

* DATE: May 01, 2023

From: Tamera Porter Wilmot on behalf of Tamera Porter Wilmot <TPorterWilmot@hjf.org>
To: argoldbe@utmb.edu
Cc: Kimberly Boxley; CHRISTOPHER BRODER; Antony Dimitrov; Geisbert, Thomas W.; Haller, Sherry; Yongkang Qiu
Subject: RE: [EXTERNAL] Fwd: Revised Format for the Annual Progress Report for 1U19AI171413-01
Date: Monday, May 1, 2023 1:24:43 PM
Attachments: Other Support_Broder Dec 2022 v5-ccb.pdf
All Personnel Report 67058.pdf
Other Support_Amaya v5_signed.pdf
image001.png

Good Afternoon,

Please find the completed all personnel report and signed other support documents for Drs. Broder and Amaya. Please let us know if any additional information is needed.

Thank you,

Tammy

Tamera Porter Wilmot - *Currently teleworking*
Grants Specialist - Office of Sponsored Programs
Research Administration and Innovation Management (RAIM)
Phone: 240-694-2008
Email: tporterwilmot@hjf.org

Henry M. Jackson Foundation for the
Advancement of Military Medicine
6720A Rockledge Drive, Suite 100
Bethesda, MD 20817

> ---------- Forwarded message ---------
> From: **Goldberg, Alisha R.** <argoldbe@utmb.edu>
> Date: Wed, Apr 12, 2023 at 1:41 PM
> Subject: RE: Fw: URGENT: Revised Format for the Annual Progress Report for 1U19AI171413-01
> To: Boxley, Kimberly <kimberly.boxley.ctr@usuhs.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
> Cc: Geisbert, Thomas W. <twgeisbe@utmb.edu>, Haller, Sherry <shhaller@utmb.edu>
>
> Thanks – I talked to Kit at Novartis, who is compiling input for the Project 3 report. I think that she is on track getting the input she needs for Form Page 5 by next week and we'll handle Form pages 1, and 6. We will need updated Other Support if either Chris or Moushimi have changes to report. And I'm checking to make sure, but I think we'll have to ask you to fill out Form page 7 – the All Personnel Report. For those two pieces, we've set the week of May 1st as a target date.
>
> Alisha

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Wednesday, April 12, 2023 11:45 AM
To: Boxley, Kimberly <kimberly.boxley.ctr@usuhs.edu>; CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Goldberg, Alisha R. <argoldbe@UTMB.EDU>; Haller, Sherry <shhaller@UTMB.EDU>
Cc: Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: Fw: URGENT: Revised Format for the Annual Progress Report for 1U19AI171413-01

Bringing Alisha and Sherry into the loop on this.

From: Boxley, Kimberly <kimberly.boxley.ctr@usuhs.edu>
Sent: Wednesday, April 12, 2023 11:19 AM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; Schuenke, Kimberly <kischuen@utmb.edu>; Shi, Pei yong <peshi@UTMB.EDU>
Cc: Antony Dimitrov <antony.dimitrov.ctr@usuhs.edu>
Subject: Re: Fw: URGENT: Revised Format for the Annual Progress Report for 1U19AI171413-01

Hello all,

I don't see a due date for us to have these materials to you. I inquired previously but want to make sure we are on time.

Thanks much .... Kim

On Thu, Mar 2, 2023 at 10:30 AM Broder, Christopher <christopher.broder@usuhs.edu> wrote:

> fysa
>
> ---------- Forwarded message ---------
> From: **Geisbert, Thomas W.** <twgeisbe@utmb.edu>
> Date: Thu, Mar 2, 2023 at 10:26 AM
> Subject: Fw: URGENT: Revised Format for the Annual Progress Report for 1U19AI171413-01
> To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, Haller, Sherry <shhaller@utmb.edu>, Stutz, Sonja J. <sjstutz@utmb.edu>
>
> FYI
>
> ---
>
> **From:** Shi, Pei yong <peshi@UTMB.EDU>

Sent: Thursday, March 2, 2023 9:24 AM
To: Chen, Ping (NIH/NIAID) [E <chenpi@niaid.nih.gov>
Cc: Kitsoulis, Regina (NIH/NIAID) [E <regina.kitsoulis@nih.gov>; Inbox <Inbox2@UTMB.EDU>; Diagana, Thierry <thierry.diagana@novartis.com>; Menachery, Vineet <vimenach@UTMB.EDU>; Jarrousse, Nadine <nadine.jarrousse@novartis.com>; Goldberg, Alisha R. <argoldbe@UTMB.EDU>; Chan, Kit <kit.chan@novartis.com>; Xie, Xuping <xuxie@UTMB.EDU>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Subject: FW: URGENT: Revised Format for the Annual Progress Report for 1U19AI171413-01

Dear Dr. Chen,

Thank you for the new information about the AViDD progress report. We will prepare accordingly.

Best, Pei-Yong

Pei-Yong Shi, Ph.D.

John Sealy Distinguished Chair in Innovations in Molecular Biology

Director, Institute for Drug Discovery

Associate Chief Research Officer

Vice President for Research Innovation

University of Texas Medical Branch at Galveston

Phone: 409-772-6370

From: Chen, Ping (NIH/NIAID) [E] <chenpi@niaid.nih.gov>
Sent: Thursday, March 2, 2023 9:14 AM
To: Shi, Pei yong <peshi@UTMB.EDU>
Cc: Kitsoulis, Regina (NIH/NIAID) [E] <regina.kitsoulis@nih.gov>; Inbox <Inbox2@UTMB.EDU>; Inbox <Inbox2@UTMB.EDU>
Subject: URGENT: Revised Format for the Annual Progress Report for 1U19AI171413-01
Importance: High

Dear Dr. Shi and Authorized Institutional Official,

NIH has identified issues with the submission of Multi-Year Funded Research Performance Progress Reports (RPPRs) using the eRA Commons, consequently **all AViDD awardees must now use the PHS 2590 format** instead of the eRA Commons to prepare and submit the Annual Progress Report for Year 1. **Please disregard the instructions previously transmitted via the email sent in November 2022**.

Please see the attached instructions for the PHS 2590 format and the Revised AViDD Progress Report Guidelines. The additional information being requested in the Revised AViDD Progress Report Guidelines is the same as the previous version, however, for the PHS 2590 format you will now provide this information using Form page 5 instead of sections noted in the previous version.

The PHS 2590 forms can be downloaded from: https://grants.nih.gov/grants/funding/2590/2590.htm

The Annual Progress Report will contain separate reports for each component of your AViDD. You will create a separate PHS 2590 report for the AViDD Overall progress, each Research Project, and each Core, and these will be combined to generate the Annual Progress Report.

The separate reports for each component will contain:

Form Page 1: Face Page – leave Items 8a and 8b blank

Form Page 1-continued: Additional form if Multiple PIs are involved

Project/Performance Site Format Page - use only if additional space is needed

Biographical Sketch: Complete a Biographical Sketch for all new senior/key personnel since the previous submission. Use form available from https://grants.nih.gov/grants/forms/biosketch.htm

Other Support: Include other active support for all senior/key personnel whose support has changed during the past year and indicate what the change has occurred. Use form available from https://grants.nih.gov/grants/forms/othersupport.htm

Form Page 5: Progress Report Summary - this form will be used to provide both the additional information required for AViDD awards as well as the standard information required for a Progress Report. Please refer to the Revised AViDD Progress Report Guidelines for the additional information required to be included in the Progress Summary for the AViDD Overall, Research Project and Core reports.

Use the Continuation page after Form page 5 is full for this section and any other sections that require additional pages after the initial form.

Form Page 6: Checklist

Form Page 7: All Personnel Report

If Human Subjects Research is being conducted - include the PHS Inclusion Enrollment Report

Please refer to the attached Revised AViDD Progress Report Guidelines and the phs2590 General Instructions found on pages 5 through 17 for guidance about the information required for each section.

Because your AViDD award is a Multi-Year Funded award you are not required to submit Detailed Budget for Next Budget Period or Budget Justification pages as part of the Annual Progress Report. In addition, carry forward requests are not required during the remaining time of the Multi-Year Funded project period. However, if the situation arises, please note, prior approval is still required for compliance with the significant rebudgeting policy as stated in the NIH Grants Policy Statement https://grants.nih.gov/policy/nihgps/index.htm:

Significant rebudgeting, whether or not the particular expenditure(s) require prior approval: Significant rebudgeting occurs when expenditures in a single direct cost budget category deviate (increase or decrease) from the categorical commitment level established for the budget period by 25 percent or more of the total direct costs awarded. For example, if the award budget for total direct costs is $200,000, any rebudgeting that would result in an increase or decrease of more than $50,000 in a budget category is considered significant rebudgeting

To submit the report, your Authorized Institutional Official will email the entire Annual Progress Report to Regina Kitsoulis, who is the NIAID Grants Management Official for this award, and me.

These reports are due by the project anniversary date (05/16/2023) for your award and can be submitted anytime between now and then. As noted above, please read through the Revised AViDD Report Guidelines and the PHS 2590 instructions. If questions remain, then please have your business official contact Regina Kitsoulis and me.

We apologize for the inconvenience.

Sincerely yours,

Ping

Ping Chen PhD

Program Officer

Drug Development Section

Office of Biodefense Research Resources and Translational Research

Division of Microbiology and Infectious Diseases

National Institute of Allergy and Infectious Diseases

National Institutes of Health

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Department of Health and Human Services

Part 1. Overview Information

Participating Organization(s)

National Institutes of Health (NIH (http://www.nih.gov))

Components of Participating Organizations

National Institute of Allergy and Infectious Diseases (NIAID (https://www.niaid.nih.gov/))

Funding Opportunity Title

Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers for Bunyavirales, Paramyxoviridae and Picornaviridae (U19 Clinical Trial Not Allowed)

Activity Code

U19 (//grants.nih.gov/grants/funding/ac_search_results.htm?text_curr=u19&Search.x=0&Search.y=0&sort=ac&Search_Type=Activity&text_prev=) Research Program – Cooperative Agreements

Announcement Type

New

Related Notices

NOT-OD-22-189 (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-22-189.html) - Implementation Details for the NIH Data Management and Sharing Policy

NOT-OD-22-195 (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-22-195.html) - New NIH "FORMS-H" Grant Application Forms and Instructions Coming for Due Dates on or after January 25, 2023

NOT-OD-22-198 (https://grants.nih.gov/grants/guide/notice-files/not-od-22-198.html) - Implementation Changes for Genomic Data Sharing Plans Included with Applications Due on or after January 25, 2023

NOT-OD-23-012 (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-23-012.html) - Reminder: FORMS-H Grant Application Forms & Instructions Must be Used for Due Dates On or After January 25, 2023 - New Grant Application Instructions Now Available

Funding Opportunity Announcement (FOA) Number

RFA-AI-23-020

Companion Funding Opportunity

RFA-AI-23-019 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-019.html), U19 (https://grants.nih.gov/grants/funding/ac_search_results.htm?text_curr=U19&&Search.x=0&&Search.y=0&&Search_Type=Activity) Research Program (Cooperative Agreement)
RFA-AI-23-021 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-021.html), UG3 (https://grants.nih.gov/grants/funding/ac_search_results.htm?text_curr=UG3&&Search.x=0&&Search.y=0&&Search_Type=Activity)/ UH3 (https://grants.nih.gov/grants/funding/ac_search_results.htm?text_curr=UH3&&Search.x=0&&Search.y=0&&Search_Type=Activity) Phase 1 Exploratory/Developmental Cooperative Agreement/Exploratory/Developmental Cooperative Agreement Phase II

Number of Applications

See Section III. 3. Additional Information on Eligibility.

Assistance Listing Number(s)

93.855

Funding Opportunity Purpose

This Funding Opportunity Announcement (FOA) solicits applications to participate in the Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Network. The purpose of this FOA is to establish comprehensive, cooperative basic and translational research Centers to carry out in-depth research on prototype members of select virus families that have the potential to emerge as pandemic pathogens. The goal of these Centers will be to develop vaccine and monoclonal antibody strategies for prototype pathogen(s) that can be applied to closely related family members based on shared functional and structural properties. This FOA solicits for centers proposing research on virus families from Bunyavirales, Paramyxoviridae and Picornaviridae to be part of the ReVAMPP Network.

Key Dates

Posted Date

March 16, 2023

Open Date (Earliest Submission Date)

May 08, 2023

Letter of Intent Due Date(s)

30 days prior to the application due date

Application Due Dates			Review and Award Cycles		
New	**Renewal / Resubmission / Revision (as allowed)**	**AIDS**	**Scientific Merit Review**	**Advisory Council Review**	**Earliest Start Date**
June 08, 2023	Not Applicable	Not Applicable	November 2023	January 2024	March 2024

All applications are due by 5:00 PM local time of applicant organization.

Applicants are encouraged to apply early to allow adequate time to make any corrections to errors found in the application during the submission process by the due date.

No late applications will be accepted for this Funding Opportunity Announcement.

Expiration Date

June 09, 2023

Due Dates for E.O. 12372

Not Applicable

Required Application Instructions

It is critical that applicants follow the Multi-Project (M) Instructions in the SF424 (R&R) Application Guide (https://grants.nih.gov/grants/guide/url_redirect.htm?id=82400), except where instructed to do otherwise (in this FOA or in a Notice from the *NIH Guide for Grants and Contracts* (//grants.nih.gov/grants/guide/url_redirect.htm?id=11164)). Conformance to all requirements (both in the Application Guide and the FOA) is required and strictly enforced. Applicants must read and follow all application instructions in the Application Guide as well as any program-specific instructions noted in Section IV. When the program-specific instructions deviate from those in the Application Guide, follow the program-specific instructions. **Applications that do not comply with these instructions may be delayed or not accepted for review.**

There are several options available to submit your application through Grants.gov to NIH and Department of Health and Human Services partners. You **must** use one of these submission options to access the application forms for this opportunity.

1. Use the NIH ASSIST system to prepare, submit and track your application online.

   Apply Online Using ASSIST

2. Use an institutional system-to-system (S2S) solution to prepare and submit your application to Grants.gov and eRA Commons (https://public.era.nih.gov/commons/) to track your application. Check with your institutional officials regarding availability.

Table of Contents

Part 2. Full Text of Announcement

Section I. Funding Opportunity Description

The National Institute of Allergy and Infectious Diseases (NIAID) supports complementary research programs to understand, control and prevent viral diseases and related pandemics. As part of pandemic preparedness planning, this Funding Opportunity Announcement (FOA) solicits applications to establish comprehensive, cooperative basic and translational research centers to 1) advance scientific knowledge needed to develop vaccines and monoclonal antibodies (mAbs) for prototype viral pathogens within virus families that have pandemic potential; and 2) leverage this information to develop and evaluate generalizable approaches for vaccines and mAbs for the prototype pathogens and other related family members based on shared functional and structural properties. Centers proposing research on select virus families from Bunyavirales, Paramyxoviridae and Picornaviridae are included in this FOA.

The ReVAMPP Network is comprised of ReVAMPP Centers from both this RFA (Bunyavirales, Paramyxoviridae and Picornaviridae) and its companion FOAs soliciting for ReVAMPP Centers focusing on Flaviviridae and Togaviridae (see RFA-AI-23-019 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-019.html)) and the ReVAMPP Coordinating and Data Sharing Center (CDSC) (see RFA-AI-23-021 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-021.html)). ReVAMPP Centers will conduct independent research project(s) and are expected to share information and collaborate within the Network under the direction of the ReVAMPP CDSC which is responsible for establishing and maintaining a collaborative ReVAMPP Network platform for data sharing and overall collaboration among the ReVAMPP Centers.

In the event of an outbreak, the ReVAMPP Centers will be poised to leverage the expertise and resources within the network to assist in a coordinated research response. This new NIAID Network will align with the goals of the American Pandemic Preparedness Plan: Transforming Our Capabilities (AP3) (https://www.whitehouse.gov/wp-content/uploads/2021/09/American-Pandemic-Preparedness-Transforming-Our-Capabilities-Final-For-Web.pdf), which was announced in September 2021, and recognizes the need for a trans-government investment and response to combat future pandemics and NIAID's Pandemic Preparedness Plan (https://www.niaid.nih.gov/sites/default/files/pandemic-preparedness-plan.pdf).

Background

The emergence and re-emergence of infectious diseases continues to threaten the health of Americans and people worldwide. Over the past two decades, the public health community has responded to emerging infectious diseases including those caused by Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-1), the 2009 H1N1 influenza virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Ebola virus, Zika virus, and most recently, SARS-CoV-2. The global pandemic caused by SARS-CoV-2 further underscores the continual threat of newly emerging and re-emerging pathogens and the critical value of basic and translational research for pandemic preparedness. The unprecedented rapid development of vaccines and mAbs for SARS-CoV-2 was enabled by decades of foundational research on related coronaviruses which allowed scientists to quickly and effectively respond once SARS-CoV-2 emerged. In recent years, considerable resources have been invested in vaccine and mAb development for coronavirus and influenza viruses to prepare for the next pandemic from these families. However, viruses from other families also propose substantial risk of causing a pandemic. Thus, continuing to build a robust basic research portfolio and advancing translational science for other viral families with pandemic potential is essential for biomedical countermeasure preparedness. In addition to known threats, effective preparedness must also account for unexpected emerging disease threats. To mitigate risks associated with these yet-unknown pathogens, NIAID's intent for the ReVAMPP Network is to promote focused research needed to develop vaccines and mAbs for prototype-pathogens from viral families known to infect humans (Graham BS and Corbett KS J Clin Invest. 2020; 130(7):3348-3349 (https://www.jci.org/articles/view/139601), Cassetti MC, et al., JID. D022; jiac296). (https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiac296/6649664?login=true) As defined in this FOA, a prototype pathogen is a representative virus from which research on the virology, pathology and immunology of the prototype will generate generalizable knowledge, and in turn vaccine and mAb strategies, which can be applied to other members of the viral family. As the prototype pathogen approach proposes, viruses are organized into families based upon shared functional and structural similarities and thus candidate vaccine strategies developed against a prototype pathogen may similarly work against other members in the same family. Through targeted basic and applied research on these prototype pathogens, a solid foundation of knowledge will enable a rapid response when a previously unknown (or known but understudied) pathogen emerges or spreads from any of the known high-risk viral families. This anticipatory approach will increase the knowledge base needed for preparedness and enable rapid development and translation of candidate vaccines and mAbs into clinical trials and large-scale production.

Research Objectives and Scope

The objective of this FOA is to establish multidisciplinary research Centers to be part of the highly collaborative ReVAMPP Network focused on in-depth basic and translational research on prototype members of certain virus families that have the potential to emerge as pandemic pathogens, namely Paramyxoviridae, Picornaviridae and Bunyavirales including Arenaviridae, Hantaviridae, Nairoviridae, Phenuiviridae, and Peribunyaviridae. The major goal of these Centers will be to develop vaccine strategies for prototype pathogens that can be applied to other closely related family members based on shared functional and structural properties. Additionally, these Centers may perform basic research to expand foundational knowledge of virology, pathology, and immunology, or perform early development activities for mAbs for prototype viruses. Towards these goals, each Center will encompass a multi-project multidisciplinary research program that employs innovative virology, structural biology, and immunology to identify strategies for vaccine design. Each Center will collaborate across the ReVAMPP Network through the sharing of data, reagents, protocols, and animal models to facilitate advancement toward pandemic preparedness for all virus families.

Priority Viral Families for Pandemic Preparedness

A prototype pathogen is a representative virus from which research on the virology, pathology and immunology of the prototype will generate generalizable knowledge, and in turn vaccine and mAb strategies, which can be applied to other members of the virus family. For this FOA, each ReVAMPP Center will support a multi-project research program directed towards enabling basic and translational research and development of vaccines, and optionally early development of mAbs, against prototype viruses from one or more of the following selected virus families of pandemic potential, and Centers are encouraged to work on more than one virus family (listed alphabetically, all of equivalent priority):

- Bunyavirales
    - Arenaviridae (e.g., Lassa virus, Junin virus)
    - Hantaviridae (e.g., Andes virus, Sin Nombre virus, Hantaan orthohantavirus virus)
    - Nairoviridae (e.g., Crimean-Congo Hemorrhagic Fever virus (CCHF), Hazara virus)
    - Phenuiviridae (e.g., Rift Valley Fever virus, Severe Fever with thrombocytopenia syndrome virus, Punta Toro virus)
    - Peribunyaviridae (e.g., LaCrosse virus (LAC), Cache Valley virus)
- Paramyxoviridae (e.g., Menangle virus, HPIV1, HPIV3, Canine Distemper virus, Cedar virus)
- Picornaviridae (e.g., Enterovirus A71, Enterovirus D68, Echovirus B29, Rhinovirus C)

Centers are encouraged to include research projects focused on multiple virus families and may include a single prototype or multiple prototypes from a family. The investigators will determine which virus should serve as a prototype for a given family, but prototype selection must be justified. Considerations may include, but are not limited to, how well the prototype reflects properties shared by other members of the family, whether a single prototype is sufficient or whether multiple prototypes are needed to address differences amongst family members, the ease of working with the prototype virus, and whether vaccines against the prototype would have potential value for current public health needs.

These selected priority virus families of pandemic potential were identified by NIAID based on the ability to infect humans, the potential to cause a pandemic, and the current resources invested. The Coronaviridae and Orthomyxovirdae virus families were not included in this list as vaccine development and preparedness research for these families is supported through other mechanisms such as NOT-AI-21-002: Emergency Awards: Notice of Special Interest (NOSI) on Pan-Coronavirus Vaccine Development Program Projects (https://grants.nih.gov/grants/guide/notice-files/NOT-AI-21-002.html) and NIAID Centers of Excellence for Influenza Research and Surveillance (https://www.niaidceirs.org/), among others. In November 2021, NIAID convened a workshop titled "NIAID Workshop on Pandemic Preparedness: The Prototype Pathogen Approach to Accelerate Medical Countermeasures - Vaccines and Monoclonal Antibodies" where experts summarized current knowledge of the basic and translational research landscape, described research and intervention gaps, and proposed suitable prototype pathogens for further study and medical countermeasure development (Graham BS and Corbett KS J Clin Invest. 2020; 130(7):3348-3349 (https://www.jci.org/articles/view/139601), Cassetti MC, et al. JID. 2022;jiac296. (https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiac296/6649664)) This workshop highlighted the critical need to continue to expand basic research efforts and advance translational science for nine of the selected virus families. NIAID's intent for the ReVAMPP Network is to promote focused and coordinated research needed for the development of vaccines, and optionally early development of mAbs, for prototype-pathogens from virus families known to infect humans.

Research Areas

This FOA will support basic research such as virology studies to better understand cell tropism and receptor/entry requirements and to determine replication mechanisms, pathogenesis, and capacity for antigenic diversity. It will also support structural biology research to define the atomic-level details of surface proteins likely to be antigenic/immunologic targets. It will support research to assess the immune response in humans to natural infections and existing vaccines as well as determine correlates of immune protection, establish robust animal models, and develop reagents and new immunological assays.

This FOA will also support early and Investigational New Drug Application (IND)-enabling translational research for vaccines. Translational activities may include antigen/immunogen design and evaluation, screening technology platforms or adjuvants for immunogenicity and efficacy in animal models, development of assays and reagents, identification of correlates or surrogate markers of protection, lead optimization, stability, and manufacturability testing, and/or early process development. Once lead vaccine candidates/strategies have been identified, the same generalizable approach will be applied to other viruses within in the same family to validate the overall strategy for the virus family. Given the need to respond rapidly to emerging threats, the vaccine strategies should incorporate technologies that are amendable to antigen interchange and rapid manufacturing such as "plug and play" platforms.

The development of prototype vaccine strategies and structure-function characterization of the immune response to infection and vaccination is likely to result in the identification of mAbs with therapeutic potential. Thus, this FOA will support functional characterization of candidate mAbs and early translational activities for mAb development for prototype viruses. These activities may include discovery, *in vitro* characterization including epitope identification, neutralization potency, effector function analysis and structural studies, mAb optimization, determination of mechanism of action, *in vivo* evaluation including efficacy, dose titration, and route of administration studies in animal models, and candidate down-selection. If early mAb development is included, following the identification of lead mAb candidates for the prototype virus, it should be determined if antibodies with similar epitopes and/or properties are effective against other viruses within the same family.

Milestones

This FOA will utilize a bi-phasic, milestone-driven cooperative agreement award mechanism with Phase I consisting of the first 3 years, and Phase II consisting of years 4 and 5. Although applicants will apply for five years of funding, near the end of year 3 grantees will submit a transition package which will be evaluated by NIAID program staff for progress of research towards development of a generalizable vaccine strategy for the proposed viral family(ies), and if applicable early mAb development, and contribution to the ReVAMPP Network through data sharing and collaboration. The administrative review for funding of years 4 and 5 will be based on successful achievement of milestones included in the application and negotiated with the recipient prior to award, overall feasibility of program advancement,compliance with the ReVAMPP Network data sharing and CDSC requests, evidence of collaboration with other ReVAMPP Centers, programmatic priorities, and the availability of funding.

Industry Partnership

Each ReVAMPP Center is expected to have an established, or have plans to establish when appropriate, collaboration with an industry partner which will provide access to vaccine expertise in manufacturing, clinical development, and regulatory pathways. For Centers proposing IND-enabling translational research, an industry partnership is required. For the purpose of this FOA, "industry" is defined as a large or small, domestic, or foreign, pharmaceutical, biotechnology, or bioengineering company, or a related non-profit entity. The establishment of these public-private-partnerships is expected to extend the reach of the Center's comprehensive translational efforts, helping to ensure a focused, critical path through early-phase clinical trials for the most promising candidates. Centers will be encouraged to create partnerships/in-licensing opportunities and intellectual property strategies in compliance with NIH Intellectual Property Policy (https://grants.nih.gov/policy/intell-property.htm) to support advancing promising vaccine, and if applicable mAb, candidates into the clinic and to allow for hand-off to industry for advanced development. Centers are encouraged to develop and use intellectual property strategies that promote accessibility, similar to efforts from the World Health Organization (WHO), NIAID, and Bill & Melinda Gates Foundation (BMGF) to make COVID-19 vaccine and mAbs technologies accessible for the developing world (COVID-19 technology access pool (https://www.who.int/initiatives/covid-19-technology-access-pool)).

NIAID Resources

Each ReVAMPP Center is expected to provide lead candidates for comparative studies using NIAID's preclinical services or other gap filling mechanisms. It is anticipated that after award NIAID's comprehensive suite of preclinical services (Resources for Researchers | NIH: National Institute of Allergy and Infectious Diseases (https://www.niaid.nih.gov/research/resources)), NIAID's Division of Allergy, Immunology, and Transplantation (DAIT) programs (Adjuvant Discovery Program (https://www.niaid.nih.gov/research/adjuvant-discovery-program), B cell and T cell Epitope Discovery program, etc.) and repositories could be leveraged as needed to support ReVAMPP objectives including reagent storage/development, assay and animal model harmonization and evaluation, and development of lead vaccine and mAb candidates developed under this Network. NIAID program officials will connect ReVAMPP investigators to these services.

ReVAMPP Network interactions

Collaboration and data sharing among ReVAMPP Centers and with external partners is key to successful achievement of the ReVAMPP network goals. Therefore, each ReVAMPP Center must adopt FAIR data principles (https://www.go-fair.org/fair-principles/) as per the NIH Data Management Sharing Plan (DMSP (https://sharing.nih.gov/data-management-and-sharing-policy)) and manage and rapidly share data within the Network of ReVAMPP Centers under the direction of the ReVAMPP CDSC. This data will be shared confidentially within the Network to harmonize reagents, assays, and animal models and exchange knowledge on structure/function-based vaccine solutions and antigen/immunogen designs as well as assess the utility of vaccine technology platforms for virus families. To assist in the administration and management of information exchange, a separate ReVAMPP CDSC will be directing these efforts for the Network. The ReVAMPP CDSC will facilitate collaboration within and outside the Network, and each ReVAMPP Center will be expected to comply in accordance with network-wide timelines. As such, the CDSC will develop network-wide data sharing platforms and templates for all types of data generated by the ReVAMPP Centers. This may include information/data related to reagents, tools, assays, models, vaccine technology platforms, immune epitope design and/or correlates of protection. The CDSC will also develop, in conjunction with the Centers, a network wide ReVAMPP governance structure, provide guidance as to engagement with stakeholders within and outside the research centers, and collate information and facilitate exchange with other NIAID Programs, U.S. Government partners and other key stakeholders, including the WHO, BMGF, and Coalition for Epidemic Preparedness Innovations (CEPI), among others as appropriate.

ReVAMPP Center Structure

Each Center in the ReVAMPP Network will be organized around a multidisciplinary research program with interrelated projects focused on the prototype member(s) of virus families that have the potential to emerge as pandemic pathogens to inform a strategy for development of vaccines for the prototype pathogen and other closely related family members based on shared functional and structural properties, with the objective of translating research results to product development. Each Center is expected to include the following components:

Administrative Core

An Administrative Core will manage, coordinate, and supervise all Center activities under the direction of the Program Director(s)/Principal Investigator(s) (PD(s)/PI(s). The Administrative Core will also ensure seamless communication across the projects through regular meetings of Center participants and the ReVAMPP network as directed by the ReVAMPP CDSC. In addition, the Administrative Core will coordinate detailed communication of Center efforts and progress with NIAID program staff, including organizing annual ReVAMPP Center progress meetings with NIAID, and participating and assisting with ReVAMPP Network meetings as necessary virtually and/or at NIAID. The Administrative Core will also be responsible for leading coordination and collaboration efforts with the CDSC and other Centers within the Network and ensuring the Center program complies with requests from the CDSC and NIAID program staff.

Scientific Advisory Board

Each Center will include a Scientific Advisory Board (SAB) that will act as an independent, external advisory body for the PD(s)/PI(s) but will not be involved in the day-to-day activities of the Center. The SAB will facilitate Go/No-Go decision making and recommend new research directions as appropriate. The SAB will participate in annual ReVAMPP Center progress meetings at NIAID to review Center activities and evaluate progress, adherence to milestones and timelines, and the continued relevance of each Research Project, including those within industry partnerships, to the overall Center objective(s). If requested by the PD(s)/PI(s) and NIAID Project Scientist, the SAB will provide a summary written evaluation of the group's activities and recommendations following the annual ReVAMPP Center progress meeting. The SAB will include at least 5 non-conflicted external advisors. Centers must have at least 2 of the external advisors who have demonstrated and relevant industry-level expertise. SAB membership will be established in consultation with NIAID program staff. *Potential external SAB members MUST NOT be named in the application or contacted prior to completion of review activities.*

Data Management Core

A Data Management Core will be responsible for housing data generated by the Center and managing the transfer of data within the Center and to the ReVAMPP Network as directed by the ReVAMPP CDSC, including the upload of data to the ReVAMPP Network data sharing platforms. The Data Management Core will also be responsible for collating and collecting information from the Center as requested by the ReVAMPP CDSC or NIAID program staff in accordance with network-wide timelines, and ensuring the Center complies with network data sharing policies.

Scientific Cores

A Center may include up to three Scientific Cores to support resources and/or facilities that are essential for the activities of two or more Research Projects, but inclusion of Scientific Cores is not required. Scientific Cores are intended to only serve the needs of Center project researchers and they may not conduct research independent of the Research Projects. In lieu of a Scientific Core, use of existing institutional core facilities may be included in specific Research Projects.

Research Projects

Each Center must include at least 2 and no more than 5 interdependent Research Projects focused on prototype members of the priority virus families of pandemic potential listed above. At least one Research Project must focus on development of vaccines, and other Research Projects may focus on additional vaccine development, early mAb development or foundational research in virology, immunology, pathology, and structural biology necessary for such development. Each Research Project must clearly and directly contribute to the Center's approach and objective(s). The Center PD(s)/PI(s) will monitor all Research Projects and actively promote efforts that foster integration, collaboration, and synergy across the program. Research Project Leaders may be affiliated with either an academic organization or industry.

Research Projects are expected to incorporate state-of-the-art technology and approaches and may include consortium arrangements for required activities. Applicants are encouraged to carefully consider the scope and range of research proposed and develop a Center that is coherent overall and consistent with available resources and personnel.

Example ReVAMPP Centers:

Center programs and objectives may range from research and development of single or multiple prototype vaccines targeting one or more of the priority virus families, and activities may range from early basic research aimed at gaining the foundational knowledge needed to design a generalizable vaccine strategy, to mAb discovery and characterization to late-stage preclinical vaccine development with industry participation. Examples of hypothetical ReVAMPP Centers follow:

Example 1:

Center for Countermeasure Discovery for Bunyaviruses

Administrative Core

Data Management Core

Scientific Core: Virology, Reagents, and Assays

Scientific Core: Structural Biology of Viral Proteins, Vaccines, and Monoclonal Antibodies

Research Project 1: Viral receptor discovery and characterization for Nairoviruses and Peribunyaviruses

Research Project 2: Characterization of the human immune response and antibody discovery

Research Project 3: Antigen design and immunogenicity evaluation of Nairovirus vaccines

Research Project 4: Animal model development and determination of CCHF and LAC pathogenesis

Research Project 5: Antigen design and immunogenicity evaluation of Peribunyavirus vaccines

Example 2:

Paramyxovirus and Arenavirus Vaccine and Monoclonal Antibody Development Center

Administrative Core

Data Management Core

Scientific Core: Animal model development and candidate vaccine and monoclonal antibody evaluation

Research Project 1: Antigen design and immunogenicity testing of Paramyxovirus vaccine candidates

Research Project 2: Antigen design and immunogenicity testing of Arenavirus vaccine candidates

Research Project 3: Determination of correlates of protection for different vaccine platforms

Research Project 4: Preclinical evaluation of monoclonal antibodies targeting paramyxoviruses and arenaviruses

Applications including the following types of studies will be considered non-responsive and will not be reviewed:

- Clinical trials: Clinical research may be supported but not clinical trials, as defined by the NIH (https://grants.nih.gov/policy/clinical-trials/definition.htm (https://grants.nih.gov/policy/clinical-trials/definition.htm)).
- Centers proposing only monoclonal antibody discovery and development without vaccine development.
- Projects proposing toxicology studies or GMP manufacturing for vaccines.
- Projects proposing later-stage development of monoclonal antibodies including process development, tissue cross-reactivity studies, toxicology studies, or GMP manufacturing.
- Centers proposing research on Coronaviridae, Orthomyxoviridae or other virus families not listed under *Priority Viral Families for Pandemic Preparedness*.
- Centers proposing research on Flaviviridae or Togaviridae families which are covered in the companion RFA-AI-22-019 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-22-019.html)

- Applications that do not include a clear section on Milestones with Go/No-Go criteria for the Overall Program and each individual project.

This FOA supports research on virus families from Bunyavirales, Paramyxoviridae and Picornaviridae. For ReVAMPP Center programs proposing research on virus families from Flaviviridae and Togaviridae see the companion FOA, RFA-AI-23-019 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-019.html). The ReVAMPP Coordinating and Data Sharing Center (CDSC) will oversee data coordination and sharing for the ReVAMPP Centers in the ReVAMPP Network (companion FOA, RFA-AI-23-021 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-021.html)).

For additional information about the Research and Development of Vaccines and Monoclonal Antibodies for Pandemic Preparedness (ReVAMPP) Centers), see the "Frequently Asked Questions (FAQ)" link here: https://www.niaid.nih.gov/grants-contracts/questions-and-answers-revampp-funding-opportunities (https://www.niaid.nih.gov/grants-contracts/questions-and-answers-revampp-funding-opportunities).

Webinar Announcement

NIAID plans to hold a pre-application informational webinar for this FOA. Details about webinar registration will be available at this same FAQ link shortly after FOA publication. Participation in the webinar is not required to submit an application in response to this FOA.
See Section VIII. Other Information for award authorities and regulations.

Section II. Award Information

Funding Instrument

Cooperative Agreement: A support mechanism used when there will be substantial Federal scientific or programmatic involvement. Substantial involvement means that, after award, NIH scientific or program staff will assist, guide, coordinate, or participate in project activities. See Section VI.2 for additional information about the substantial involvement for this FOA.

Application Types Allowed

New

The OER Glossary (//grants.nih.gov/grants/guide/url_redirect.htm?id=11116) and the SF424 (R&R) Application Guide provide details on these application types. Only those application types listed here are allowed for this FOA.

Clinical Trial?

Not Allowed: Only accepting applications that do not propose clinical trials.

Need help determining whether you are doing a clinical trial? (https://grants.nih.gov/grants/guide/url_redirect.htm?id=82370)

Funds Available and Anticipated Number of Awards

NIAID intends to commit ~$70-85M in FY2024 to fund 5-6 awards. Funding in subsequent years is subject to the availability of funds.

Award Budget

Application budgets are not expected to exceed $10M direct costs/year and need to reflect the actual needs of the proposed project.

Award Project Period

The scope of the proposed project should determine the project period. The maximum period is 5 years.

NIH grants policies as described in the NIH Grants Policy Statement (//grants.nih.gov/grants/guide/url_redirect.htm?id=11120) will apply to the applications submitted and awards made from this FOA.

Section III. Eligibility Information

1. Eligible Applicants

Eligible Organizations

Higher Education Institutions

- Public/State Controlled Institutions of Higher Education
- Private Institutions of Higher Education

The following types of Higher Education Institutions are always encouraged to apply for NIH support as Public or Private Institutions of Higher Education:

- Hispanic-serving Institutions
- Historically Black Colleges and Universities (HBCUs)
- Tribally Controlled Colleges and Universities (TCCUs)
- Alaska Native and Native Hawaiian Serving Institutions
- Asian American Native American Pacific Islander Serving Institutions (AANAPISIs)

Nonprofits Other Than Institutions of Higher Education

- Nonprofits with 501(c)(3) IRS Status (Other than Institutions of Higher Education)
- Nonprofits without 501(c)(3) IRS Status (Other than Institutions of Higher Education)

For-Profit Organizations

- Small Businesses
- For-Profit Organizations (Other than Small Businesses)

Local Governments

- State Governments
- County Governments
- City or Township Governments
- Special District Governments
- Indian/Native American Tribal Governments (Federally Recognized)
- Indian/Native American Tribal Governments (Other than Federally Recognized)

Federal Governments

- Eligible Agencies of the Federal Government
- U.S. Territory or Possession

Foreign Institutions

Non-domestic (non-U.S.) Entities (Foreign Institutions) **are** eligible to apply.

Non-domestic (non-U.S.) components of U.S. Organizations **are** eligible to apply.

Foreign components, as defined in the *NIH Grants Policy Statement* (//grants.nih.gov/grants/guide/url_redirect.htm?id=11118), **are** allowed.

Required Registrations

Applicant organizations

Applicant organizations must complete and maintain the following registrations as described in the SF 424 (R&R) Application Guide to be eligible to apply for or receive an award. All registrations must be completed prior to the application being submitted. Registration can take 6 weeks or more, so applicants should begin the registration process as soon as possible. The NIH Policy on Late Submission of Grant Applications (//grants.nih.gov/grants/guide/notice-files/NOT-OD-15-039.html) states that failure to complete registrations in advance of a due date is not a valid reason for a late submission.

- System for Award Management (SAM) (https://grants.nih.gov/grants/guide/url_redirect.htm?id=82390) – Applicants must complete and maintain an active registration, **which requires renewal at least annually**. The renewal process may require as much time as the initial registration. SAM registration includes the assignment of a Commercial and Government Entity (CAGE) Code for domestic organizations which have not already been assigned a CAGE Code.
    - NATO Commercial and Government Entity (NCAGE) Code (//grants.nih.gov/grants/guide/url_redirect.htm?id=11176) – Foreign organizations must obtain an NCAGE code (in lieu of a CAGE code) in order to register in SAM.
    - Unique Entity Identifier (UEI) - A UEI is issued as part of the SAM.gov registration process. The same UEI must be used for all registrations, as well as on the grant application.
- eRA Commons (https://era.nih.gov/) - Once the unique organization identifier is established, organizations can register with eRA Commons in tandem with completing their Grants.gov registration; all registrations must be in place by time of submission. eRA Commons requires organizations to identify at least one Signing Official (SO) and at least one Program Director/Principal Investigator (PD/PI) account in order to submit an application.
- Grants.gov (//grants.nih.gov/grants/guide/url_redirect.htm?id=82300) – Applicants must have an active SAM registration in order to complete the Grants.gov registration.

Program Directors/Principal Investigators (PD(s)/PI(s))

All PD(s)/PI(s) must have an eRA Commons account. PD(s)/PI(s) should work with their organizational officials to either create a new account or to affiliate their existing account with the applicant organization in eRA Commons. If the PD/PI is also the organizational Signing Official, they must have two distinct eRA Commons accounts, one for each role. Obtaining an eRA Commons account can take up to 2 weeks.

Eligible Individuals (Program Director/Principal Investigator)

Any individual(s) with the skills, knowledge, and resources necessary to carry out the proposed research as the Program Director(s)/Principal Investigator(s) (PD(s)/PI(s)) is invited to work with his/her organization to develop an application for support. Individuals from diverse backgrounds, including underrepresented racial and ethnic groups, individuals with disabilities, and women are always encouraged to apply for NIH support. See, Reminder: Notice of NIH's Encouragement of Applications Supporting Individuals from Underrepresented Ethnic and Racial Groups as well as Individuals with Disabilities, NOT-OD-22-019 (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-22-019.html).

For institutions/organizations proposing multiple PDs/PIs, visit the Multiple Program Director/Principal Investigator Policy and submission details in the Senior/Key Person Profile (Expanded) Component of the SF424 (R&R) Application Guide.

Applicants listed as a PD(s)/PI(s) for this FOA will not be eligible to be listed as PD(s)/PI(s) on applications submitted to the companion FOA (RFA-AI-23-019) (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-019.html) ReVAMPP Centers for Flaviviridae and Togaviridae but can participate as collaborators on subcomponents of those Centers. Applicants to this FOA will not be eligible to submit to or participate in the companion ReVAMPP CDSC FOA (RFA-AI-23-021 (https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-23-021.html)) due to the centralized role of the CDSC in the Network coordination and communication.

2. Cost Sharing

This FOA does not require cost sharing as defined in the NIH Grants Policy Statement (//grants.nih.gov/grants/guide/url_redirect.htm?id=11126).

3. Additional Information on Eligibility

Number of Applications

Applicant organizations may submit more than one application, provided that each application is scientifically distinct.

The NIH will not accept duplicate or highly overlapping applications under review at the same time, per 2.3.7.4 Submission of Resubmission Application (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_2/2.3.7_policies_affecting_applications.htm#Submissi). This means that the NIH will not accept:

- A new (A0) application that is submitted before issuance of the summary statement from the review of an overlapping new (A0) or resubmission (A1) application.
- A resubmission (A1) application that is submitted before issuance of the summary statement from the review of the previous new (A0) application.
- An application that has substantial overlap with another application pending appeal of initial peer review (see 2.3.9.4 Similar, Essentially Identical, or Identical Applications (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_2/2.3.9_application_receipt_information_and_deadlines.htm#Similar,)).

Section IV. Application and Submission Information

1. Requesting an Application Package

The application forms package specific to this opportunity must be accessed through ASSIST or an institutional system-to-system solution. A button to apply using ASSIST is available in Part 1 of this FOA. See your administrative office for instructions if you plan to use an institutional system-to-system solution.

2. Content and Form of Application Submission

It is critical that applicants follow the Multi-Project (M) Instructions in the SF424 (R&R) Application Guide (https://grants.nih.gov/grants/guide/url_redirect.htm?id=82400), except where instructed in this funding opportunity announcement to do otherwise and where instructions in the Application Guide are directly related to the Grants.gov downloadable forms currently used with most NIH opportunities. Conformance to the requirements in the Application Guide is required and strictly enforced. Applications that are out of compliance with these instructions may be delayed or not accepted for review.

Letter of Intent

Although a letter of intent is not required, is not binding, and does not enter into the review of a subsequent application, the information that it contains allows IC staff to estimate the potential review workload and plan the review.

By the date listed in Part 1. Overview Information, prospective applicants are asked to submit a letter of intent that includes the following information:

- Descriptive title of proposed activity
- Name(s), address(es), and telephone number(s) of the PD(s)/PI(s)
- Names of other key personnel
- Participating institution(s)
- Number and title of this funding opportunity

The letter of intent should be sent to:

Frank De Silva, Ph.D.
Telephone: 240-669-5023
Email: fdesilva@niaid.nih.gov (mailto:fdesilva@niaid.nih.gov)

Page Limitations

All page limitations described in the SF424 Application Guide and the Table of Page Limits (//grants.nih.gov/grants/guide/url_redirect.htm?id=11133) must be followed.

Component	Component Type for Submission	Page Limit	Required/Optional	Minimum	Maximum
Overall	Overall	12	Required	1	1
Admin Core	Admin Core	12	Required	1	1
Data Management Core	Data Management Core	6	Required	1	1
Core	Core	6	Optional	0	3
Project	Project	12	Required	2	5

Instructions for the Submission of Multi-Component Applications

The following section supplements the instructions found in the SF424 (R&R) Application Guide and should be used for preparing a multi-component application.

The application should consist of the following components:

Overall: required

Administrative Core: required

Data Management Core: required

Scientific Cores: optional, maximum 3, each Scientific Core must support at least two Research Projects

Projects: required, minimum 2, maximum 5

Overall Component

When preparing your application, use Component Type ‘Overall’.

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

SF424(R&R) Cover (Overall)

Complete entire form.

PHS 398 Cover Page Supplement (Overall)

Note: Human Embryonic Stem Cell lines from other components should be repeated in cell line table in Overall component.

Research & Related Other Project Information (Overall)

Follow standard instructions.

Facilities & Other Resources

Describe any unique features in the environment and/or resources that make this a strong research program.

In the " Facilities & Other Resources" attachment include a clearly marked section titled “BSL3/4 facilities” detailing availability of adequate access to BSL3/4 biocontainment facilities to support the proposed Center program, if applicable. Applicants must identify all Research Projects within the application that will require BSL3/4 containment facilities and provide a description of facilities including that are available currently or planned at either the applicant institution or though consortium institutions. A table format may be used to list each activity that requires BSL3/4 access and the likely facilities to be used. All information on BSL3/4 facilities should be contained in the Overall and not within individual Research Projects.

If institutional core facilities will be utilized, in a clearly marked section titled "Institutional Core Facilities" describe how institutional core facilities will be used to support the Research Projects.

Project/Performance Site Locations (Overall)

Enter primary site only.

A summary of Project/Performance Sites in the Overall section of the assembled application image in eRA Commons compiled from data collected in the other components will be generated upon submission.

Research and Related Senior/Key Person Profile (Overall)

Include only the Project Director/Principal Investigator (PD/PI) and any multi-PDs/PIs (if applicable to this FOA) for the entire application.

A summary of Senior/Key Persons followed by their Biographical Sketches in the Overall section of the assembled application image in eRA Commons will be generated upon submission.

Budget (Overall)

The only budget information included in the Overall component is the Estimated Project Funding section of the SF424 (R&R) Cover.

A budget summary in the Overall section of the assembled application image in eRA Commons compiled from detailed budget data collected in the other components will be generated upon submission.

PHS 398 Research Plan (Overall)

Specific Aims: List in priority order, the broad, long-range objectives, and goals of the proposed Center. Concisely describe the Center objectives.

Research Strategy: This narrative section summarizes the overall research plan for the multi-project application. The multi-project application should be viewed as a confederation of interrelated research projects, each capable of standing on its own scientific merit, but complementary to one another. This is an important section for it provides the group of investigators an opportunity to give conceptual wholeness to the overall program – by giving a statement of the general problem area and by laying out a broad strategy for attacking the problems.

Applicants should clearly define the Center program and its significance regarding the scientific approach in terms of discovery and development of vaccines, and if applicable early development of mAbs, against prototype viruses from families with high pandemic potential. Discuss the rationale behind the overall approach and prototype selected for research and development including the public health need and benefit of a successful effort, and the range of activities being pursued. For the prototype selection justification, considerations may include, but are not limited to, how well the prototype reflects properties shared by other members of the family, whether a single prototype is sufficient or whether multiple prototypes are needed to address differences amongst family members, the ease of working with the prototype virus, and whether vaccines against the prototype would have potential value for current public health needs. Include a discussion of the current state of foundational knowledge and maturity of product development for the proposed virus families, and how this Center program will advance vaccine development, and if applicable, early development of mAbs, and enhance the ability to respond rapidly to an emerging virus from the prototype virus's family. Additionally, each application must detail how each Research Project and Core contributes to the Center program, objectives, and project interdependence. Applications should outline expected synergies provided by the proposed Center structure and any other special features that make this application strong or unique.

Milestone Plan: In a clearly labeled section titled "Program Milestones and Timelines", applicants should describe specific quantifiable milestones for the overall program, including detailed quantitative and qualitative criteria for Go/No-Go decisions by annum and include annual timelines for the overall research program and for tracking progress from individual research projects and Cores. This plan must include Go/No-Go criteria to be met by the end of Year 3 of the award for continuation to Phase II. Milestones must specify the outcome(s) for each activity. Milestones should be quantifiable and scientifically justified, and include major milestones from the individual research projects and Cores. Include any milestones that are integrated from independent research projects or Cores. Milestone criteria should not simply be a restatement of the specific aims. Using a Gantt chart or equivalent tool, describe the associated timelines and identified outcomes for the research Center.

Resource Sharing Plan:
Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide.

Other Plan(s):

Note: Effective for due dates on or after January 25, 2023, the Data Management and Sharing Plan will be attached in the Other Plan(s) attachment in FORMS-H application forms packages. If required, the Data Management and Sharing (DMS) Plan must be provided in the Overall component.

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions:

- All applicants planning research (funded or conducted in whole or in part by NIH) that results in the generation of scientific data are required to comply with the instructions for the Data Management and Sharing Plan. All applications, regardless of the amount of direct costs requested for any one year, must address a Data Management and Sharing Plan.
- Investigators must develop data structures that are FAIR (FAIR Principles - GO FAIR (go-fair.org) (https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.go-fair.org%2Ffair-principles%2F&data=05%7C01%7Cchelsea.boyd%40nih.gov%7C8247f78a75c04c5ea99208db1b643066%7C14b77578977342d58507251ca2dc2b06%7C0%7C0%7C638133
This will produce data sets that are harmonized and facilitate progressive data sharing models. Applications must provide a well-thought-out plan for how data will be shared using the FAIR principles.

Appendix:

Only limited items are allowed in the Appendix. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide; any instructions provided here are in addition to the SF424 (R&R) Application Guide instructions.

PHS Human Subjects and Clinical Trials Information (Overall)

When involving human subjects research, clinical research, and/or NIH-defined clinical trials follow all instructions for the PHS Human Subjects and Clinical Trials Information form in the SF424 (R&R) Application Guide, with the following additional instructions:

If you answered "Yes" to the question "Are Human Subjects Involved?" on the R&R Other Project Information form, there must be at least one human subjects study record using the **Study Record: PHS Human Subjects and Clinical Trials Information** form or a **Delayed Onset Study** record within the application. The study record(s) must be included in the component(s) where the work is being done, unless the same study spans multiple components. To avoid the creation of duplicate study records, a single study record with sufficient information for all involved components must be included in the Overall component when the same study spans multiple components.

Study Record: PHS Human Subjects and Clinical Trials Information

All instructions in the SF424 (R&R) Application Guide must be followed.

Delayed Onset Study

Note: Delayed onset (https://grants.nih.gov/grants/glossary.htm#DelayedOnsetStudy) does NOT apply to a study that can be described but will not start immediately (i.e., delayed start).All instructions in the SF424 (R&R) Application Guide must be followed.

PHS Assignment Request Form (Overall)

All instructions in the SF424 (R&R) Application Guide must be followed.

Administrative Core

When preparing your application, use Component Type ‘Admin Core.’

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

Note: Effective for due dates on or after January 25, 2023, the Data Management and Sharing Plan will be attached in the Other Plan(s) attachment in FORMS-H application forms packages. If required, the Data Management and Sharing (DMS) Plan must be provided in the Overall component.

SF424 (R&R) Cover (Administrative Core)

Complete only the following fields:

- Applicant Information
- Type of Applicant (optional)
- Descriptive Title of Applicant’s Project
- Proposed Project Start/Ending Dates

PHS 398 Cover Page Supplement (Administrative Core)

Enter Human Embryonic Stem Cells in each relevant component.

Research & Related Other Project Information (Administrative Core)

Human Subjects: Answer only the ‘Are Human Subjects Involved?’ and 'Is the Project Exempt from Federal regulations?’ questions.

Vertebrate Animals: Answer only the ‘Are Vertebrate Animals Used?’ question.

Project Narrative: Do not complete. Note: ASSIST screens will show an asterisk for this attachment indicating it is required. However, eRA systems only enforce this requirement in the Overall component and applications will not receive an error if omitted in other components.

Project /Performance Site Location(s) (Administrative Core)

List all performance sites that apply to the specific component.

Note: The Project Performance Site form allows up to 300 sites, prior to using additional attachment for additional entries.

Research & Related Senior/Key Person Profile (Administrative Core)

- In the Project Director/Principal Investigator section of the form, use Project Role of ‘Other’ with Category of ‘Project Lead’ and provide a valid eRA Commons ID in the Credential field.
- In the additional Senior/Key Profiles section, list Senior/Key persons that are working in the component.
- Include a single Biographical Sketch for each Senior/Key person listed in the application regardless of the number of components in which they participate. When a Senior/Key person is listed in multiple components, the Biographical Sketch can be included in any one component.
- If more than 100 Senior/Key persons are included in a component, the Additional Senior Key Person attachments should be used.

Budget (Administrative Core)

Budget forms appropriate for the specific component will be included in the application package.

- The Core Lead must commit at least 0.6 person months effort per year to these responsibilities.
- Include funds for the overall administrative effort, collaborative activities, communications, and publications.
- Include costs related to Regulatory expertise as defined effort or periodic consultation.
- Include funds for the PD(s)/PI(s), Project Leaders, additional Center Key Personnel and postdocs/researchers/students (at the discretion of the PD(s)/PI(s)), to travel and attend annual ReVAMPP Network-wide review meetings to be held over an approximately 1-3 full days in the Rockville, MD area or other NIAID-approved site for data presentation, progress evaluation and related activities.
- Include funds for the PD(s)/PI(s), Project Leaders, external SAB members, and additional Center Key Personnel (at the discretion of the PD/PI) to travel and attend annual mandatory ReVAMPP Center progress meetings at NIAID in Years 1-4 of the project period.

Note: The R&R Budget form included in many of the component types allows for up to 100 Senior/Key Persons in section A and 100 Equipment Items in section C prior to using attachments for additional entries. All other SF424 (R&R) instructions apply.

PHS 398 Research Plan (Administrative Core)

Specific Aims: List in priority order, the broad, long-range objectives, and goals of the proposed Core. In addition, state the Core's relationship to the Center’s program and how it relates to the individual Research Projects or other Cores in the application.

Research Strategy: The Administrative Core must include a Management Plan that identifies and discusses: The Administrative Core organizational structure, the roles of Administrative Core personnel, the facilitation of communications throughout the Center, including with industry partners and how a strong collaborative environment will be established within the Center. The plan should specifically address continual evaluation of research and development progress, communications, group meetings and teleconferences, the identification and proposed resolution of problems and engagement of the NIAID staff as appropriate. A description of how consortia (subcontracts) will be managed should be provided and should include how communications such as periodic meetings and conference calls will be organized, managed, and documented. The plan should also detail how Center and research-related travel will be managed.

Describe how the Center will coordinate communication and collaborations with the CDSC, NIAID Staff, and other Centers within the Network. Describe how the Administrative Core will ensure the Center program complies in a timely manner with requests from the CDSC and NIAID program staff.

Each Administrative Core must include the following:

Scientific Advisory Board: Describe the composition and duties of the Scientific Advisory Board (SAB), including the categories of expertise to be represented on the SAB and how the SAB will be utilized to guide Center activities. The description should include a discussion of how the proposed expertise of the SAB will be integrated into the operations of the Center. Describe the procedures and approaches for obtaining SAB input via teleconferences, ad hoc and annual meetings, review of written materials/data, etc. The SAB must include at least two members with relevant industry-level experience and procedures for identification and selection of the SAB should be included. *Candidates for the SAB MUST NOT be named in the application or contacted prior to completion of review activities.*

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide.

Appendix:

Only limited items are allowed in the Appendix. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide; any instructions provided here are in addition to the SF424 (R&R) Application Guide instructions.

PHS Human Subjects and Clinical Trials Information (Administrative Core)

When involving human subjects research, clinical research, and/or NIH-defined clinical trials follow all instructions for the PHS Human Subjects and Clinical Trials Information form in the SF424 (R&R) Application Guide, with the following additional instructions:

If you answered "Yes" to the question "Are Human Subjects Involved?" on the R&R Other Project Information form, you must include at least one human subjects study record using the **Study Record: PHS Human Subjects and Clinical Trials Information form** or a **Delayed Onset Study** record.

Study Record: PHS Human Subjects and Clinical Trials Information

All instructions in the SF424 (R&R) Application Guide must be followed

Delayed Onset Study

Note: Delayed onset (https://grants.nih.gov/grants/glossary.htm#DelayedOnsetHumanSubjectStudy) does NOT apply to a study that can be described but will not start immediately (i.e., delayed start). All instructions in the SF424 (R&R) Application Guide must be followed.

Data Management Core

When preparing your application, use Component Type 'Data Management Core.'

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

Note: Effective for due dates on or after January 25, 2023, the Data Management and Sharing Plan will be attached in the Other Plan(s) attachment in FORMS-H application forms packages. If required, the Data Management and Sharing (DMS) Plan must be provided in the Overall component.

SF424 (R&R) Cover (Data Management Core)

Complete only the following fields:

- Applicant Information
- Type of Applicant (optional)
- Descriptive Title of Applicant's Project
- Proposed Project Start/Ending Dates

PHS 398 Cover Page Supplement (Data Management Core)

Enter Human Embryonic Stem Cells in each relevant component.

Research & Related Other Project Information (Data Management Core)

Human Subjects: Answer only the 'Are Human Subjects Involved?' and 'Is the Project Exempt from Federal regulations?' questions.

Vertebrate Animals: Answer only the 'Are Vertebrate Animals Used?' question.

Project Narrative: Do not complete. Note: ASSIST screens will show an asterisk for this attachment indicating it is required. However, eRA systems only enforce this requirement in the Overall component and applications will not receive an error if omitted in other components.

Project /Performance Site Location(s) (Data Management Core)

List all performance sites that apply to the specific component.

Note: The Project Performance Site form allows up to 300 sites, prior to using additional attachment for additional entries.

Research & Related Senior/Key Person Profile (Data Management Core)

- In the Project Director/Principal Investigator section of the form, use Project Role of 'Other' with Category of 'Core Lead' and provide a valid eRA Commons ID in the Credential field.
- In the additional Senior/Key Profiles section, list Senior/Key persons that are working in the component.
- Include a single Biographical Sketch for each Senior/Key person listed in the application regardless of the number of components in which they participate. When a Senior/Key person is listed in multiple components, the Biographical Sketch can be included in any one component.
- If more than 100 Senior/Key persons are included in a component, the Additional Senior Key Person attachments should be used.

Budget (Data Management Core)

Budget forms appropriate for the specific component will be included in the application package.

The Core Leader should commit to the Core at least 1.2 person months of effort.

Note: The R&R Budget form included in many of the component types allows for up to 100 Senior/Key Persons in section A and 100 Equipment Items in section C prior to using attachments for additional entries. All other SF424 (R&R) instructions apply.

PHS 398 Research Plan (Data Management Core)

Specific Aims: List in priority order, the broad, long-range objectives, and goals of the proposed Core. In addition, state the Core's relationship to the Center program and how it relates to the individual Research Projects or other Cores in the application.

Research Strategy: Describe the organizational structure and role of the Data Management Core in the overall Center research activities and include a Strategy for Management of Data Activities Plan that describes internal and external data acquisition strategies to achieve harmonization of systems and procedures for data management, data quality, data analyses, and dissemination for all data and data-related materials generated by the Center to the ReVAMPP CDSC. Describe the quality control procedures for the data, and how to identify and resolve issues with quality control that maintains data integrity. Describe how the Data Management Core will ensure compliance with the ReVAMPP network-wide data sharing.

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide.

Appendix:

Only limited items are allowed in the Appendix. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide; any instructions provided here are in addition to the SF424 (R&R) Application Guide instructions.

PHS Human Subjects and Clinical Trials Information (Data Management Core)

When involving human subjects research, clinical research, and/or NIH-defined clinical trials follow all instructions for the PHS Human Subjects and Clinical Trials Information form in the SF424 (R&R) Application Guide, with the following additional instructions:

If you answered “Yes” to the question “Are Human Subjects Involved?” on the R&R Other Project Information form, you must include at least one human subjects study record using the **Study Record: PHS Human Subjects and Clinical Trials Information** form or a **Delayed Onset Study** record.

Study Record: PHS Human Subjects and Clinical Trials Information

All instructions in the SF424 (R&R) Application Guide must be followed

Delayed Onset Study

Note: Delayed onset (https://grants.nih.gov/grants/glossary.htm#DelayedOnsetStudy) does NOT apply to a study that can be described but will not start immediately (i.e., delayed start). All instructions in the SF424 (R&R) Application Guide must be followed.

Scientific Core

When preparing your application, use Component Type ‘Core.’

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

Note: Effective for due dates on or after January 25, 2023, the Data Management and Sharing Plan will be attached in the Other Plan(s) attachment in FORMS-H application forms packages. If required, the Data Management and Sharing (DMS) Plan must be provided in the Overall component.

SF424 (R&R) Cover (Scientific Core)

Complete only the following fields:

- Applicant Information
- Type of Applicant (optional)
- Descriptive Title of Applicant’s Project
- Proposed Project Start/Ending Dates

PHS 398 Cover Page Supplement (Scientific Core)

Enter Human Embryonic Stem Cells in each relevant component.

Research & Related Other Project Information (Scientific Core)

Human Subjects: Answer only the ‘Are Human Subjects Involved?’ and 'Is the Project Exempt from Federal regulations?’ questions.

Vertebrate Animals: Answer only the ‘Are Vertebrate Animals Used?’ question.

Project Narrative: Do not complete. Note: ASSIST screens will show an asterisk for this attachment indicating it is required. However, eRA systems only enforce this requirement in the Overall component and applications will not receive an error if omitted in other components.

Project /Performance Site Location(s) (Scientific Core)

List all performance sites that apply to the specific component.

Note: The Project Performance Site form allows up to 300 sites, prior to using additional attachment for additional entries.

Research & Related Senior/Key Person Profile (Scientific Core)

- In the Project Director/Principal Investigator section of the form, use Project Role of ‘Other’ with Category of ‘Core Lead’ and provide a valid eRA Commons ID in the Credential field.
- In the additional Senior/Key Profiles section, list Senior/Key persons that are working in the component.
- Include a single Biographical Sketch for each Senior/Key person listed in the application regardless of the number of components in which they participate. When a Senior/Key person is listed in multiple components, the Biographical Sketch can be included in any one component.
- If more than 100 Senior/Key persons are included in a component, the Additional Senior Key Person attachments should be used.

Budget (Scientific Core)

Budget forms appropriate for the specific component will be included in the application package.

The Core Leader should commit to the Core at least 1.2 person months of effort.

Note: The R&R Budget form included in many of the component types allows for up to 100 Senior/Key Persons in section A and 100 Equipment Items in section C prior to using attachments for additional entries. All other SF424 (R&R) instructions apply.

PHS 398 Research Plan (Scientific Core)

Specific Aims: List in priority order, the broad, long-range objectives, and goals of the proposed Core. In addition, state the Core's relationship to the Center program and how it relates to the individual Research Projects or other Cores in the application.

Research Strategy: Describe and justify the role of the Core in the overall Center research activities, describe how the proposed Core activities will contribute to meeting the Center's goals and objectives, specify how a proposed scientific Core provides a unique service that cannot be obtained through institutional or commercial means, and explain the rationale for selection of the general methods and approaches proposed to accomplish the specific aims. Describe the facilities or services that will be provided by the Core including procedures, techniques, and quality control to ensure high quality outputs. In addition, this section should indicate the relevance of the Core to the primary objectives of the application. Provide details of the services or resources provided by the optional Cores to at least two Research Projects and clarify how the optional Cores are not duplicative of other services or facilities. Additionally, plans for staffing, managing, and prioritizing use of the Cores must be provided, as well as plans for determining fees to users if charging fees is necessary.

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide.

Appendix:

Only limited items are allowed in the Appendix. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide; any instructions provided here are in addition to the SF424 (R&R) Application Guide instructions.

PHS Human Subjects and Clinical Trials Information (Scientific Core)

When involving human subjects research, clinical research, and/or NIH-defined clinical trials follow all instructions for the PHS Human Subjects and Clinical Trials Information form in the SF424 (R&R) Application Guide, with the following additional instructions:

If you answered “Yes” to the question “Are Human Subjects Involved?” on the R&R Other Project Information form, you must include at least one human subjects study record using the **Study Record: PHS Human Subjects and Clinical Trials Information** form or a **Delayed Onset Study** record.

Study Record: PHS Human Subjects and Clinical Trials Information

All instructions in the SF424 (R&R) Application Guide must be followed

Delayed Onset Study

Note: Delayed onset (https://grants.nih.gov/grants/glossary.htm#DelayedOnsetStudy) does NOT apply to a study that can be described but will not start immediately (i.e., delayed start). All instructions in the SF424 (R&R) Application Guide must be followed.

Research Project

When preparing your application, use Component Type ‘Project.’

All instructions in the SF424 (R&R) Application Guide must be followed, with the following additional instructions, as noted.

Note: Effective for due dates on or after January 25, 2023, the Data Management and Sharing Plan will be attached in the Other Plan(s) attachment in FORMS-H application forms packages. If required, the Data Management and Sharing (DMS) Plan must be provided in the Overall component.

SF424 (R&R) Cover (Research Project)

Complete only the following fields:

- Applicant Information
- Type of Applicant (optional)
- Descriptive Title of Applicant’s Project
- Proposed Project Start/Ending Dates

PHS 398 Cover Page Supplement (Research Project)

Enter Human Embryonic Stem Cells in each relevant component.

Research & Related Other Project Information (Research Project)

Human Subjects: Answer only the ‘Are Human Subjects Involved?’ and 'Is the Project Exempt from Federal regulations? questions.

Vertebrate Animals: Answer only the ‘Are Vertebrate Animals Used?’ question.

Project Narrative: Do not complete. Note: ASSIST screens will show an asterisk for this attachment indicating it is required. However, eRA systems only enforce this requirement in the Overall component and applications will not receive an error if omitted in other components.

Project /Performance Site Location(s) (Research Project)

List all performance sites that apply to the specific component.

Note: The Project Performance Site form allows up to 300 sites, prior to using additional attachment for additional entries.

Research & Related Senior/Key Person Profile (Research Project)

- In the Project Director/Principal Investigator section of the form, use Project Role of ‘Other’ with Category of ‘Project Lead’ and provide a valid eRA Commons ID in the Credential field.
- In the additional Senior/Key Profiles section, list Senior/Key persons that are working in the component.
- Include a single Biographical Sketch for each Senior/Key person listed in the application regardless of the number of components in which they participate. When a Senior/Key person is listed in multiple components, the Biographical Sketch can be included in any one component.
- If more than 100 Senior/Key persons are included in a component, the Additional Senior Key Person attachments should be used.

Budget (Research Project)

Budget forms appropriate for the specific component will be included in the application package.

Each project leader must commit at least 1.2 person months effort to their project per year.

Note: The R&R Budget form included in many of the component types allows for up to 100 Senior/Key Persons in section A and 100 Equipment Items in section C prior to using attachments for additional entries. All other SF424 (R&R) instructions apply.

PHS 398 Research Plan (Research Project)

Specific Aims: List, in priority order, the broad long-range objectives and goals of the proposed project. Concisely describe the research activities to be performed. In addition, concisely state the individual Research Project's relationship to the Center program and how it relates to other projects or cores.

Research Strategy: Use this section to describe how the proposed research will contribute to meeting the Center objectives and explain the rationale for selecting the methods to accomplish the specific aims, and the biological significance of the research.

At least one Research Project must focus on development of vaccines, and other Research Projects may focus on additional vaccine development, early mAb development or foundational research in virology, immunology, pathology, and structural biology necessary for such development.

Describe the research design, conceptual procedures, and analyses to be used to accomplish the specific aims of the project. Describe any new methodology and its advantage over existing methodologies. Describe any novel concepts, approaches, techniques, methodologies, tools, or technologies for the proposed studies that will fundamentally advance how vaccines, and if applicable mAbs, will be developed. Discuss whether traditional approaches will be used in a new, novel way and how reliable, validated methods that mitigate risk will be balanced with innovative new approaches to expand foundational knowledge and advance product development. Describe plans for how the vaccine strategies and other research findings from the prototype virus research will be applied broadly to other viruses within the family to assess the breadth of the approach within and outside of the virus family to validate the generalizability of the approach. Discuss how the vaccine technologies or platforms used can be rapidly adapted for response to known or novel emerging viruses within the same family. If proposing early mAb development, describe plans for the assessment of whether antibodies with similar epitopes and/or properties of identified lead mAb candidates for the prototype virus are effective against other viruses within the same family. Discuss the potential difficulties and limitations of the proposed procedures and alternative approaches to achieve the aims.

Milestone Plan: In a clearly labeled section titled "Project Milestones and Timelines" include a clear delineation of goals with measurable milestones, including detailed quantitative and qualitative criteria for Go/No-Go decision-making, and a timeline for the attainment of each goal and milestone and should be reflected in the Milestone Plan for the overall Program. This plan must include Go/No-Go criteria to be met by the end of Year 3 of the award for continuation to Phase II. Milestones must specify the outcome(s) for each activity. Milestones should be quantifiable and scientifically justified, and include the completion of major research study activities, for example, identification of protective epitopes, animal model development, vaccine or mAb candidate down-selection, identification of correlates of protection, validation of vaccine or mAb strategies for other family members, and analysis, sharing and publication of final data. Milestone criteria should not simply be a restatement of the specific aims. Using a Gantt chart or equivalent tool, describe the associated timelines and identified outcomes for the research Center.

Industry Expertise and Regulatory Considerations: For projects proposing early vaccine development, describe how industry partners will be identified and incorporated into the proposed project including a timeline for inclusion. For projects proposing IND-enabling later stage vaccine development, NIAID requires Centers to include active participation of an industry partner to ensure access to vaccine technology platforms, expertise in manufacturing, clinical development, and regulatory pathways. Applicants should describe the role of this partner in the proposed project and/or team to facilitate discovery, candidate evaluation and/or product development. For the purpose of this FOA, "industry" is defined as a large or small, domestic or foreign, pharmaceutical, biotechnology, bioengineering, or chemical company, or a related non-profit entity.

Additionally, each Center is expected to consider anticipated regulatory barriers for the targeted vaccine technology, particularly for new technology platforms for which there are no precedents for FDA approval. Describe anticipated regulatory barriers and propose research and/or strategies to resolve or overcome these barriers. For a project where multiple and/or complementary expertise is required, discuss plans for coordination among investigators and other collaborators including industry partners and the process to overcome obstacles to achieve the Center aims.

Letter of Support: For projects proposing later stage vaccine development where an industry partner is required, provide a Letter of Support from the Industry partner(s). For projects without later stage vaccine development, a Letter of Support from Industry partners may be included. Provide any additional letters of support that are specific to this project.

Resource Sharing Plan: Individuals are required to comply with the instructions for the Resource Sharing Plans as provided in the SF424 (R&R) Application Guide.

Appendix:

Only limited items are allowed in the Appendix. Follow all instructions for the Appendix as described in the SF424 (R&R) Application Guide; any instructions provided here are in addition to the SF424 (R&R) Application Guide instructions.

PHS Human Subjects and Clinical Trials Information (Research Project)

When involving human subjects research, clinical research, and/or NIH-defined clinical trials follow all instructions for the PHS Human Subjects and Clinical Trials Information form in the SF424 (R&R) Application Guide, with the following additional instructions:

If you answered "Yes" to the question "Are Human Subjects Involved?" on the R&R Other Project Information form, you must include at least one human subjects study record using the **Study Record: PHS Human Subjects and Clinical Trials Information** form or a **Delayed Onset Study** record.

Study Record: PHS Human Subjects and Clinical Trials Information

All instructions in the SF424 (R&R) Application Guide must be followed

Delayed Onset Study

Note: Delayed onset (https://grants.nih.gov/grants/glossary.htm#DelayedOnsetStudy) does NOT apply to a study that can be described but will not start immediately (i.e., delayed start). All instructions in the SF424 (R&R) Application Guide must be followed.

Foreign Institutions

Foreign (non-U.S.) institutions must follow policies described in the *NIH Grants Policy Statement* (//grants.nih.gov/grants/guide/url_redirect.htm?id=11137), and procedures for foreign institutions described throughout the SF424 (R&R) Application Guide.

3. Unique Entity Identifier and System for Award Management (SAM)

See Part 1. Section III.1 for information regarding the requirement for obtaining a unique entity identifier and for completing and maintaining active registrations in System for Award Management (SAM), NATO Commercial and Government Entity (NCAGE) Code (if applicable), eRA Commons, and Grants.gov

4. Submission Dates and Times

Part I. Overview Information contains information about Key Dates and times. Applicants are encouraged to submit applications before the due date to ensure they have time to make any application corrections that might be necessary for successful submission. When a submission date falls on a weekend or Federal holiday (https://grants.nih.gov/grants/guide/url_redirect.htm?id=82380), the application deadline is automatically extended to the next business day.

Organizations must submit applications to Grants.gov (//grants.nih.gov/grants/guide/url_redirect.htm?id=11128) (the online portal to find and apply for grants across all Federal agencies) using ASSIST or other electronic submission systems. Applicants must then complete the submission process by tracking the status of the application in the eRA Commons (//grants.nih.gov/grants/guide/url_redirect.htm?id=11123), NIH's electronic system for grants administration. NIH and Grants.gov systems check the application against many of the application instructions upon submission. Errors must be corrected and a changed/corrected application must be submitted to Grants.gov on or before the application due date and time. If a Changed/Corrected application is submitted after the deadline, the application will be considered late. Applications that miss the due date and time are subjected to the NIH Policy on Late Application Submission.

Applicants are responsible for viewing their application before the due date in the eRA Commons to ensure accurate and successful submission.

Information on the submission process and a definition of on-time submission are provided in the SF424 (R&R) Application Guide.

5. Intergovernmental Review (E.O. 12372)

This initiative is not subject to intergovernmental review (https://grants.nih.gov/grants/policy/nihgps/html5/section_10/10.10.1_executive_orders.htm).

6. Funding Restrictions

All NIH awards are subject to the terms and conditions, cost principles, and other considerations described in the *NIH Grants Policy Statement* (//grants.nih.gov/grants/guide/url_redirect.htm?id=11120).

Pre-award costs are allowable only as described in the *NIH Grants Policy Statement* (//grants.nih.gov/grants/guide/url_redirect.htm?id=11143).

7. Other Submission Requirements and Information

Applications must be submitted electronically following the instructions described in the SF424 (R&R) Application Guide. Paper applications will not be accepted.

For information on how your application will be automatically assembled for review and funding consideration after submission go to: http://grants.nih.gov/grants/ElectronicReceipt/files/Electronic_Multi-project_Application_Image_Assembly.pdf (//grants.nih.gov/grants/ElectronicReceipt/files/Electronic_Multi-project_Application_Image_Assembly.pdf).

Applicants must complete all required registrations before the application due date. Section III. Eligibility Information contains information about registration.

For assistance with your electronic application or for more information on the electronic submission process, visit How to Apply – Application Guide (https://grants.nih.gov/grants/how-to-apply-application-guide.html). If you encounter a system issue beyond your control that threatens your ability to complete the submission process on-time, you must follow the Dealing with System Issues (https://grants.nih.gov/grants/how-to-apply-application-guide/due-dates-and-submission-policies/dealing-with-system-issues.htm) guidance. For assistance with application submission, contact the Application Submission Contacts in Section VII.

> **Important reminders:**
>
> All PD(s)/PI(s) and component Project Leads must include their eRA Commons ID in the Credential field of the Senior/Key Person Profile form**.** Failure to register in the Commons and to include a valid PD/PI Commons ID in the credential field will prevent the successful submission of an electronic application to NIH.
>
> The applicant organization must ensure that the unique entity identifier provided on the application is the same identifier used in the organization's profile in the eRA Commons and for the System for Award Management. Additional information may be found in the SF424 (R&R) Application Guide (https://grants.nih.gov/grants/guide/url_redirect.htm?id=82400).
>
> See more tips (//grants.nih.gov/grants/guide/url_redirect.htm?id=11146) for avoiding common errors.

Upon receipt, applications will be evaluated for completeness and compliance with application instructions by the Center for Scientific Review and responsiveness by NIAID, NIH. Applications that are incomplete, non-compliant and/or nonresponsive will not be reviewed.

Post Submission Materials

Applicants are required to follow the instructions for post-submission materials, as described in the policy (//grants.nih.gov/grants/guide/url_redirect.htm?id=82299).

Section V. Application Review Information

1. Criteria

Only the review criteria described below will be considered in the review process. Applications submitted to the NIH in support of the NIH mission (//grants.nih.gov/grants/guide/url_redirect.htm?id=11149) are evaluated for scientific and technical merit through the NIH peer review system.

Overall Impact - Overall

Reviewers will provide an overall impact score to reflect their assessment of the likelihood for the Program to exert a sustained, powerful influence on the research field(s) involved, in consideration of the following review criteria and additional review criteria (as applicable for the Program proposed).

Scored Review Criteria - Overall

Reviewers will consider each of the review criteria below in the determination of scientific merit and give a separate score for each. An application does not need to be strong in all categories to be judged likely to have major scientific impact. For example, a project that by its nature is not innovative may be essential to advance a field.

Significance

Does the project address an important problem or a critical barrier to progress in the field? Is the prior research that serves as the key support for the proposed project rigorous? If the aims of the project are achieved, how will scientific knowledge, technical capability, and/or clinical practice be improved? How will successful completion of the aims change the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field?

Specific to this FOA:

How well does the application describe a single clearly defined and scientifically justified program that supports the development of vaccine strategies for prototype pathogens that can be applied to other family members? Understanding that the different virus families have different levels of existing foundational knowledge and different states of maturity for product development, how appropriate is the research to increase knowledge to advance vaccine, and if applicable mAb, development for the proposed virus family? If successful, how likely will the proposed Center program enhance the ability to rapidly respond to an emerging known or currently unknown virus from the prototype virus's family?

How well does the Center as a whole leverage scientific gains and synergy by combining the component projects into a multi-project program beyond the gains achievable if each project were pursued independently? To what extent is the program cohesive and do the Research Projects and Cores relate to a common objective demonstrating cohesion, multidisciplinary interactions, and coordination? How well are the scientific cores justified relative to the Overall?

Investigator(s)

Are the PD(s)/PI(s), collaborators, and other researchers well suited to the project? If Early Stage Investigators or those in the early stages of independent careers, do they have appropriate experience and training? If established, have they demonstrated an ongoing record of accomplishments that have advanced their field(s)? If the project is collaborative or multi-PD/PI, do the investigators have complementary and integrated expertise; are their leadership approach, governance and organizational structure appropriate for the project?

Specific to this FOA:

To what extent is there an appropriate balance between investigators with expertise studying the virus families and investigators with product development experience? To what extent is there appropriate and adequate representation of investigators with the necessary vaccine discovery and development experience and expertise? For projects involving IND-enabling later stage activities, how appropriate are industry partnerships proposed to support this effort? For early development projects, how appropriate is the plan and timeline for identification and incorporation of industry partnership to facilitate rapid transition of vaccines into clinical development? How sufficiently are the academic and industry partners coordinated to facilitate discovery, candidate evaluation and/or product development? How adequate is the level of commitment of the PD(s)/PI(s) and key personnel to manage the overall Program?

Innovation

Does the application challenge and seek to shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches or methodologies, instrumentation, or interventions? Are the concepts, approaches or methodologies, instrumentation, or interventions novel to one field of research or novel in a broad sense? Is a refinement, improvement, or new application of theoretical concepts, approaches or methodologies, instrumentation, or interventions proposed?

Specific to this FOA:

To what extent is there an effective balance between reliable, validated methods that mitigate risk with innovative, new approaches that could expand the field? How well have the investigators used traditional approaches in new, novel ways? To what extent are there techniques, methodologies, concepts, or processes which fundamentally advance how vaccines, and if applicable mAbs, are developed?

Approach

Are the overall strategy, methodology, and analyses well-reasoned and appropriate to accomplish the specific aims of the project? Have the investigators included plans to address weaknesses in the rigor of prior research that serves as the key support for the proposed project? Have the investigators presented strategies to ensure a robust and unbiased approach, as appropriate for the work proposed? Are potential problems, alternative strategies, and benchmarks for success presented? If the project is in the early stages of development, will the strategy establish feasibility and will particularly risky aspects be managed? Have the investigators presented adequate plans to address relevant biological variables, such as sex, for studies in vertebrate animals or human subjects?

If the project involves human subjects and/or NIH-defined clinical research, are the plans to address:

1) the protection of human subjects from research risks, and
2) inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion or exclusion of individuals of all ages (including children and older adults), justified in terms of the scientific goals and research strategy proposed?

Specific to this FOA:

How well is the selection of the prototype(s) justified, and likely to generate generalizable knowledge that can be applied to develop vaccines for related viruses? To what extent do the investigators propose a well-reasoned plan to evaluate their vaccine approach broadly to other viruses within a given family to validate the generalizability of the approach? If proposing early mAb development, to what extent do the investigators propose a well-reasoned plan to validate the generalizability of the approach by assessing whether mAbs with similar epitopes and/or properties of lead candidate mAbs against the prototype virus are effective against other viruses within a given family?

To what extent does the approach use technologies and platforms that could be rapidly adapted for response to known or novel emerging viruses within the same family? How appropriate is the proposed project given the current level of knowledge and vaccine development landscape for the virus family? For programs that include IND-enabling translational research, how well does the approach take into consideration the anticipated regulatory process and any anticipated regulatory barriers and resolutions?

How well are the overall Timelines and proposed Milestones defined with quantifiable measures and criteria that are appropriate for enabling clear Go/No-Go decisions and assessing the success of the overall program? How well do the overall milestones support the goal of advancing generalizable vaccine, and if applicable, mAb solutions? How realistic are the timelines proposed for achieving these overall milestones?

How well do the individual projects contribute, either directly or through generation of essential resources or foundational knowledge, to the identification of generalizable vaccine, and if applicable mAb, approaches for a given virus family? How well are the Research Project milestones defined with quantifiable measures and appropriate for enabling clear Go/No-Go decisions and assessing the success of the individual Research Projects? How well do the investigators provide a clear plan for achieving defined Research Project milestones and timelines? To what extent are the timelines proposed for achieving these Research Project milestones realistic or inclusive of necessary steps?

Environment

Will the scientific environment in which the work will be done contribute to the probability of success? Are the institutional support, equipment and other physical resources available to the investigators adequate for the project proposed? Will the project benefit from unique features of the scientific environment, subject populations, or collaborative arrangements?

Specific to this FOA: To what extent do the investigators have access to facilities with the appropriate biocontainment and capacity and resources for the proposed research?

Additional Review Criteria - Overall, Administrative Core, Data Management Core, Scientific Core, and Research Projects

As applicable for the project proposed, reviewers will evaluate the following additional items while determining scientific and technical merit, and in providing an overall impact score, but will not give separate scores for these items.

Administrative Core

How appropriate is the administrative and organizational structure and adequate to achieve the goals of the proposed program? How appropriate is the Management Plan for fiscal accountability and communication within the program? How appropriate are the plans for coordination and the establishment of a strong collaborative environment for the program? How adequate are the plans for communication among the Centers and with the CDSC to facilitate collaborative activities? How appropriate is the plan for collaboration with industry partners clear given the state of the program? How sufficient is the time and effort committed by the PD/PI and Key Personnel to adequately manage the Program? To what extent do the investigators provide a well-thought-out plan for coordination, communication, and collaborations with the CDSC, NIAID Staff, and other Centers within the Network? How well have the applicants developed a plan for ensuring timely communication and collaboration with the CDSC, NIAID Staff, and other Centers within the Network?

Data Management Core

How appropriate and adequate is the organizational structure to achieve the goals of the proposed program? How appropriate is the Strategy for Management of Data Activities Plan for the type of data generated by the research program? How sufficient are the described data management activities sufficient? To what extent do the investigators provide a well-thought-out plan for network-wide sharing of the data generated by the Center?

Scientific Core

How sufficiently is the Core justified? To what extent does it support at least two Research Projects? How well is the core connected to the central focus of the overall program? To what extent are the facilities or services provided by the core (including procedures, techniques, and quality control) high quality and well-justified? How effectively will the services be used? To what extent are the core leader and key personnel well qualified and is there an adequate commitment of time?

Protections for Human Subjects

For research that involves human subjects but does not involve one of the categories of research that are exempt under 45 CFR Part 46, the committee will evaluate the justification for involvement of human subjects and the proposed protections from research risk relating to their participation according to the following five review criteria: 1) risk to subjects, 2) adequacy of protection against risks, 3) potential benefits to the subjects and others, 4) importance of the knowledge to be gained, and 5) data and safety monitoring for clinical trials.

For research that involves human subjects and meets the criteria for one or more of the categories of research that are exempt under 45 CFR Part 46, the committee will evaluate: 1) the justification for the exemption, 2) human subjects involvement and characteristics, and 3) sources of materials. For additional information on review of the Human Subjects section, please refer to the Guidelines for the Review of Human Subjects (//grants.nih.gov/grants/guide/url_redirect.htm?id=11175).

Inclusion of Women, Minorities, and Individuals Across the Lifespan

When the proposed project involves human subjects and/or NIH-defined clinical research, the committee will evaluate the proposed plans for the inclusion (or exclusion) of individuals on the basis of sex/gender, race, and ethnicity, as well as the inclusion (or exclusion) of individuals of all ages (including children and older adults) to determine if it is justified in terms of the scientific goals and research strategy proposed. For additional information on review of the Inclusion section, please refer to the Guidelines for the Review of Inclusion in Clinical Research (//grants.nih.gov/grants/guide/url_redirect.htm?id=11174).

Vertebrate Animals

The committee will evaluate the involvement of live vertebrate animals as part of the scientific assessment according to the following criteria: (1) description of proposed procedures involving animals, including species, strains, ages, sex, and total number to be used; (2) justifications for the use of animals versus alternative models and for the appropriateness of the species proposed; (3) interventions to minimize discomfort, distress, pain and injury; and (4) justification for euthanasia method if NOT consistent with the AVMA Guidelines for the Euthanasia of Animals. Reviewers will assess the use of chimpanzees as they would any other application proposing the use of vertebrate animals. For additional information on review of the Vertebrate Animals section, please refer to the Worksheet for Review of the Vertebrate Animals Section (//grants.nih.gov/grants/guide/url_redirect.htm?id=11150).

Biohazards

Reviewers will assess whether materials or procedures proposed are potentially hazardous to research personnel and/or the environment, and if needed, determine whether adequate protection is proposed.

Resubmissions

Not Applicable

Renewals

Not Applicable

Revisions

Not Applicable

Additional Review Considerations - Overall, Administrative Core, Data Management Core, Scientific Core, and Research Projects

As applicable for the project proposed, reviewers will consider each of the following items, but will not give scores for these items, and should not consider them in providing an overall impact score.

Applications from Foreign Organizations

Reviewers will assess whether the project presents special opportunities for furthering research programs through the use of unusual talent, resources, populations, or environmental conditions that exist in other countries and either are not readily available in the United States or augment existing U.S. resources.

Select Agent Research

Reviewers will assess the information provided in this section of the application, including 1) the Select Agent(s) to be used in the proposed research, 2) the registration status of all entities where Select Agent(s) will be used, 3) the procedures that will be used to monitor possession use and transfer of Select Agent(s), and 4) plans for appropriate biosafety, biocontainment, and security of the Select Agent(s).

Resource Sharing Plans

Reviewers will comment on whether the Resource Sharing Plan(s) (e.g., Sharing Model Organisms (https://sharing.nih.gov/other-sharing-policies/model-organism-sharing-policy#policy-overview)) or the rationale for not sharing the resources, is reasonable.

Authentication of Key Biological and/or Chemical Resources:

For projects involving key biological and/or chemical resources, reviewers will comment on the brief plans proposed for identifying and ensuring the validity of those resources.

Budget and Period of Support

Reviewers will consider whether the budget and the requested period of support are fully justified and reasonable in relation to the proposed research.

2. Review and Selection Process

Applications will be evaluated for scientific and technical merit by (an) appropriate Scientific Review Group(s) convened by the National Institute of Allergy and Infectious Diseases, in accordance with NIH peer review policy and procedures (//grants.nih.gov/grants/guide/url_redirect.htm?id=11154), using the stated review criteria. Assignment to a Scientific Review Group will be shown in the eRA Commons.

As part of the scientific peer review, all applications will receive a written critique.

Applications may undergo a selection process in which only those applications deemed to have the highest scientific and technical merit (generally the top half of applications under review) will be discussed and assigned an overall impact score.

Appeals (https://grants.nih.gov/grants/policy/nihgps/html5/section_2/2.4.2_appeals_of_initial_scientific_review.htm) of initial peer review will not be accepted for applications submitted in response to this FOA

Applications will be assigned to the appropriate NIH Institute or Center. Applications will compete for available funds with all other recommended applications submitted in response to this FOA. Following initial peer review, recommended applications will receive a second level of review by the National Advisory Allergy and Infectious Diseases Council. The following will be considered in making funding decisions:

- Scientific and technical merit of the proposed project as determined by scientific peer review.
- Availability of funds.
- Relevance of the proposed project to program priorities.

3. Anticipated Announcement and Award Dates

After the peer review of the application is completed, the PD/PI will be able to access his or her Summary Statement (written critique) via the eRA Commons (//grants.nih.gov/grants/guide/url_redirect.htm?id=11123). Refer to Part 1 for dates for peer review, advisory council review, and earliest start date.

Information regarding the disposition of applications is available in the NIH Grants Policy Statement (//grants.nih.gov/grants/guide/url_redirect.htm?id=11120).

Section VI. Award Administration Information

1. Award Notices

If the application is under consideration for funding, NIH will request "just-in-time" information from the applicant as described in the NIH Grants Policy Statement (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_2/2.5.1_just-in-time_procedures.htm).

A formal notification in the form of a Notice of Award (NoA) will be provided to the applicant organization for successful applications. The NoA signed by the grants management officer is the authorizing document and will be sent via email to the recipient's business official.

Recipients must comply with any funding restrictions described in Section IV.6. Funding Restrictions. Selection of an application for award is not an authorization to begin performance. Any costs incurred before receipt of the NoA are at the recipient's risk. These costs may be reimbursed only to the extent considered allowable pre-award costs.

Any application awarded in response to this FOA will be subject to terms and conditions found on the Award Conditions and Information for NIH Grants (https://grants.nih.gov/grants/policy/nihgps/HTML5/part_ii_subpart_b.htm) website. This includes any recent legislation and policy applicable to awards that is highlighted on this website.

Institutional Review Board or Independent Ethics Committee Approval: Grantee institutions must ensure that protocols are reviewed by their IRB or IEC. To help ensure the safety of participants enrolled in NIH-funded studies, the recipient must provide NIH copies of documents related to all major changes in the status of ongoing protocols.

2. Administrative and National Policy Requirements

All NIH grant and cooperative agreement awards include the NIH Grants Policy Statement (//grants.nih.gov/grants/guide/url_redirect.htm?id=11120) as part of the NoA. For these terms of award, see the NIH Grants Policy Statement Part II: Terms and Conditions of NIH Grant Awards, Subpart A: General (//grants.nih.gov/grants/guide/url_redirect.htm?id=11157) and Part II: Terms and Conditions of NIH Grant Awards, Subpart B: Terms and Conditions for Specific Types of Grants, Recipients, and Activities (//grants.nih.gov/grants/guide/url_redirect.htm?id=11159), including of note, but not limited to:

- Federal wide Research Terms and Conditions (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_3/3.1_federalwide_standard_terms_and_conditions_for_research_grants.htm)
- Prohibition on Certain Telecommunications and Video Surveillance Services or Equipment (https://grants.nih.gov/grants/guide/notice-files/NOT-OD-21-041.html)
- Acknowledgment of Federal Funding (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_4/4.2.1_acknowledgement_of_federal_funding.htm)

If a recipient is successful and receives a Notice of Award, in accepting the award, the recipient agrees that any activities under the award are subject to all provisions currently in effect or implemented during the period of the award, other Department regulations and policies in effect at the time of the award, and applicable statutory provisions.

Should the applicant organization successfully compete for an award, recipients of federal financial assistance (FFA) from HHS will be required to complete an HHS Assurance of Compliance form (HHS 690) (https://ocrportal.hhs.gov/ocr/aoc/instruction.jsf) in which the recipient agrees, as a term and condition of receiving the grant, to administer their programs in compliance with federal civil rights laws that prohibit discrimination on the basis of race, color, national origin, age, sex and disability, and agreeing to comply with federal conscience laws, where applicable. This includes ensuring that entities take meaningful steps to provide meaningful access to persons with limited English proficiency; and ensuring effective communication with persons with disabilities. Where applicable, Title XI and Section 1557 prohibit discrimination on the basis of sexual orientation, and gender identity. The HHS Office for Civil Rights provides guidance on complying with civil rights laws enforced by HHS. Please see https://www.hhs.gov/civil-rights/for-providers/provider-obligations/index.html (https://www.hhs.gov/civil-rights/for-providers/provider-obligations/index.html) and https://www.hhs.gov/civil-rights/for-individuals/nondiscrimination/index.html (https://www.hhs.gov/civil-rights/for-individuals/nondiscrimination/index.html)

HHS recognizes that research projects are often limited in scope for many reasons that are nondiscriminatory, such as the principal investigator’s scientific interest, funding limitations, recruitment requirements, and other considerations. Thus, criteria in research protocols that target or exclude certain populations are warranted where nondiscriminatory justifications establish that such criteria are appropriate with respect to the health or safety of the subjects, the scientific study design, or the purpose of the research. For additional guidance regarding how the provisions apply to NIH grant programs, please contact the Scientific/Research Contact that is identified in Section VII under Agency Contacts of this FOA.

- Recipients of FFA must ensure that their programs are accessible to persons with limited English proficiency. For guidance on meeting the legal obligation to take reasonable steps to ensure meaningful access to programs or activities by limited English proficient individuals see https://www.hhs.gov/civil-rights/for-individuals/special-topics/limited-english-proficiency/fact-sheet-guidance/index.html (https://www.hhs.gov/civil-rights/for-individuals/special-topics/limited-english-proficiency/fact-sheet-guidance/index.html)andhttps://www.lep.gov (https://www.lep.gov/).

- For information on an institution’s specific legal obligations for serving qualified individuals with disabilities, including providing program access, reasonable modifications, and to provide effective communication, see https://www.hhs.gov/civil-rights/for-individuals/disability/index.html (https://www.hhs.gov/civil-rights/for-individuals/disability/index.html).

- HHS funded health and education programs must be administered in an environment free of sexual harassment, see https://www.hhs.gov/civil-rights/for-individuals/sex-discrimination/index.html (https://www.hhs.gov/civil-rights/for-individuals/sex-discrimination/index.html). For information about NIH's commitment to supporting a safe and respectful work environment, who to contact with questions or concerns, and what NIH's expectations are for institutions and the individuals supported on NIH-funded awards, please see https://grants.nih.gov/grants/policy/harassment.htm (https://grants.nih.gov/grants/policy/harassment.htm).

- For guidance on administering programs in compliance with applicable federal religious nondiscrimination laws and applicable federal conscience protection and associated anti-discrimination laws see https://www.hhs.gov/conscience/conscience-protections/index.html (https://www.hhs.gov/conscience/conscience-protections/index.html) and https://www.hhs.gov/conscience/religious-freedom/index.html (https://www.hhs.gov/conscience/religious-freedom/index.html).

Please contact the HHS Office for Civil Rights for more information about obligations and prohibitions under federal civil rights laws at https://www.hhs.gov/ocr/about-us/contact-us/index.html (https://www.hhs.gov/ocr/about-us/contact-us/index.html) or call 1-800-368-1019 or TDD 1-800-537-7697.

In accordance with the statutory provisions contained in Section 872 of the Duncan Hunter National Defense Authorization Act of Fiscal Year 2009 (Public Law 110-417), NIH awards will be subject to the Federal Awardee Performance and Integrity Information System (FAPIIS) requirements. FAPIIS requires Federal award making officials to review and consider information about an applicant in the designated integrity and performance system (currently FAPIIS) prior to making an award. An applicant, at its option, may review information in the designated integrity and performance systems accessible through FAPIIS and comment on any information about itself that a federal agency previously entered and is currently in FAPIIS. The Federal awarding agency will consider any comments by the applicant, in addition to other information in FAPIIS, in making a judgement about the applicant’s integrity, business ethics, and record of performance under Federal awards when completing the review of risk posed by applicants as described in 45 CFR Part 75.205 and 2 CFR Part 200.206 “Federal awarding agency review of risk posed by applicants.” This provision will apply to all NIH grants and cooperative agreements except fellowships.”

Cooperative Agreement Terms and Conditions of Award

The following special terms of award are in addition to, and not in lieu of, otherwise applicable U.S. Office of Management and Budget (OMB) administrative guidelines, U.S. Department of Health and Human Services (DHHS) grant administration regulations at 45 CFR Part 75 and 2 CFR Part 200, and other HHS, PHS, and NIH grant administration policies.

The administrative and funding instrument used for this program will be the cooperative agreement, an "assistance" mechanism (rather than an "acquisition" mechanism), in which substantial NIH programmatic involvement with the recipients is anticipated during the performance of the activities. Under the cooperative agreement, the NIH purpose is to support and stimulate the recipients' activities by involvement in and otherwise working jointly with the recipients in a partnership role; it is not to assume direction, prime responsibility, or a dominant role in the activities. Consistent with this concept, the dominant role and prime responsibility resides with the recipients for the project as a whole, although specific tasks and activities may be shared among the recipients and the NIH as defined below.

The PD(s)/PI(s) will have the primary responsibility for:

PD(s)/PI(s) will have the primary responsibility for coordinating the Projects and Cores within the overall Program. Specifically, the PD(s)/PI(s) have primary responsibility as described below.

- The PD(s)/PI(s) will be responsible for defining the research objectives, approaches, and details of the projects within the guidelines of the FOA and retains primary responsibility for the planning, directing, and executing the proposed scientific activities
- The PD(s)/PI(s) will monitor all Research Projects and actively promote efforts that foster integration, collaboration, and synergy across the projects.
- The PD(s)/PI(s) will be responsible for ensuring timely compliance with ReVAMPP Network policies for template usage, data sharing, and collaboration.
- The PD(s)/PI(s) are responsible for ensuring that appropriate systems are in place to provide for biosafety and security of materials, data, facilities and resources, including compliance with regard to Select Agent Regulations, Biosafety in Microbiology and Biomedical Laboratories (BMBL) Guidelines, Centers for Disease Control and Prevention and the National Institutes of Health, sixth Edition; U.S. Code of Federal Regulations 42 C.F.R. Part 73, 7 C.F.R. Part 331, and 9 C.F.R. Part 121.

In addition, the PD(s)/PI(s) will be responsible for:

- Organizing and chairing annual ReVAMPP Center Progress meeting activities. The annual ReVAMPP Center progress meetings are anticipated to be held at a location at/near Rockville, MD or at another NIAID-approved site and will last up to 2 days.
- Advertising the availability of the Program generated resources through outreach activities.

Recipients will retain custody of and have primary rights to the data and software developed under these awards, subject to Government rights of access consistent with current HHS, PHS, and NIH policies.

NIH staff have substantial programmatic involvement that is above and beyond the normal stewardship role in awards, as described below:

The role of the NIAID Project Scientist is to support and encourage the recipient's activities by substantial involvement as partners and facilitators in the process without assuming responsibilities that remain with the PDs/PIs.

- The NIAID Project Scientist will work closely with the PD(s)/PI(s) and other Program member scientists to facilitate collaborations and to leverage the resources available to the ReVAMPP Network.
- The NIAID Project Scientist will monitor the progress of the Center, help coordinate research approaches among all Centers funded through the FOA and contribute to the shaping of research projects or approaches as warranted. The NIAID Project Scientist will support and facilitate this process but will not direct it.
- Near the end of year 3 of the award, Program Staff will assess the progress towards development of generalizable approaches for vaccine, and if applicable early development of mAbs, for virus families of pandemic concern through the accomplishment of the milestones and overall feasibility of program advancement. The assessment will be based on the first three annual reports, the milestones included in the application and negotiated with the recipient prior to award, any additional information that the PD/PI elects to submit, evidence of collaboration with other ReVAMPP Centers, compliance with the ReVAMPP Network data sharing and CDSC requests, programmatic priorities, and the availability of funding.
- The NIAID Project Scientist will keep the ReVAMPP Centers informed about other ongoing studies supported by NIAID to avoid duplication of effort and encourage sharing/collaboration in infectious diseases research.
- The NIAID Project Scientist will coordinate access for the recipients to other NIAID resources, as well as assist the research efforts of the Program by facilitating access to fiscal and intellectual resources provided by industry, private foundations, NIH intramural scientists and other federal government agencies as appropriate.

In addition to the NIAID Project Scientist, an agency program official or IC program director will be responsible for the normal scientific and programmatic stewardship of the award and will be named in the award notice.

Areas of Joint Responsibility include:

- The NIAID Project Scientist and the PD(s)/PI(s) will hold regular program-wide discussions to facilitate the achievement of program goals.
- The PD(s)/PI(S) and the NIAID Project Scientist will collaborate in the establishment of the Scientific Advisory Board
- The NIAID Project Scientist and the PD/PI will collaborate during the course of the award to revise and/or update project milestones as appropriate.

Dispute Resolution

Any disagreements that may arise in scientific or programmatic matters (within the scope of the award) between award recipients and the NIH may be brought to Dispute Resolution. A Dispute Resolution Panel composed of three members will be convened. It will have three members: a designee of the Steering Committee chosen without NIH staff voting, one NIH designee, and a third designee with expertise in the relevant area who is chosen by the other two; in the case of individual disagreement, the first member may be chosen by the individual recipient. This special dispute resolution procedure does not alter the recipient's right to appeal an adverse action that is otherwise appealable in accordance with PHS regulation 42 CFR Part 50, Subpart D and DHHS regulation 45 CFR Part 16.

3. Data Management and Sharing

Note: The NIH Policy for Data Management and Sharing is effective for due dates on or after January 25, 2023.

Consistent with the NIH Policy for Data Management and Sharing, when data management and sharing is applicable to the award, recipients will be required to adhere to the Data Management and Sharing requirements as outlined in the NIH Grants Policy Statement (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_8/8.2.3_sharing_research_resources.htm). Upon the approval of a Data Management and Sharing Plan, it is required for recipients to implement the plan as described.

Recipients will be required to adhere to the FAIR Principles (FAIR Principles - GO FAIR (go-fair.org) (https://gcc02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.go-fair.org%2Ffair-principles%2F&data=05%7C01%7Cchelsea.boyd%40nih.gov%7C8247f78a75c04c5ea99208db1b643066%7C14b77578977342d58507251ca2dc2b06%7C0%7C0%7C63813388934
for data management and sharing.

4. Reporting

When multiple years are involved, recipients will be required to submit the Research Performance Progress Report (RPPR) (//grants.nih.gov/grants/rppr/index.htm) annually and financial statements as required in the NIH Grants Policy Statement. (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_8/8.4.1_reporting.htm)

A final RPPR, invention statement, and the expenditure data portion of the Federal Financial Report are required for closeout of an award, as described in the NIH Grants Policy Statement (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_8/8.6_closeout.htm). NIH FOAs outline intended research goals and objectives. Post award, NIH will review and measure performance based on the details and outcomes that are shared within the RPPR, as described at 45 CFR Part 75.301 and 2 CFR Part 200.301.

The Federal Funding Accountability and Transparency Act of 2006 (Transparency Act), includes a requirement for recipients of Federal grants to report information about first-tier subawards and executive compensation under Federal assistance awards issued in FY2011 or later. All recipients of applicable NIH grants and cooperative agreements are required to report to the Federal Subaward Reporting System (FSRS) available at www.fsrs.gov (//grants.nih.gov/grants/guide/url_redirect.htm?id=11170) on all subawards over the threshold. See the NIH Grants Policy Statement (https://grants.nih.gov/grants/policy/nihgps/HTML5/section_4/4.1.8_federal_funding_accountability_and_transparency_act__ffata_.htm) for additional information on this reporting requirement.

In accordance with the regulatory requirements provided at 45 CFR 75.113and 2 CFR Part 200.113 and Appendix XII to 45 CFR Part 75 and 2 CFR Part 200, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts from all Federal awarding agencies with a cumulative total value greater than $10,000,000 for any period of time during the period of performance of a Federal award, must report and maintain the currency of information reported in the System for Award Management (SAM)about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently FAPIIS). This is a statutory requirement under section 872 of Public Law 110-417, as amended (41 U.S.C. 2313). As required by section 3010 of Public Law 111-212, all information posted in the designated integrity and performance system on or after April 15, 2011, except past performance reviews required for Federal procurement contracts, will be publicly available. Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75and 2 CFR Part 200 – Award Term and Condition for Recipient Integrity and Performance Matters.

Section VII. Agency Contacts

We encourage inquiries concerning this funding opportunity and welcome the opportunity to answer questions from potential applicants.

Application Submission Contacts

eRA Service Desk (Questions regarding ASSIST, eRA Commons, application errors and warnings, documenting system problems that threaten submission by the due date, and post-submission issues)

Finding Help Online: https://www.era.nih.gov/need-help (https://www.era.nih.gov/need-help) (preferred method of contact)
Telephone: 301-402-7469 or 866-504-9552 (Toll Free)

General Grants Information (Questions regarding application instructions, application processes, and NIH grant resources)
Email: GrantsInfo@nih.gov (mailto:GrantsInfo@nih.gov) (preferred method of contact)
Telephone: 301-637-3015

Grants.gov Customer Support (Questions regarding Grants.gov registration and Workspace)
Contact Center Telephone: 800-518-4726
Email: support@grants.gov (mailto:support@grants.gov)

Scientific/Research Contact(s)

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Section VIII. Other Information

Recently issued trans-NIH policy notices (//grants.nih.gov/grants/guide/url_redirect.htm?id=11163) may affect your application submission. A full list of policy notices published by NIH is provided in the NIH Guide for Grants and Contracts (//grants.nih.gov/grants/guide/url_redirect.htm?id=11164). All awards are subject to the terms and conditions, cost principles, and other considerations described in the NIH Grants Policy Statement (//grants.nih.gov/grants/guide/url_redirect.htm?id=11120).

Authority and Regulations

Awards are made under the authorization of Sections 301 and 405 of the Public Health Service Act as amended (42 USC 241 and 284) and under Federal Regulations 42 CFR Part 52 and 45 CFR Part 75 and 2 CFR Part 200.

Weekly TOC for this Announcement (/grants/guide/WeeklyIndex.cfm?03-17-23)
NIH Funding Opportunities and Notices (/grants/guide/index.html)

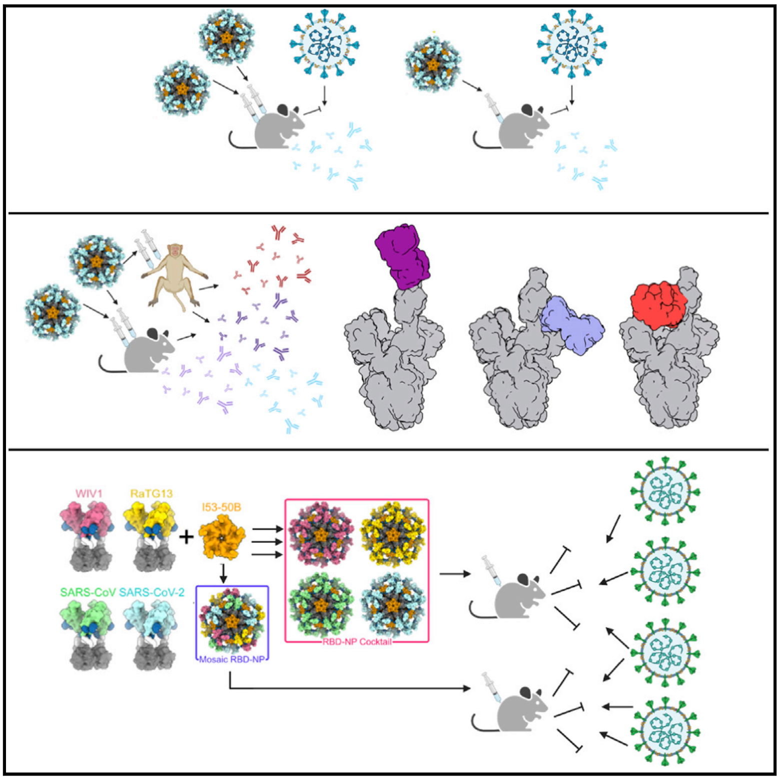
Note: For help accessing PDF, RTF, MS Word, Excel, PowerPoint, Audio or Video files, see Help Downloading Files (/grants/edocs.htm).

Cell

Article

Elicitation of broadly protective sarbecovirus immunity by receptor-binding domain nanoparticle vaccines

Graphical abstract



Highlights

- RBD-NP enables dose-sparing and protects mice after only one dose
- RBD-NP elicits diverse polyclonal antibody responses in non-human primates
- RBD-NP and HexaPro confer similar resilience to antigenic drift
- Mosaic and cocktail RBD-NPs protect against heterotypic SARS-CoV challenge

Authors

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In brief

A clinical stage multivalent SARS-CoV-2 spike receptor-binding domain nanoparticle (RBD-NP) vaccine is protective in mice after a single immunization and elicits strong antibody responses across circulating mutants and some sarbecoviruses. Multivalent sarbecovirus RBD-NP vaccines elicit heterotypic protection against sarbecoviruses.

Walls et al., 2021, Cell *184*, 5432–5447
October 14, 2021
https://doi.org/10.1016/j.cell.2021.09.015

CellPress Cell

Article

Elicitation of broadly protective sarbecovirus immunity by receptor-binding domain nanoparticle vaccines

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https://doi.org/10.1016/j.cell.2021.09.015

SUMMARY

Understanding vaccine-elicited protection against SARS-CoV-2 variants and other sarbecoviruses is key for guiding public health policies. We show that a clinical stage multivalent SARS-CoV-2 spike receptor-binding domain nanoparticle (RBD-NP) vaccine protects mice from SARS-CoV-2 challenge after a single immunization, indicating a potential dose-sparing strategy. We benchmarked serum neutralizing activity elicited by RBD-NPs in non-human primates against a lead prefusion-stabilized SARS-CoV-2 spike (HexaPro) using a panel of circulating mutants. Polyclonal antibodies elicited by both vaccines are similarly resilient to many RBD residue substitutions tested, although mutations at and surrounding position 484 have negative consequences for neutralization. Mosaic and cocktail nanoparticle immunogens displaying multiple sarbecovirus RBDs elicit broad neutralizing activity in mice and protect mice against SARS-CoV challenge even in the absence of SARS-CoV RBD in the vaccine. This study provides proof of principle that multivalent sarbecovirus RBD-NPs induce heterotypic protection and motivates advancing such broadly protective sarbecovirus vaccines to the clinic.

INTRODUCTION

The emergence of SARS-CoV-2 in late 2019 resulted in the COVID-19 pandemic that brought the world to a standstill. Moreover, the recurrent spillovers of coronaviruses in humans along with detection of SARS-CoV-2-, SARS-CoV-, and MERS-CoV-related coronaviruses in bats suggest that future zoonotic transmission events may continue to occur (Menachery et al., 2015, 2016; Zhou et al., 2020a). SARS-CoV-2 infects host cells through the attachment of the viral transmembrane spike (S) glycoprotein to angiotensin-converting enzyme 2 (ACE2), followed by fusion of the viral and host membranes (Hoffmann et al., 2020; Lan et al., 2020; Letko et al., 2020; Shang et al., 2020; Walls et al., 2020a; Wang et al., 2020; Wrapp et al., 2020; Yan et al., 2020; Zhou et al., 2020b). The SARS-CoV-2 S protein is the primary target of neutralizing antibodies (Abs), and the immunodominant receptor-binding domain (RBD) accounts for more than 90% of the neutralizing activity in COVID-19 convalescent sera and vaccinated individuals (Greaney et al., 2021a, 2021b; Piccoli et al., 2020). Numerous monoclonal Abs (mAbs) recognizing distinct antigenic sites on the RBD were isolated and shown to neutralize viral entry and protect small animals and non-human primates (NHPs) from SARS-CoV-2 challenge (Barnes et al., 2020; Baum et al., 2020a; Brouwer et al., 2020; Piccoli et al., 2020; Pinto et al., 2020; Rogers et al., 2020; Starr et al., 2021; Tortorici et al., 2020; Wec et al., 2020; Zost et al., 2020). As a result, SARS-CoV-2 S is the focus of nucleic acid, vectored, and protein subunit vaccines currently being developed and deployed (Corbett et al., 2020a, 2020b; Jackson et al., 2020; Mercado et al., 2020; Polack et al., 2020; Tostanoski et al., 2020; Yu et al., 2020).

Worldwide sequencing of SARS-CoV-2 clinical isolates has led to the identification of numerous mutations in the >1,500,000 genome sequences available to date (https://www.gisaid.org/). The SARS-CoV-2 S D614G mutation has become globally dominant and is associated with enhanced viral transmission and replication but does not significantly affect Ab-mediated neutralization (Hou et al., 2020; Korber et al., 2020; Plante et al., 2020; Yurkovetskiy et al., 2020). Conversely, some mutations found in circulating SARS-CoV-2 isolates were shown to promote escape from mAbs and to reduce neutralization by immune sera (Baum et al., 2020b; Collier et al., 2021; Li et al., 2020; McCallum et al., 2021a, 2021b; Wang et al., 2021a; Weisblum et al., 2020; Wibmer et al., 2021). As a result, formulation of mAb cocktails or the use of mAbs targeting conserved epitopes and neutralizing a broader spectrum of circulating SARS-CoV-2 variants emerged as a promising strategy to overcome this issue (Baum et al., 2020b; Cathcart et al., 2021; Dong et al., 2021; Greaney et al., 2020; Jette et al., 2021; Martinez et al., 2021a; Pinto et al., 2020; Tortorici et al., 2020, 2021). The recent emergence of several variants and variants of concern (VOCs) with numerous S mutations is especially worrisome, including B.1.1.7 (alpha), B.1.351 (beta), B.1.427/B.1.429 (epsilon), P.1 (gamma), and B.1.617.2 (delta) that originated in the UK, South Africa, the USA, Brazil, and India, respectively (Davies et al., 2020; Deng et al., 2021; Faria et al., 2021; McCallum et al., 2021b; Tegally et al., 2020). Some of these mutations lead to significant reductions in the neutralization potency of N-terminal domain (NTD)- and RBD-specific mAbs, convalescent sera, and Pfizer/BioNTech BNT162b2- or Moderna mRNA-1273-elicited sera (Collier et al., 2021; McCallum et al., 2021a, 2021b; Wang et al., 2021a).

We recently described a multivalent subunit vaccine displaying the SARS-CoV-2 RBD nanoparticle (RBD-NP) in a highly immunogenic array using a computationally designed self-assembling protein nanoparticle (Bale et al., 2016; Walls et al., 2020b). Vaccination with RBD-NP resulted in 10-fold higher neutralizing Ab titers in mice than the prefusion-stabilized S2P trimer (which is used in most current vaccines) despite a 5-fold lower dose and protected mice against mouse-adapted SARS-CoV-2 (SARS-CoV-2-MA) challenge (Dinnon et al., 2020; Walls et al., 2020b). We demonstrated that the RBD-NP vaccine elicited robust neutralizing Ab and CD4 T cell responses in NHPs when formulated with several clinic-ready adjuvants and conferred protection against SARS-CoV-2 infection in the nose, pharynges, and bronchioles (Arunachalam et al., 2021). The RBD-NP vaccine is currently being evaluated in two phase I/II clinical trials (ClinicalTrials.gov: NCT04742738 and NCT04750343) and recently received funding from the coalition for epidemic preparedness innovations for phase III clinical trials.

Although the S fusion machinery (S_2 subunit) has higher sequence conservation than the RBD (Pinto et al., 2021; Sauer et al., 2021; Tortorici and Veesler, 2019; Walls et al., 2016, 2020a), the breadth of neutralization and protection provided by RBD-based vaccines remains unknown. The isolation of RBD-specific cross-reactive mAbs neutralizing SARS-CoV-2 and SARS-CoV suggests that RBD-based vaccines could, in principle, elicit Abs that neutralize distantly related sarbecoviruses, which have future pandemic potential (Cathcart et al., 2021; Jette et al., 2021; Martinez et al., 2021a; Pinto et al., 2020; Rappazzo et al., 2021; Starr et al., 2021; Tortorici et al., 2021; Wec et al., 2020). RBD-based vaccines are also unaffected by S mutations outside of the RBD, especially in the highly variable NTD that is heavily mutated in many VOCs (Andreano et al., 2020; Avanzato et al., 2020; Choi et al., 2020; Collier et al., 2021; Wang et al., 2021a; McCallum et al., 2021a, 2021b; McCarthy et al., 2021). Here, we explored dose-sparing strategies for the RBD-NP vaccine and evaluated the impact of genetic diversity among SARS-CoV-2 clinical isolates and sarbecoviruses on vaccine-elicited Abs. We further designed mosaic and cocktail sarbecovirus RBD-NPs that elicit broad and protective Ab responses against heterologous sarbecovirus challenge, which could represent the next generation of pan-sarbecovirus vaccines.

RESULTS

Dose-sparing RBD-NP vaccination protects mice against SARS-CoV-2 challenge

Considering the unprecedented need for rapid global distribution of SARS-CoV-2 vaccines, we investigated the ability of RBD-NP to induce neutralizing and protective Ab titers at lower doses than previously evaluated. Specifically, we set out to test whether vaccine-elicited neutralizing Ab titers are altered by lowering the immunogen dose 10-fold (two 0.1 μg immunizations, RBD antigen dose) or reducing the number of doses

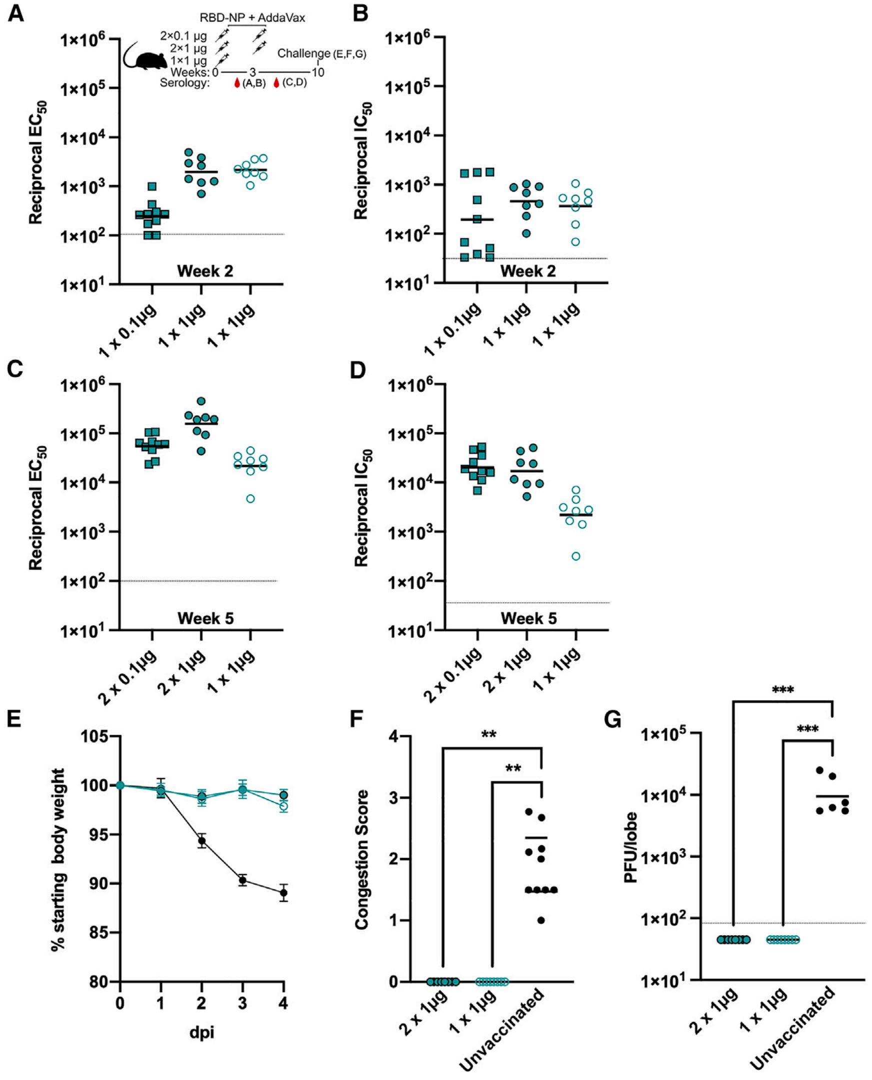


Figure 1. A single immunization with RBD-NP protects BALB/c cByJ mice from SARS-CoV-2 MA10 challenge

(A and C) S2P-binding Abs were measured 2 (A) or 5 (C) weeks post-prime; teal squares: 2 doses of 0.1 µg (n = 10); filled teal circles: 2 doses of 1 µg (n = 8); open teal circles: one dose of 1 µg (n = 8) with a limit of detection (LOD) of 1 × $10^{2\cdot}$

(B and D) Serum neutralizing Ab titers at 2 (B) or 5 (D) weeks post-prime determined using an MLV pseudotyping system with an LOD of 3.3 × 10^1.

(E) Weight loss following SARS-CoV-2 MA10 challenge up to 4 days post infection (n = 8 vaccinated; n = 6 naive mice shown as black filled circles).

(F) Congestion score following SARS-CoV-2 MA10 challenge with a score of 0 indicating unchanged lung color and 4 indicating a darkened and diseased lung.

(G) Viral titers in the mice lungs (expressed in plaque forming units [PFUs] per lobe) following challenge with an LOD of 9 × 10^1. Statistical significance was determined by Kruskal-Wallis test and shown only when significant. $^{**}p < 0.01$. LODs are shown as gray horizontal dotted lines.

Raw data curves shown in Data S1.

(a single 1 µg immunization, RBD antigen dose) compared to our initial work (Walls et al., 2020b). To test this, BALB/c cByJ mice were immunized intramuscularly at week 0 with either 0.1 µg (ten mice) or 1 µg (two groups of eight mice or sixteen mice total) of AddaVax-adjuvanted RBD-NP. Two weeks post-prime, serum binding and neutralizing Ab titers were roughly comparable for the two vaccine doses (neutralization geometric mean titer [GMT] ~2–4 × 10^2) measured using a mouse leukemia virus (MLV) pseudotyping system (Millet and Whittaker, 2016; Walls et al., 2020a) (Figures 1A and 1B; Data S1). Three weeks post prime, we boosted the mice that were immunized with 0.1 µg of the vaccine and eight of the sixteen mice immunized with 1 µg of the vaccine with the same respective dose. Two weeks post-boost, serum binding and neutralizing Ab titers were comparable for the 0.1 µg and 1 µg groups (neutralization GMT ~2 × 10^4) (Figures 1C and 1D; Data S1), suggesting that a 10-fold reduction in the RBD-NP dose does not affect serum neutralizing responses. Furthermore, we observed that the magnitude of binding and neutralizing Ab titers increased over time for the mice that received a single immunization (neutralization GMT 2 × 10^3). These results suggest that lowering the vaccine dose or reducing the number of immunizations represent possible dose-sparing strategies that did not compromise elicitation of high levels of neutralizing Ab titers using RBD-NPs.

To further evaluate vaccine efficacy, eight mice that received one dose and eight mice that received two doses of the 1-µg RBD-NP vaccine along with six unvaccinated mice were challenged ten weeks post-prime with 10^5 plaque-forming units (PFUs) of mouse-adapted SARS-CoV-2 MA10 (Leist et al., 2020). The SARS-CoV-2 MA10 model causes weight loss and lung pathology when 10^5 PFUs (the highest titer tested [Leist et al., 2020]) are used in young BALB/c mice. The mice were followed for four days to assess protection from disease. All mice in the RBD-NP-vaccinated groups were protected from weight loss throughout the duration of the experiment regardless of the number of doses, whereas control mice lost ~10% of their weight by day four (Figure 1E). Analysis of lung pathology and viral titers in lung tissues indicated that the vaccinated mice were not affected by SARS-CoV-2 MA10 challenge, whereas the control mice showed lung discoloration four days post-infection and a high viral load (~1 × 10^4 PFUs) (Figures 1F and 1G). These results indicate that one or two immunizations with 1 µg RBD-NPs results in protection against SARS-CoV-2 MA10-induced disease. Furthermore, mice vaccinated twice with a 10-fold lower dose of RBD-antigen (0.1 µg) had higher serum neutralizing Ab titers than mice receiving a single 1-µg dose, suggesting that they would likely also be protected since neutralizing Ab titers are a correlate of protection against SARS-CoV-2 (Arunachalam et al., 2021; McMahan et al., 2021). These data suggest that multiple RBD-NP dose-sparing regimens may be possible.

RBD-NP vaccination induces Abs targeting diverse antigenic sites in NHPs

To characterize the epitopes targeted by RBD-NP-elicited polyclonal Abs, we used deep mutational scanning (DMS) with sera obtained 56 days post-prime from one of two pigtail macaques immunized at days 0 and 28 with a dose of AddaVax-adjuvanted RBD-NP containing 88 µg of the SARS-CoV-2 RBD (with a linker of twelve glycine/serine (GS) residues between the RBD and the NP) (Walls et al., 2020b) (Figures 2A and 2B). These experiments rely on yeast surface display of RBD libraries covering nearly all possible amino acid mutations coupled with fluorescence-activated cell sorting (FACS) to identify RBD mutants with attenuated Ab binding compared to the wild-type (Wuhan-Hu-1) SARS-CoV-2 RBD (Greaney et al., 2020; Starr et al., 2020a). No single mutation had more than a marginal effect on serum Ab (IgG/IgM/IgA) binding, indicating broad targeting of distinct RBD epitopes, whereas several previously described COVID-19 human convalescent plasmas (HCPs) analyzed by DMS displayed greater sensitivity to individual mutations and are shown for comparison (Greaney et al., 2021b) (Figures 2B and S1; Table S1). These results show that RBD-NP vaccination elicits highly diverse polyclonal Ab responses that target multiple distinct antigenic sites and are more resilient to escape mutations reducing polyclonal Ab binding than HCP (Barnes et al., 2020; Greaney et al., 2021b; Piccoli et al., 2020; Starr et al., 2021).

To measure the magnitude of vaccine-elicited Abs against distinct RBD antigenic sites, we used quantitative competition ELISAs with human ACE2-Fc (hACE2-Fc), which binds to the receptor-binding motif (RBM) corresponding to antigenic sites Ia and Ib, as well as with structurally characterized mAbs recognizing antigenic sites II (CR3022) and IV (S309) (Huo et al., 2020; Piccoli et al., 2020; Pinto et al., 2020; Yuan et al., 2020) (Figures 2C–2E; Data S1). These experiments

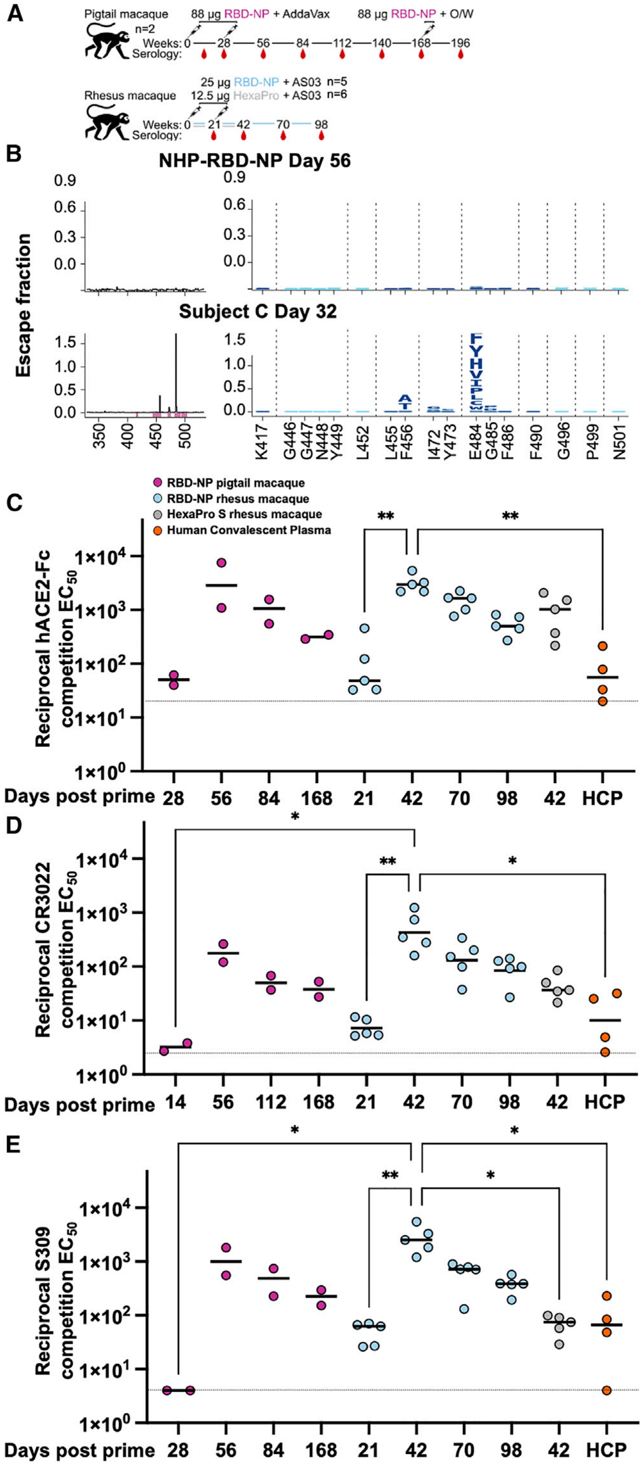


Figure 2. RBD-NP vaccination elicits high titers of Abs targeting diverse RBD antigenic sites in NHPs

(A) NHP study designs.

(B) Effect of RBD mutations on polyclonal Ab binding measured by DMS analysis of serum obtained 8 weeks post-prime from an immunized pigtail macaque (n = 1) compared to a previously reported DMS measurement from a representative COVID-19 HCP sample (reproduced here for comparison; Greaney et al., 2021b). The line plots on the left show the summed effect of all mutations at a site in the RBD on serum or plasma binding, with RBD residues on the x axis and Ab escape on the y axis (Table S1). Due to the use of sample-specific FACS gates (Figure S1), the y axes are scaled independently. Sites in the logo plots are colored dark blue if located in the receptor-binding ridge or cyan if located in the RBD 443–450 loop. Larger values indicate more Ab escape.

(C–E) Competition ELISA between 0.2-nM hACE2-Fc (LOD of 2×10^1) (C), 2-nM CR3022 mAb (LOD of 2×10^0) (D), or 0.01-nM S309 mAb (LOD of 4×10^0) (E), and RBD-NP-elicited sera in pigtail macaques (n = 2), RBD-NP-elicited sera in rhesus macaques (n = 5), or HexaPro S-elicited sera in rhesus macaques (n = 5) at various time points following vaccination, benchmarked against HCP (n = 4) (Table S2). Each plot shows the magnitude of inhibition of hACE2/mAb binding to immobilized SARS-CoV-2 S2P, expressed as reciprocal serum dilution blocking 50% of the maximum binding response. Statistical significance was determined by Kruskal-Wallis test and shown only when significant. **p < 0.01. LODs are shown as gray horizontal dotted lines.

Raw data curves shown in Data S1.

used sera from the aforementioned pigtail macaques (RBD GMT EC_{50} 5 × 10^4) followed for 168 days and sera from 5 rhesus macaques immunized twice on days 0 and 21 with AS03-adjuvanted RBD-NPs containing 25 μg of the SARS-CoV-2 RBD (with a 16-GS linker between the RBD and the NP; RBD GMT EC_{50} 1 × 10^4) and followed for 98 days (Arunachalam et al., 2021; Walls et al., 2020b; Figures 2A and S2A). These two sets of RBD-NP sera were compared to sera from 5 rhesus macaques immunized twice on days 0 and 21 with 12.5 μg of AS03-adjuvanted prefusion-stabilized HexaPro S trimer (RBD EC_{50} 1 × 10^4) and followed for 42 days (Arunachalam et al., 2021; Hsieh et al., 2020), as well as a panel of COVID-19 HCP (Table S2) (RBD EC_{50} 2 × 10^4) (Figures 2A and S2A). All NHP sera post boost had high titers of Abs targeting sites Ia and Ib in the immunodominant RBM (day 42 hACE2 competition RBD-NP GMT 3 × 10^3 and HexaPro GMT 7 × 10^2) (Figure 2C; Data S1), a correlate of neutralization potency (Piccoli et al., 2020), in agreement with the potent immunogenicity and protective efficacy of RBD-NP and HexaPro S in NHPs (Arunachalam et al., 2021). We also observed strong Ab responses against antigenic sites II (RBD-NP day 42 GMT 4 × 10^2 and HexaPro GMT 4 × 10^1) with CR3022 competition and IV (RBD-NP day 42 GMT 2.5 × 10^3 and HexaPro GMT 6.5 × 10^1) (Figures 2D and 2E; Data S1), which comprise conserved sarbecovirus epitopes recognized by broadly neutralizing sarbecovirus mAbs such as S2X259 (site II) and S309 (site IV) (Piccoli et al., 2020; Pinto et al., 2020; Tortorici et al., 2021). Ab responses were durable against all 3 antigenic sites regardless of dose or adjuvant, with a decrease of competition titers by roughly half an order to one order of magnitude 168 days (pigtail macaque) or 98 days (rhesus macaque) post-prime. RBD-NP elicited much higher peak binding titers toward all RBD antigenic sites evaluated compared to HexaPro S and HCP, showcasing the potency, durability, and multi-specificity of Ab responses induced by multivalent display of the RBD, possibly as a result of enhanced accessibility of the antigen.

RBD-NP vaccine elicits potent neutralizing Ab responses in NHPs against a panel of SARS-CoV-2 S variants

To assess the neutralization breadth of RBD-NP- and HexaPro S-elicited Abs in NHPs at peak titer (42 days post-prime), we evaluated serum neutralizing activity against a panel of pseudotyped viruses comprising wild-type (D614G) SARS-CoV-2 S and twelve single-residue SARS-CoV-2 RBD mutants detected in clinical isolates (N439K, L452R, Y453F, L455F, S477N, E484A/K/Q, F486L, S494P, N501Y, and G504D) as well as the B.1.1.7 (H69-V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H); B.1.1.7 + E484K (H69-V70 deletion, Y144 deletion, E484K, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H); B.1.351 (L18F, D80A, D215G, L242-L244 deletion, R246I, K417N, E484K, N501Y, D614G, A701V); and P.1 (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I). Several single-residue RBD mutations did not affect neutralizing titers compared to wild-type D614G SARS-CoV-2 S, for which GMTs of 1.8 × 10^3 and 2.4 × 10^3 were determined with RBD-NP- and HexaPro S-elicited sera, respectively, using a vesicular stomatitis virus (VSV) pseudotyped virus (Figure 3A; Data S1). For example, the N439K- (GMT 2.5 × 10^3 for RBD-NP and 2.7 × 10^3 for HexaPro S) and mink-associated Y453F (GMT 4 × 10^3 for RBD-NP and 5 × 10^3 for HexaPro S) mutation did not dampen the neutralization potency of either RBD-NP- or HexaPro S-elicited sera (Figure 3A; Data S1). However, the N501Y substitution (present in the B.1.1.7, P.1, and B.1.351 lineages) reduced the neutralization potency of RBD-NP- (GMT 5 × 10^2) and HexaPro S (GMT 8 × 10^2)-elicited sera 3-to-4-fold and the L452R mutation (present in the B.1.427/B.1.429, B.1.617.1, and B.1.617.22 variant) led to a 3.4-to-4.4-fold drop in neutralization potency (GMT 4 × 10^2 for RBD-NP and 7 × 10^2 for HexaPro S). These substitutions have been associated with the loss of binding and neutralization for vaccine-elicited Abs and mAbs (Collier et al., 2021; McCallum et al., 2021b; Wang et al., 2021b; Thomson et al., 2021; Wang et al., 2021c). The E484A mutation reduced serum neutralization activity up to 2-fold (GMT 1.1x10^3 for RBD-NP and 1.1x10^3 for HexaPro S) whereas E484K, present in B.1.351 and P1 (GMT 2 × 10^2 for RBD-NP and 2 × 10^2 for HexaPro S), and E484Q, found in B.1.617.1 (GMT 5 × 10^2 for RBD-NP and 3.6 × 10^2 for HexaPro S), decreased it 9-fold (E484K) and 4-fold (E484Q) for RBD-NP and 12-fold (484K) and 7-fold (484Q) for HexaPro S compared to D614G SARS-CoV-2 S. These experiments suggest that a substantial fraction of the neutralizing activity elicited by both RBD-NP and HexaPro S is focused on the RBM, especially near position 484, as further supported by the ~5-fold decrease in neutralization resulting from the F486L (GMT 3.7 × 10^2 for RBD-NP and 5 × 10^2 for HexaPro S) substitution.

To understand the impact of the full constellation of mutations present in the S proteins of the aforementioned VOCs, we evaluated RBD-NP- and HexaPro S-elicited serum neutralizing activity against corresponding HIV and VSV pseudotyped variants (D614G GMT RBD-NP HIV 5 × 10^3 and VSV RBD-NP 6 × 10^2, respectively, and D614G GMT HexaPro S HIV 3.5 × 10^3 and VSV 1.2 × 10^3) (Figures 3B and 3C). Although we did not observe major reductions in neutralization titers (up to to 2-fold) toward the B.1.1.7 VOC (GMT RBD-NP HIV 2.5 × 10^3, VSV 4.7 × 10^2 and HexaPro S HIV 1.8 × 10^3, VSV 8.8 × 10^2) (Figures 3B and 3C), addition of the E484K mutation to the B.1.1.7 background reduced neutralization 4-to-6-fold for RBD-NP- (GMT HIV 8.8 × 10^2) and HexaPro S-elicited sera (GMT HIV 8.6 × 10^2), confirming the importance of the 484 position (Figure 3B). Neutralization of B.1.351 was 10-fold lower using HIV pseudovirus (GMT RBD-NP 5 × 10^2 and HexaPro S 3.6x10^2) (Figure 3B) and ~3-fold lower using VSV pseudovirus (GMT RBD-NP 2 × 10^2 and HexaPro S 4x10^2) (Figure 3C) for both RBD-NP- and HexaPro-elicited sera, in agreement with authentic virus neutralization data (Arunachalam et al., 2021). Interestingly, RBD-specific binding Ab titers did not differ significantly between the B.1.351 RBD (GMT RBD-NP 6.5 × 10^3 and HexaPro S 3 × 10^3) and Wuhan-Hu-1 RBD (GMT RBD-NP 1.4 × 10^4 and HexaPro S 1.7 × 10^4) (Figure S2A), indicating that neutralizing activity is accounted for by a portion of total binding Abs. Reductions in neutralizing Ab titers were also observed against P.1 S VSV pseudoviruses, with a 6-fold drop for RBD-NP (GMT 1 × 10^2) and 8-fold dampening for HexaPro S (GMT 1.5 × 10^2) (Figure 3C). Furthermore, we observed a 10-fold reduction in plasma

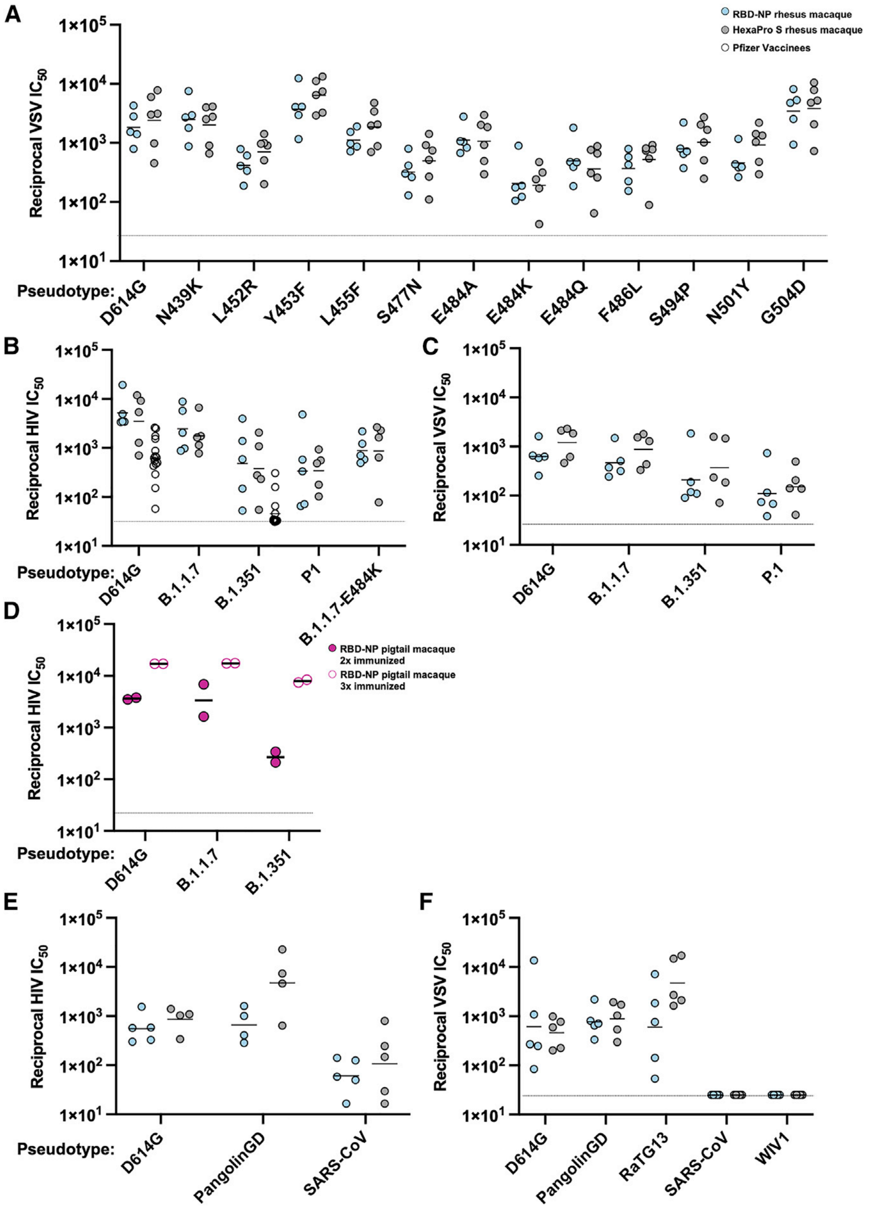


Figure 3. RBD-NP and HexaPro S elicit Abs with similar neutralization breadth toward SARS-CoV-2 variants

(A) Neutralizing Ab titers against wild-type (D614G) SARS-CoV-2 S and RBD point mutants determined using RBD-NP-elicited sera in rhesus macaques (blue, n = 5) and HexaPro S-elicited sera in rhesus macaques (gray, n = 6) with an VSV pseudotyping system with an LOD of 3.3 × 10^1. Neutralization performed once.

(B) Neutralizing Ab titers against HIV pseudotyped viruses harboring wild-type (D614G) SARS-CoV-2 S, B.1.1.7 S, B.1.1.7-E484K S, B.1.351 S, or P.1 S, determined using RBD-NP-elicited sera in rhesus macaques (blue), HexaPro S-elicited sera in rhesus macaques (gray), or plasma from individuals who received two doses of Pfizer mRNA vaccine (open circles) with an LOD of 3.3 × 10^1. Neutralization performed twice and a representative shown.

(C) Neutralizing Ab titers against VSV pseudotyped viruses harboring wild-type (D614G) SARS-CoV-2 S, B.1.1.7 S, B.1.351 S, or P.1 S, determined using RBD-NP-elicited sera in rhesus macaques (blue) or HexaPro S-elicited sera in rhesus macaques (gray) with an LOD of 2.5 × 10^1. Neutralization performed twice and a representative shown.

(D) Neutralizing Ab titers against D614G SARS-CoV-2 S, B.1.1.7, and B.1.351 S HIV pseudoviruses in pigtail macaque sera collected 28 days after a second (filled symbols, n = 2) or third (open symbols, n = 2) immunization with 88-μg RBD-NP (RBD antigen dose) with an LOD of 1 × 10^2. Neutralization performed twice and a representative shown.

(E) Neutralizing Ab titers against HIV pseudotyped viruses harboring wild-type (D614G) SARS-CoV-2 S, Pangolin-GD S, or SARS-CoV S, determined using RBD-NP-elicited sera in in rhesus macaques (blue) or HexaPro S-elicited sera in rhesus macaques (gray) with an LOD of 1 × 10^1. Neutralization performed twice and a representative shown.

(F) Neutralizing Ab titers against VSV pseudotyped viruses harboring wild-type (D614G) SARS-CoV-2 S, Pangolin-GD S, RaTG13 S, SARS-CoV S, or WIV1 S determined using SARS-CoV-2 RBD-NP-elicited sera in rhesus macaques (blue) or HexaPro S-elicited sera in rhesus macaques (gray) with an LOD of 2.5 × 10^1. Neutralization performed twice and a representative shown. Statistical significance was determined by Kruskal-Wallis test and shown in Table S3. LODs are shown as gray horizontal dotted lines. Raw data curves shown in Data S1 and GMTs in Table S4. The various pseudovirus backbones were benchmarked against NIBSC standard and are shown in Table S5.

neutralizing potency for individuals who received two doses of the Pfizer-BioNTech BNT162b2 mRNA vaccine against B.1.351 S pseudotyped virus (GMT 6 × 10^1) relative to the wild-type (D614G) SARS-CoV-2 S (GMT 6 × 10^2) pseudotyped virus (Figure 3B; Data S1). These findings show that, as is the case for many convalescent individuals (Greaney et al., 2021b; Liu et al., 2021; Piccoli et al., 2020), an important fraction of vaccine-elicited neutralizing Abs in NHPs and humans is focused on the RBM (specifically around position 484), independently of the immunogen (RBD-NP, HexaPro S, or 2P-stabilized S [Pallesen et al., 2017]) or the vaccine modality (protein subunit or mRNA). However, we note that neutralization assays may underestimate the contribution of NTD-specific or non-RBM RBD-targeted neutralizing Abs (Lempp et al., 2021; McCallum et al., 2021a; Suryadevara et al., 2021), and protection from challenge will be the ultimate readout.

To further investigate the relationships between neutralizing Ab titers and emerging SARS-CoV-2 variants, we immunized the two pigtail macaques a third time with RBD-NP, formulated with an oil-in-water (O/W) emulsion-based adjuvant, 168 days after the primary immunization (Figure 2A). This boost induced potent serum neutralizing activity against wild-type (D614G)

SARS-CoV-2 (GMT 2 × 10^4) (Figure 3D) as well as the B.1.1.7 (GMT 2 × 10^4) and B.1.351 (GMT 8 × 10^3) VOC pseudoviruses, suggesting that an overall increase in neutralizing Ab titers may be a suitable strategy to cope with emerging variants. These results are consistent with recent studies demonstrating that boosting COVID-19 convalescent individuals with a single mRNA vaccination elicited high (neutralizing) Ab titers, including against the B.1.351 variant (Abu Jabal et al., 2021; Krammer et al., 2021; Stamatatos et al., 2021), and suggest that a third vaccination of naive individuals could be a suitable strategy to limit the impact of emerging variants. This strategy is currently being evaluated for immunocompromised patients in several countries.

RBD-NP vaccine elicits cross-reactive sarbecovirus polyclonal Abs in NHPs

As RBD-NP- and HexaPro S-elicited polyclonal Abs exhibited similar resilience to a range of mutations, we next investigated cross-reactivity with a panel of sarbecovirus RBDs. RBD-NP-elicited polyclonal Abs purified from pigtail macaque serum obtained 70 days post-prime strongly cross-reacted with the SARS-CoV-2-related Pangolin-GD and RaTG13 RBDs and bound more weakly to distantly related RmYN02, SARS-CoV, WIV16, and ZXC21 RBDs (Figures S2B and S2C). Measurement of Ab binding titers using the SARS-CoV and SARS-CoV-2 S2P ectodomain trimers by ELISA showed that RBD-NPs and HexaPro S induced similar levels of cross-reactive Abs against each antigen, with responses roughly two orders of magnitude higher against SARS-CoV-2 S2P (detection antigen matching immunogen) compared to SARS-CoV S2P (Figure S2D; Data S1). The two immunogens also elicited similar peak levels of SARS-CoV S ACE2-competing Abs (GMT 2 × 10^2 RBD-NP and 5 × 10^1 HexaPro) suggesting that cross-neutralizing Abs might have been induced to similar extents between the two vaccines (Figure S2E).

Motivated by the cross-reactivity of RBD-NP-elicited polyclonal Abs with various sarbecovirus RBDs and the correlation between ACE2 competition and serum neutralization titers (Piccoli et al., 2020), we evaluated neutralization of a panel of HIV (D614G GMT 5.5 × 10^2) and VSV (D614G GMT 6 × 10^2) pseudoviruses harboring sarbecovirus S glycoproteins. RBD-NP-elicited sera efficiently neutralized pseudotyped viruses harboring the S glycoprotein of the Pangolin-GD isolate (GMT 6.6 × 10^2 and 7.7 × 10^2 VSV) and RaTG13 (GMT VSV 6 × 10^2) (Figures 3E and 3F) (Lam et al., 2020; Zhou et al., 2020b), in agreement with the close phylogenetic relationship of their RBDs with that of SARS-CoV-2 S (Figure S2B). HexaPro S-elicited NHP sera also efficiently neutralized Pangolin-GD S (GMT HIV 4.7 × 10^3 and VSV 9 × 10^2) and RaTG13 S pseudotypes (GMT VSV 4.7 × 10^3) (Figures 3E and 3F). Furthermore, we observed that both RBD-NP- and HexaPro S-induced polyclonal Abs that weakly neutralized HIV pseudovirus carrying SARS-CoV S (RBD-NP GMT 6 × 10^1 and HexaPro GMT 1 × 10^2) (Figures 3E and 3F). Collectively, these data demonstrate that both immunogens elicited comparable neutralization breadth and potency against the pseudoviruses tested, reinforcing the notion that most sarbecovirus S-directed neutralizing Abs target the RBD (Greaney et al., 2021a, 2021b; Piccoli et al., 2020).

Design, assembly, and characterization of mosaic and cocktail sarbecovirus RBD-NPs

We and others have recently evaluated nanoparticle immunogens that display multiple antigenic variants of viral glycoproteins as a potential route toward broadly protective vaccines (Boyoglu-Barnum et al., 2021; Cohen et al., 2021a, 2021b; Kanekiyo et al., 2019). Given the large number of coronaviruses circulating in zoonotic reservoirs, such vaccines could be important for understanding vaccine-induced neutralization/protection breadth and preventing future pandemics (Menachery et al., 2015, 2016). We expressed and purified four RBDs from the S proteins of SARS-CoV-2, SARS-CoV, and the bat coronaviruses WIV1 and RaTG13 genetically fused to the I53-50A trimer. An equimolar mixture of these four proteins was added to the I53-50B pentamer to assemble a mosaic RBD-NP (mRBD-NP) co-displaying the four RBDs on the same nanoparticle (Figure 4A and S3A). We also assembled a trivalent mosaic RBD-NP designated “drop out” lacking the SARS-CoV RBD (mRBD-NP-DO), as well as cocktail immunogens with three (cRBD-NP-DO; lacking the SARS-CoV RBD) or four (cRBD-NP) independently assembled nanoparticles, each displaying a single type of the aforementioned RBDs, mixed after independent assembly. Finally, we made a bivalent mosaic RBD-NP co-displaying the SARS-CoV and SARS-CoV-2 RBDs (Figure S3A) to directly confirm co-display on the nanoparticles using a sandwich binding assay. All of the nanoparticle immunogens formed the intended icosahedral architecture and retained native antigenicity, as shown by SDS-PAGE, dynamic light scattering, negative staining electron microscopy, and binding to hACE2-Fc (Figures S3B–S3E). We found that the vaccine candidates were stable for at least 4 weeks at several temperatures except the highest temperature evaluated (37°C), at which we observed a decrease in hACE2 recognition over time beginning after 7 to 14 days, presumably due to aggregation (Figures 4B–4E). Following immobilization using the SARS-CoV-2-specific mAb S2H14 (Piccoli et al., 2020), the quadrivalent, trivalent, and bivalent mRBD-NPs all bound the Fab of the SARS-CoV-specific Ab S230 (Piccoli et al., 2020; Rockx et al., 2008; Walls et al., 2019), confirming co-display, whereas the monovalent SARS-CoV-2 RBD-NP did not (Figure S3G). We determined that the reactivity of the trivalent mRBD-NP derived from the inclusion of the WIV1 RBD in this vaccine, as the monovalent WIV1 RBD-NP also bound the S230 Fab after immobilization with hACE2 (as expected [Menachery et al., 2016]), whereas the monovalent SARS-CoV-2 and RaTG13 RBD-NPs did not (Figure S3H). Collectively, these data demonstrate that all of the nanoparticles were stable and (co-)displayed the various RBDs as intended.

Cocktail and mosaic RBD-NP vaccines elicit cross-reactive and broadly neutralizing sarbecovirus Abs

The mosaic and cocktail nanoparticle immunogens were compared to monovalent RBD-NP vaccines and a non-assembling control vaccine comprising all four RBD-I53-50A trimeric components and a non-assembling I53-50B pentamer (Figure S3F) in an immunization study in BALB/c cByJ mice (Figures 4A and S3A). All immunizations comprised 1 μg of total RBD antigen, such that ~0.25 μg of each RBD was given in each administration of the quadrivalent vaccines

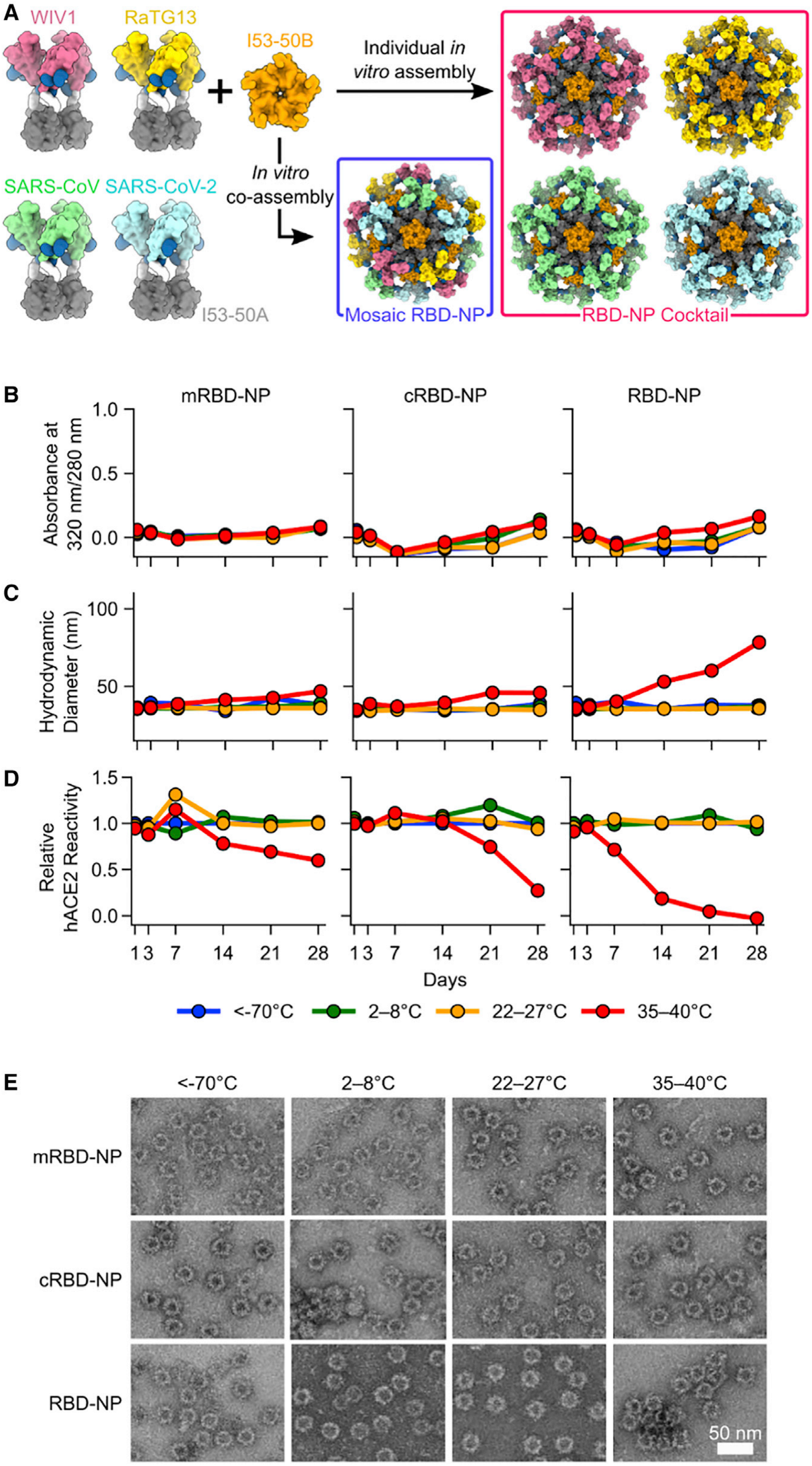


Figure 4. *In vitro* assembly and accelerated stability studies of mosaic and cocktail nanoparticle immunogens

(A) Schematic of *in vitro* assembly of mRBD-NPs and cRBD-NPs.

(B–E) The physical and antigenic stability of mRBD-NP, cRBD-NP, and (SARS-CoV-2) RBD-NP samples incubated at four different temperatures was followed for four weeks.

(B) The ratio of UV/vis absorbance at 320 nm/280 nm is a measure of turbidity (proxy for aggregation).

(C) Hydrodynamic diameter of the nanoparticles measured using dynamic light scattering.

(D) hACE2-Fc binding measured by comparing the peak amplitude of hACE2-Fc binding for each sample to a reference sample stored at $< -70^\circ C$ using biolayer interferometry.

(E) Electron micrographs of negatively stained samples after incubation for 28 days at the indicated temperatures. Scale bar, 50 nm.

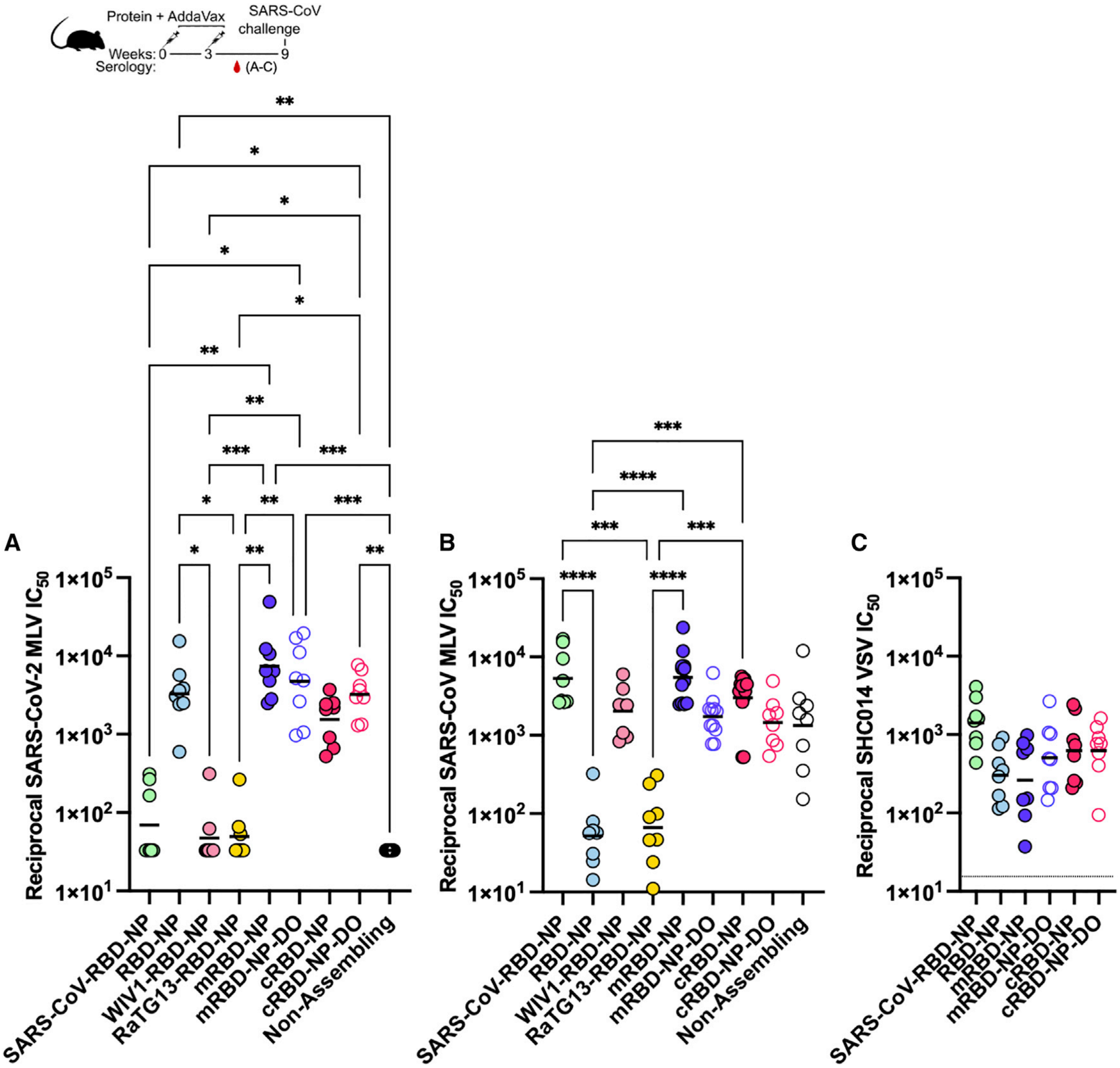


Figure 5. Mosaic and cocktail RBD-NP vaccines elicit neutralizing Abs against multiple sarbecoviruses

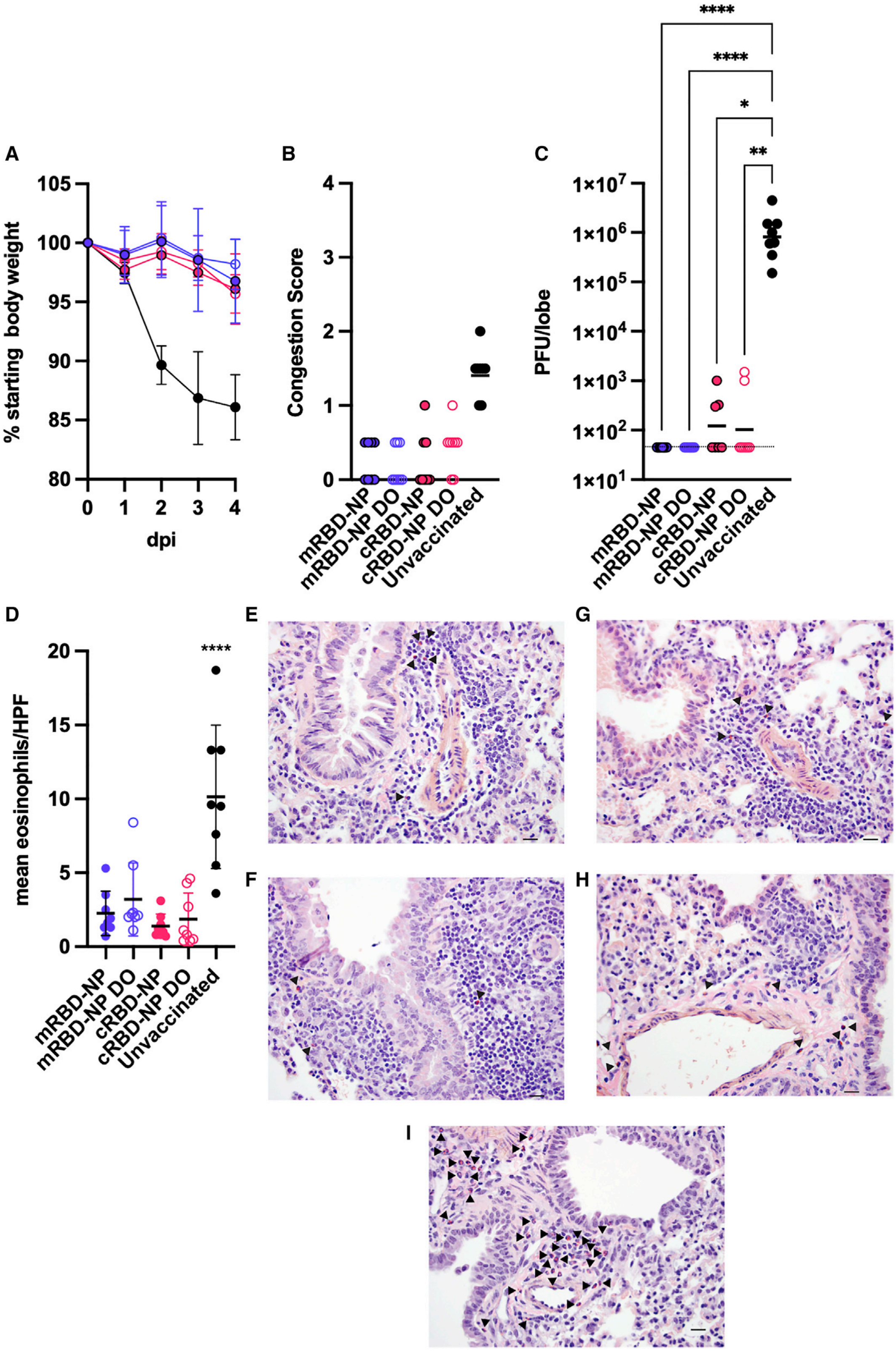
(A) Neutralizing Ab titers in mice (n = 8) against wild-type (D614G) SARS-CoV-2 S MLV pseudovirus five weeks post-prime elicited by monovalent, mosaic, and cocktail RBD-NPs with an LOD of 1 × 10^1.

(B) Neutralizing Ab titers in mice against SARS-CoV S MLV pseudovirus five weeks post-prime elicited by monovalent, mosaic, and cocktail RBD-NPs with an LOD of 1 × 10^1.

(C) Neutralizing Ab titers in mice against SHC014 VSV pseudovirus five weeks post-prime elicited by monovalent, mosaic, and cocktail RBD-NPs with an LOD of 1.7 × 10^1.

Raw data curves shown in Data S1. Statistical significance was determined by Kruskal-Wallis test and shown only when significant. $^{**}p<0.01$. LOD is shown as a gray horizontal dotted line in (C).

and ~0.33 μg of each RBD in the trivalent vaccines. After two immunizations, all four mosaic or cocktail RBD-NP vaccines elicited strong binding (GMT ~1–5 × 10^4) (Figure S4A; Data S1) and potent serum neutralizing (GMT 2–8 × 10^3; Figure 5A; Data S1) Ab titers against wild-type (D614G) SARS-CoV-2 S pseudovirus. Competition ELISAs using hACE2, CR3022, S309, and S2X259 demonstrated that all mosaic and cocktail vaccine candidates elicited robust Ab titers targeting all antigenic sites tested, showcasing the diversity of RBD-specific Abs elicited (Figures S4B–S4E; Data S1). The neutralizing SARS-CoV-2 S (D614G) Ab responses were slightly higher for mRBD-NPs and slightly lower for cRBD-NPs than that of the monovalent RBD-NP (GMT 3 × 10^3) (Figure 5A), similar to the aforementioned low-dose RBD-NP immunization study (Figure 1D) and suggesting that the dose of strain-matched antigen in the multivalent vaccines is not a limiting factor of the magnitude of neutralizing Ab responses. The neutralizing activity against SARS-CoV-2 elicited by the other monovalent RBD-NPs and the non-assembling control were ~2 orders of magnitude lower (GMT < 5 × 10^1) than for the monovalent RBD-NP, indicating poor elicitation of cross-neutralizing Abs by these heterologous monovalent RBD-NPs.



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Although ELISA binding titers were comparable across mosaic and cocktail groups against SARS-CoV S2P (GMT 3–10 × 10^3), the corresponding pseudovirus neutralization titers showed more nuanced patterns (Figures 5B and S4F; Data S1). Tetravalent mosaic and cocktail RBD-NPs elicited potent neutralizing activity (GMT 3–5.5 × 10^3) with magnitudes roughly comparable to that of the monovalent SARS-CoV-RBD-NP (GMT 5 × 10^3) (Figure 5B). Strikingly, the trivalent nanoparticle immunogens (mRBD-NP-DO and cRBD-NP-DO)—which did not contain the SARS-CoV RBD—also elicited potent neutralization (GMT ~1–2 × 10^3). This cross-neutralization likely arose from the inclusion of the WIV1 RBD (Figure S4G; Data S1) in the trivalent immunogens, as WIV1 cross-reacts with a SARS-CoV-specific mAb (Figure S3H) (Menachery et al., 2016), and the monovalent WIV1-RBD-NP induced similar levels of pseudovirus neutralization (GMT 2 × 10^3) (Figure 5B; Data S1). The non-assembling control immunogen, which contains all four RBD-I53-50A trimeric components, also elicited substantial neutralizing activity against SARS-CoV (GMT 1 × 10^3) but not against SARS-CoV-2. Furthermore, we show that sera elicited by the monovalent, mRBD-NPs, and cRBD-NPs neutralize SHC014 pseudotyped virus, a distantly related sarbecovirus sharing 76%, 76%, 82%, and 84% amino acid sequence identity with the SARS-CoV-2, RaTG13, SARS-CoV, and WIV1 RBDs, respectively (Figure 5C; Data S1). These data show that both mRBD-NPs and cRBD-NPs are promising vaccine candidates for eliciting broad sarbecovirus immunity, in agreement with previous findings using a different nanoparticle platform (Cohen et al., 2021a) or chimeric sarbecovirus S glycoproteins (Martinez et al., 2021b) and recent results obtained with influenza virus hemagglutinin nanoparticle vaccines (Boyoglu-Barnum et al., 2021).

Mosaic RBD-NPs protect mice against heterotypic SARS-CoV challenge

To gauge the ability of the multivalent RBD-NPs to confer protection against vaccine-matched and heterotypic sarbecoviruses, we challenged groups of eight mice with a high dose (10^5 PFUs) of the mouse-adapted SARS-CoV MA15 virus (Roberts et al., 2007). In agreement with the pseudovirus neutralization data, animals immunized with mRBD-NP, mRBD-NP-DO, cRBD-NP, and cRBD-NP-DO were protected from weight loss (ranging between 1% and 5%) and serious lung pathology throughout the four days of the experiment (Figures 6A and 6B). Unvaccinated mice exhibited up to 15% average weight loss and signs of lung pathology (congestion score ~1.5) that were only minorly seen in all other vaccinated groups (Figures 6A and 6B). mRBD-NP- and mRBD-NP-DO-vaccinated mice were completely protected from viral replication in the lungs (Figure 6C). Overall, the trivalent mRBD-NP-DO provided protection that was indistinguishable from the tetravalent mRBD-NP, despite lacking the SARS-CoV RBD. Five out of eight and six out of eight cRBD-NP- and cRBD-NP-DO-vaccinated mice were completely protected from viral replication in the lungs, respectively, with viral burden ranging between 10^2 and 10^3 PFUs/lobe for the remaining animals (compared to unvaccinated mice with ~10^6 PFUs/lobe, Figure 6C). To assess whether the RBD-NP platform causes vaccine-induced immune pathology, which was previously described with double-inactivated SARS-CoV vaccines (Bolles et al., 2011), we investigated eosinophil infiltration and inflammation in the lungs following SARS-CoV MA15 challenge. Although infection induced inflammation and infiltration of eosinophils in the lungs of unvaccinated mice (Figures 6D, 6I, and S5A), no signs of eosinophilia were detected for any animals in the vaccinated groups using histological analysis of stained lung sections (Figures 6D–6I and S5A). These data show that mRBD-NP, mRBD-NP-DO, cRBD-NP, and cRBD-NP-DO protect against weight loss, lung injury, and viral replication following challenge, with no detectable vaccine-induced immune pathology.

To identify the gain in protective breadth provided by multivalent display of sarbecovirus RBD-NP, relative to monovalent RBD-NPs, we performed a second SARS-CoV MA15 challenge experiment including monovalent RBD-NP and SARS-CoV-RBD-NP (Figure S5B). In agreement with the pseudovirus neutralization data and first challenge (Figures 4B–4D and 6A–6C), animals immunized with the SARS-CoV-RBD-NP, mosaic, and cocktail RBD-NP formulations, as well as the non-assembling control immunogen, were protected from weight loss and serious lung pathology throughout the four days of the experiment. The animals receiving the monovalent RBD-NP vaccine experienced up to 12% average weight loss, whereas the unvaccinated mice exhibited further (up to 15%) average weight loss and signs of lung pathology (congestion score ~2) that were only minorly seen in all other vaccinated groups (Figures S5B and S5C). All mice vaccinated with SARS-CoV-RBD-NP, RBD-NP, and mRBD-NP were protected from viral replication in the lungs, whereas we detected ~$10^{2.5}$ and 10^6 PFUs/lobe for half of the mice receiving the non-assembling control immunogen and unvaccinated mice, respectively (Figure S5D). These results provide proof-of-principle that mosaic and cocktail RBD nanoparticle vaccines elicit broad protection against heterotypic sarbecovirus challenge and could represent the next generation of vaccines developed in anticipation of future spillovers.

Figure 6. Mosaic and cocktail RBD-NP vaccines protect against heterotypic SARS-CoV-MA15 challenge in 15-week-old BALB/c cByJ mice

(A) Weight loss following SARS-CoV MA15 challenge up to 4 days post infection (n = 8). Unvaccinated animals are shown as black circles.

(B) Congestion score following SARS-CoV MA15 infection with a score of 0 indicating unchanged lung color and 4 indicating a darkened and diseased lung (n = 8).

(C) Viral titers in mice lungs (expressed in PFUs per lobe) following challenge (n = 8) with an LOD of 9 × 10^1. LOD is shown as a gray horizontal dotted line. Statistical significance was determined by Kruskal-Wallis and shown when significant. **p < 0.01.

(D) Mean eosinophils per high power field (HPF) per sample run over 10 HPF per lung stained with congo red. Significance was determined using one-way ANOVA and shown where significant.

(E–I) Histological analysis of stained lung sections for mRBD-NP (E), mRBD-NP-DO (F), cRBD-NP (G), cRBD-NP-DO (H), and unvaccinated mice (I). Arrowheads indicate eosinophils. Scale bar, 20 μm.

DISCUSSION

The data presented here show that two ultra-low dose immunizations or a single immunization with RBD-NP produces potent neutralizing Ab responses in mice. The latter strategy confers protection against SARS-CoV-2 MA10 challenge, suggesting that the nanoparticle platform could enable dose-sparing regimens to achieve global vaccination, especially given the high shelf stability of this vaccine (Walls et al., 2020b). NHP vaccination with RBD-NP was concurrently shown to also elicit CD4 T cell responses and to protect from viral replication (Arunachalam et al., 2021). Moreover, RBD-NP vaccination, which is currently under phase I/II evaluation in the clinic, elicits diverse Ab responses neutralizing a broad spectrum of SARS-CoV-2 variants detected in clinical isolates with similar potency to those elicited by HexaPro S vaccination. Several RBM mutations, including at position E484, however, lead to reductions in neutralizing activity elicited by either RBD-NP or HexaPro S in NHPs. Although both RBD-NP- and HexaPro S-elicited sera robustly neutralize the B.1.1.7 S variant, which does not contain the E484 substitution, neutralization of the B.1.351, P.1, and B.1.1.7/E484K variants was dampened similarly to sera from individuals vaccinated twice with the Pfizer-BioNTech BNT162b2 mRNA. These findings are in agreement with reports showing that the serum neutralizing activity against the B.1.351 variant from mRNA-1273-vaccinated individuals was comparably reduced (Wu et al., 2021), as was also the case for neutralization of authentic SARS-CoV-2 B.1.351 by HCP (Wibmer et al., 2021). Collectively, these data indicate that a significant fraction of vaccine-elicited neutralizing activity is directed to the RBM, which is the target of potent neutralizing Abs, irrespective of the antigen design strategy (RBD- or S-based), the vaccine modality (protein subunit or mRNA), or species (NHPs or humans). We show that receiving a third immunization, even of an unaltered vaccine, improves the neutralizing Ab responses to VOCs, which is reminiscent of what has been shown in vaccinated humans previously infected with SARS-CoV-2 (Abu Jabal et al., 2021; Krammer et al., 2021; Stamatatos et al., 2021), suggesting that further booster immunizations may limit the impact of VOCs without vaccine update.

The ongoing global spread of SARS-CoV-2 and the circulation of a large number of sarbecoviruses in bats (Menachery et al., 2015, 2016) and other animal reservoirs strongly motivate the development of vaccines that protect against a broad spectrum of coronaviruses. We observed that vaccination of NHPs with RBD-NPs or HexaPro S induced comparable but moderate neutralization breadth against genetically distinct sarbecoviruses. We demonstrate here that co-display (mRBD-NP) and co-immunization (cRBD-NP) of multivalently arrayed sarbecovirus RBDs elicit robust neutralizing Ab responses against SARS-CoV-2, SARS-CoV, and SHC014 pseudotyped viruses and outperform their monovalent vaccine counterparts. The observation that mosaic and cocktail RBD-NPs elicit greater titers of Abs competing with the broadly neutralizing sarbecovirus mAb S2X259 than monovalent vaccines suggest that this vaccine design strategy could enhance neutralization breadth. Accordingly, mice vaccinated with multivalent vaccines were protected from disease upon SARS-CoV MA15 challenge, including with formulations that did not include the SARS-CoV RBD, whereas RBD-NP-vaccinated mice experienced weight loss. Previous studies showed that double-inactivated SARS-CoV vaccines could lead to eosinophil infiltration in the lungs and subsequent immune pathology (Bolles et al., 2011). Our data show that none of the multivalent vaccine candidates evaluated here induced eosinophilia whereas SARS-CoV MA15 challenge did, pointing to the safety and efficacy of these immunogens. Our data highlight the potential of this approach to achieve broad sarbecovirus immunity, overcoming both the emergence of SARS-CoV-2 variants and putative future zoonosis of genetically distinct sarbecoviruses not included in the vaccine. Based on the RBD-centric nature of neutralizing Ab responses resulting from infection and vaccination (Dejnirattisai et al., 2021; Greaney et al., 2021a, 2021b; Piccoli et al., 2020), irrespective of immunogen format or vaccine modality, and the enhanced elicitation of Abs targeting all RBD antigenic sites for multivalent RBD-NPs compared to S-based vaccines, this study paves the way for advancing RBD pan-sarbecovirus subunit vaccines to the clinic.

Limitations of study

Due to the cost of challenge and BSL-3 vivarium limitations, the mice vaccinated with a low dose of RBD-NP were not challenged, and therefore we can only infer the likelihood of protection from their sera's ability to neutralize SARS-CoV-2 pseudovirus better than other mice (i.e., those given a single vaccine dose) that were challenged. Neutralization is a correlate of protection in NHPs (Arunachalam et al., 2021), but we recognize that neutralization assays using HEK-ACE2 cell lines may underestimate the effects of non-RBM targeting Abs (Lempp et al., 2021; McCallum et al., 2021a; Suryadevara et al., 2021). Due to the limitations of the number of animals and the cost of challenge, we cannot challenge with all desired point mutant or variant strains and must use neutralization as the best correlate for interpreting the expected challenge outcome.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cell.2021.09.015.

ACKNOWLEDGMENTS

We thank Helen Chu and Marion Pepper for coordination of HCP samples and Ratika Krishnamurty for program management. The authors thank Kathryn Guerriero, Brieann Brown, Solomon Wangari, Joel Ahrens, Naoto Iwayama, and William Garrison for their technical assistance with the pigtail macaque study. This study was supported by the Bill and Melinda Gates Foundation (INV–017592 to J.S.M. and OPP1156262 to N.P.K. and D.V.); generous gifts from the Audacious Project, Jodi Green and Mike Halperin, and the Hanauer family; the Defense Threat Reduction Agency (HDTRA1-18-1-0001 to N.P.K.); the National Institute of General Medical Sciences(R01GM120553 to D.V.); National Institute of Health Office of Research Infrastructure Programs (P51-OD010425, Washington National Primate Research Center); the National Institute of Allergy and Infectious Diseases (R01-AI127521 to J.S.M. and DP1AI158186 and HHSN272201700059C to D.V.); the National Institute of General Medical Sciences (R01GM120553 to D.V. and 5T32GM008268-32 to S.K.Z.); a Pew Biomedical Scholars Award (D.V.); Investigators in the Pathogenesis of Infectious Disease Awards from the Burroughs Wellcome Fund (D.V.); Fast Grants (D.V.); Animal Models contracts HHSN272201700036I-75N93020F00001 and U01 AI149644 (R.S.B.); and the North Carolina Policy Collaboratory at the University of North Carolina at Chapel Hill with funding from the North Carolina Coronavirus Relief Fund, established and appropriated by the North Carolina General Assembly (R.S.B.). D.R.M. is currently supported by a Burroughs Wellcome Fund Postdoctoral Enrichment Program Award and a Hanna H. Gray Fellowship from the Howard Hughes Medical Institute and was supported by an NIH NIAID T32 AI007151 and an NIAID F32 AI152296.

AUTHOR CONTRIBUTIONS

Conceptualization, A.C.W., P.S.A., H.K., B.P., N.P.K., D.V.; modeling and design, A.C.W., N.P.K., D.V.; formal analysis, A.C.W., M.C.M., A.S., A.G., M.A.T., J.B., B.F., M.J.N., S.W., T.S., R.R., C.S., L.C., N.P.K, D.V.; resources, M.A.O., P.S.A., S.W.T., R.R., D.T.O., R.V.D.M., R.S.B., W.V.W., D.C., C.-L.H., J.S.M., D.H.F., F.V., B.P.; writing – original draft, A.C.W., N.P.K., D.V.; writing – review & editing, all authors; visualization, A.C.W., M.C.M., A.S., A.G., N.B., N.P.K., R.S.B., D.V.; supervision, D.H.F., J.S.M., L.C., R.S.B., T.P.S., B.P., N.P.K., D.V.; funding acquisition, J.S.M., R.S.B., N.P.K., D.V.

DECLARATION OF INTERESTS

A.C.W., N.P.K., and D.V. are named as inventors on patent applications filed by the University of Washington based on the studies presented in this paper. N.P.K. is a co-founder, shareholder, paid consultant, and chair of the scientific advisory board of Icosavax, Inc. and has received an unrelated sponsored research agreement from Pfizer. D.C. is an employee of Vir Biotechnology and may hold shares in Vir Biotechnology. D.V. is a consultant for and has received an unrelated sponsored research agreement from Vir Biotechnology, Inc. R.R., D.T.O., and R.V.D.M. are employees of GlaxoSmithKline. C.-L.H. and J.S.M. are inventors on US patent application no. 63/032,502, “Engineered Coronavirus Spike (S) Protein and Methods of Use Thereof”. R.S.B. has collaborative research agreements with Takeda, Pfizer, Eli Lily, Gilead, Ridgeback Biosciences, and VaxArt, unrelated to the current research. The other authors declare no competing interests.

Received: April 5, 2021
Revised: June 18, 2021
Accepted: September 9, 2021
Published: September 15, 2021

REFERENCES

Abu Jabal, K., Ben-Amram, H., Beiruti, K., Batheesh, Y., Sussan, C., Zarka, S., and Edelstein, M. (2021). Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine: real-world evidence from healthcare workers, Israel, December 2020 to January 2021. Euro Surveill. *26*, 2100096.

Andreano, E., Piccini, G., Licastro, D., Casalino, L., Johnson, N.V., Paciello, I., Monego, S.D., Pantano, E., Manganaro, N., Manenti, A., et al. (2020). SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. bioRxiv, 2020.12.28.424451.

Arunachalam, P.S., Walls, A.C., Golden, N., Atyeo, C., Fischinger, S., Li, C., Aye, P., Navarro, M.J., Lai, L., Edara, V.V., et al. (2021). Adjuvanting a subunit COVID-19 vaccine to induce protective immunity. Nature *594*, 253–258.

Avanzato, V.A., Matson, M.J., Seifert, S.N., Pryce, R., Williamson, B.N., Anzick, S.L., Barbian, K., Judson, S.D., Fischer, E.R., Martens, C., et al. (2020). Case Study: Prolonged infectious SARS-CoV-2 shedding from an asymptomatic immunocompromised cancer patient. Cell *183*, 1901, 1012.e9.

Bale, J.B., Gonen, S., Liu, Y., Sheffler, W., Ellis, D., Thomas, C., Cascio, D., Yeates, T.O., Gonen, T., King, N.P., and Baker, D. (2016). Accurate design of megadalton-scale two-component icosahedral protein complexes. Science *353*, 389–394.

Barnes, C.O., Jette, C.A., Abernathy, M.E., Dam, K.A., Esswein, S.R., Gristick, H.B., Malyutin, A.G., Sharaf, N.G., Huey-Tubman, K.E., Lee, Y.E., et al. (2020). SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. Nature *588*, 682–687.

Baum, A., Ajithdoss, D., Copin, R., Zhou, A., Lanza, K., Negron, N., Ni, M., Wei, Y., Mohammadi, K., Musser, B., et al. (2020a). REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. Science *370*, 1110–1115.

Baum, A., Fulton, B.O., Wloga, E., Copin, R., Pascal, K.E., Russo, V., Giordano, S., Lanza, K., Negron, N., Ni, M., et al. (2020b). Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. Science *369*, 1014–1018.

Bolles, M., Deming, D., Long, K., Agnihothram, S., Whitmore, A., Ferris, M., Funkhouser, W., Gralinski, L., Totura, A., Heise, M., and Baric, R.S. (2011). A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J. Virol. *85*, 12201–12215.

Boyoglu-Barnum, S., Ellis, D., Gillespie, R.A., Hutchinson, G.B., Park, Y.-J., Moin, S.M., Acton, O.J., Ravichandran, R., Murphy, M., Pettie, D., et al. (2021). Quadrivalent influenza nanoparticle vaccines induce broad protection. Nature *592*, 623–628.

Brouwer, P.J.M., Caniels, T.G., van der Straten, K., Snitselaar, J.L., Aldon, Y., Bangaru, S., Torres, J.L., Okba, N.M.A., Claireaux, M., Kerster, G., et al. (2020). Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science *369*, 643–650.

Cathcart, A.L., Havenar-Daughton, C., Lempp, F.A., Ma, D., Schmid, M., Agostini, M.L., Guarino, B., Di iulio, J., Rosen, L., Tucker, H., et al. (2021). The dual function monoclonal antibodies VIR-7831 and VIR-7832 demonstrate potent in vitro and in vivo activity against SARS-CoV-2. bioRxiv. https://doi.org/10.1101/2021.03.09.434607.

Choi, B., Choudhary, M.C., Regan, J., Sparks, J.A., Padera, R.F., Qiu, X., Solomon, I.H., Kuo, H.H., Boucau, J., Bowman, K., et al. (2020). Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. N. Engl. J. Med. *383*, 2291–2293.

Cohen, A.A., Gnanapragasam, P.N.P., Lee, Y.E., Hoffman, P.R., Ou, S., Kakutani, L.M., Keeffe, J.R., Wu, H.-J., Howarth, M., West, A.P., et al. (2021a). Mosaic nanoparticles elicit cross-reactive immune responses to zoonotic coronaviruses in mice. Science *371*, 735–741.

Cohen, A.A., Yang, Z., Gnanapragasam, P.N.P., Ou, S., Dam, K.A., Wang, H., and Bjorkman, P.J. (2021b). Construction, characterization, and immunization of nanoparticles that display a diverse array of influenza HA trimers. PLoS ONE *16*, e0247963.

Collier, D.A., De Marco, A., Ferreira, I.A.T.M., Meng, B., Datir, R.P., Walls, A.C., Kemp, S.A., Bassi, J., Pinto, D., Silacci-Fregni, C., et al.; CITIID-NIHR BioResource COVID-19 Collaboration; COVID-19 Genomics UK (COG-UK) Consortium (2021). Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies. Nature *593*, 136–141.

Corbett, K.S., Edwards, D.K., Leist, S.R., Abiona, O.M., Boyoglu-Barnum, S., Gillespie, R.A., Himansu, S., Schäfer, A., Ziwawo, C.T., DiPiazza, A.T., et al. (2020a). SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature *586*, 567–571.

Corbett, K.S., Flynn, B., Foulds, K.E., Francica, J.R., Boyoglu-Barnum, S., Werner, A.P., Flach, B., O'Connell, S., Bock, K.W., Minai, M., et al. (2020b). Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. N. Engl. J. Med. *383*, 1544–1555.

Crawford, K.H.D., Eguia, R., Dingens, A.S., Loes, A.N., Malone, K.D., Wolf, C.R., Chu, H.Y., Tortorici, M.A., Veesler, D., Murphy, M., et al. (2020). Protocol and Reagents for Pseudotyping Lentiviral Particles with SARS-CoV-2 Spike Protein for Neutralization Assays. Viruses *12*, 513.

Davies, N.G., Barnard, R.C., Jarvis, C.I., Kucharski, A.J., Munday, J., Pearson, C.A.B., Russell, T.W., Tully, D.C., Abbott, S., Gimma, A., et al. (2020). Estimated transmissibility and severity of novel SARS-CoV-2 Variant of Concern 202012/01 in England. medRxiv. https://doi.org/10.1101/2020.12.24.20248822.

Dejnirattisai, W., Zhou, D., Ginn, H.M., Duyvesteyn, H.M.E., Supasa, P., Case, J.B., Zhao, Y., Walter, T.S., Mentzer, A.J., Liu, C., et al. (2021). The antigenic anatomy of SARS-CoV-2 receptor binding domain. Cell *184*, 2183–2200.e22.

Deng, X., Garcia-Knight, M.A., Khalid, M.M., Servellita, V., Wang, C., Morris, M.K., Sotomayor-González, A., Glasner, D.R., Reyes, K.R., Gliwa, A.S., et al. (2021). Transmission, infectivity, and neutralization of a spike L452R SARS-CoV-2 variant. Cell *184*, 3426–3437.e8.

Dinnon, K.H., 3rd, Leist, S.R., Schäfer, A., Edwards, C.E., Martinez, D.R., Montgomery, S.A., West, A., Yount, B.L., Jr., Hou, Y.J., Adams, L.E., et al. (2020). A mouse-adapted model of SARS-CoV-2 to test COVID-19 countermeasures. Nature *586*, 560–566.

Dong, J., Zost, S.J., Greaney, A.J., Starr, T.N., Dingens, A.S., Chen, E.C., Chen, R.E., Case, J.B., Sutton, R.E., Gilchuk, P., et al. (2021). Genetic and structural basis for recognition of SARS-CoV-2 spike protein by a two-antibody cocktail. bioRxiv. https://doi.org/10.1101/2021.01.27.428529.

Faria, N.R., Claro, I.M., Candido, D., Moyses Franco, L.A., Andrade, P.S., Coletti, T.M., Silva, C.A.M., Sales, F.C., Manuli, E.R., Aguiar, R.S., et al. (2021). Genomic characterisation of an emergent SARS-CoV-2 lineage in Manaus: preliminary findings. https://virological.org/t/genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-manaus-preliminary-findings/586.

Gibson, D.G., Young, L., Chuang, R.Y., Venter, J.C., Hutchison, C.A., 3rd, and Smith, H.O. (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat. Methods *6*, 343–345.

Greaney, A.J., Starr, T.N., Gilchuk, P., Zost, S.J., Binshtein, E., Loes, A.N., Hilton, S.K., Huddleston, J., Eguia, R., Crawford, K.H.D., et al. (2020). Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. Cell Host Microbe *29*, 44–57.e9.

Greaney, A.J., Loes, A.N., Gentles, L.E., Crawford, K.H.D., Starr, T.N., Malone, K.D., Chu, H.Y., and Bloom, J.D. (2021a). Antibodies elicited by mRNA-1273 vaccination bind more broadly to the receptor binding domain than do those from SARS-CoV-2 infection. Sci. Transl. Med. *13*, 13.

Goddard, T.D., Huang, C.C., Meng, E.C., Pettersen, E.F., Couch, G.S., Morris, J.H., and Ferrin, T.E. (2018). UCSF ChimeraX: Meeting modern challenges in visualization and analysis. Protein Sci. *27*, 14–25.

Greaney, A.J., Loes, A.N., Crawford, K.H.D., Starr, T.N., Malone, K.D., Chu, H.Y., and Bloom, J.D. (2021b). Comprehensive mapping of mutations in the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human plasma antibodies. Cell Host Microbe *29*, 463–476.e6.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., et al. (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell *181*, 271–280.e8.

Hou, Y.J., Chiba, S., Halfmann, P., Ehre, C., Kuroda, M., Dinnon, K.H., 3rd, Leist, S.R., Schäfer, A., Nakajima, N., Takahashi, K., et al. (2020). SARS-CoV-2 D614G variant exhibits efficient replication ex vivo and transmission in vivo. Science *370*, 1464–1468.

Hsieh, C.L., Goldsmith, J.A., Schaub, J.M., DiVenere, A.M., Kuo, H.C., Javanmardi, K., Le, K.C., Wrapp, D., Lee, A.G., Liu, Y., et al. (2020). Structure-based design of prefusion-stabilized SARS-CoV-2 spikes. Science *369*, 1501–1505.

Huo, J., Zhao, Y., Ren, J., Zhou, D., Duyvesteyn, H.M.E., Ginn, H.M., Carrique, L., Malinauskas, T., Ruza, R.R., Shah, P.N.M., et al. (2020). Neutralisation of SARS-CoV-2 by destruction of the prefusion Spike. Cell Host Microbe *28*, 445–454.e6.

Jackson, L.A., Anderson, E.J., Rouphael, N.G., Roberts, P.C., Makhene, M., Coler, R.N., McCullough, M.P., Chappell, J.D., Denison, M.R., Stevens, L.J., et al. (2020). An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N. Engl. J. Med. *383*, 1920–1931.

Jette, C.A., Cohen, A.A., Gnanapragasam, P.N.P., Muecksch, F., Lee, Y.E., Huey-Tubman, K.E., Schmidt, F., Hatziioannou, T., Bieniasz, P.D., Nussenzweig, M.C., et al. (2021). Broad cross-reactivity across sarbecoviruses exhibited by a subset of COVID-19 donor-derived neutralizing antibodies. bioRxiv. https://doi.org/10.1101/2021.04.23.441195.

Kaname, Y., Tani, H., Kataoka, C., Shiokawa, M., Taguwa, S., Abe, T., Moriishi, K., Kinoshita, T., and Matsuura, Y. (2010). Acquisition of complement resistance through incorporation of CD55/decay-accelerating factor into viral particles bearing baculovirus GP64. J. Virol. *84*, 3210–3219.

Kanekiyo, M., Joyce, M.G., Gillespie, R.A., Gallagher, J.R., Andrews, S.F., Yassine, H.M., Wheatley, A.K., Fisher, B.E., Ambrozak, D.R., Creanga, A., et al. (2019). Mosaic nanoparticle display of diverse influenza virus hemagglutinins elicits broad B cell responses. Nat. Immunol. *20*, 362–372.

Korber, B., Fischer, W.M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., Hengartner, N., Giorgi, E.E., Bhattacharya, T., Foley, B., et al.; Sheffield COVID-19 Genomics Group (2020). Tracking Changes in SARS-CoV-2 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. Cell *182*, 812–827.e19.

Krammer, F., Srivastava, K., and the PARIS team, and Simon, V. (2021). Robust spike antibody responses and increased reactogenicity in seropositive individuals after a single dose of SARS-CoV-2 mRNA vaccine. medRxiv. https://doi.org/10.1101/2021.01.2921250653.

Lam, T.T., Jia, N., Zhang, Y.W., Shum, M.H., Jiang, J.F., Zhu, H.C., Tong, Y.G., Shi, Y.X., Ni, X.B., Liao, Y.S., et al. (2020). Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature *583*, 282–285.

Lan, J., Ge, J., Yu, J., Shan, S., Zhou, H., Fan, S., Zhang, Q., Shi, X., Wang, Q., Zhang, L., and Wang, X. (2020). Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature *581*, 215–220.

Leist, S.R., Dinnon, K.H., 3rd, Schäfer, A., Tse, L.V., Okuda, K., Hou, Y.J., West, A., Edwards, C.E., Sanders, W., Fritch, E.J., et al. (2020). A Mouse-Adapted SARS-CoV-2 Induces Acute Lung Injury and Mortality in Standard Laboratory Mice. Cell *183*, 1070–1085.e12.

Lempp, F.A., Soriaga, L., Montiel-Ruiz, M., Benigni, F., Noack, J., Park, Y.-J., Bianchi, S., Walls, A.C., Bowen, J.E., Zhou, J., et al. (2021). Membrane lectins enhance SARS-CoV-2 infection and influence the neutralizing activity of different classes of antibodies. bioRxiv. https://doi.org/10.1101/2021.04.03.438258.

Letko, M., Marzi, A., and Munster, V. (2020). Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat. Microbiol. *5*, 562–569.

Li, Q., Wu, J., Nie, J., Zhang, L., Hao, H., Liu, S., Zhao, C., Zhang, Q., Liu, H., Nie, L., et al. (2020). The Impact of Mutations in SARS-CoV-2 Spike on Viral Infectivity and Antigenicity. Cell *182*, 1284–1294.e9.

Liu, Z., VanBlargan, L.A., Bloyet, L.-M., Rothlauf, P.W., Chen, R.E., Stumpf, S., Zhao, H., Errico, J.M., Theel, E.S., Liebeskind, M.J., et al. (2021). Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. Cell Host Microbe *29*, 477–488.e4.

Martinez, D.R., Schaefer, A., Gobeil, S., Li, D., De la Cruz, G., Parks, R., Lu, X., Barr, M., Manne, K., Mansouri, K., et al. (2021a). A broadly neutralizing antibody protects against SARS-CoV, pre-emergent bat CoVs, and SARS-CoV-2 variants in mice. bioRxiv. https://doi.org/10.1101/2021.04.27.441655.

Martinez, D.R., Schäfer, A., Leist, S.R., De la Cruz, G., West, A., Atochina-Vasserman, E.N., Lindesmith, L.C., Pardi, N., Parks, R., Barr, M., et al. (2021b). Chimeric spike mRNA vaccines protect against Sarbecovirus challenge in mice. Science *373*, 991–998.

McCallum, M., De Marco, A., Lempp, F.A., Tortorici, M.A., Pinto, D., Walls, A.C., Beltramello, M., Chen, A., Liu, Z., Zatta, F., et al. (2021a). N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. Cell *184*, 2332–2347.e16.

McCallum, M., Bassi, J., De Marco, A., Chen, A., Walls, A.C., Di Iulio, J., Tortorici, M.A., Navarro, M.-J., Silacci-Fregni, C., Saliba, C., et al. (2021b). SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. Science *373*, 648–654.

McCarthy, K.R., Rennick, L.J., Nambulli, S., Robinson-McCarthy, L.R., Bain, W.G., Haidar, G., and Duprex, W.P. (2021). Recurrent deletions in the SARS-CoV-2 spike glycoprotein drive antibody escape. Science *371*, 1139–1142.

McMahan, K., Yu, J., Mercado, N.B., Loos, C., Tostanoski, L.H., Chandrashekar, A., Liu, J., Peter, L., Atyeo, C., Zhu, A., et al. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. Nature *590*, 630–634.

Menachery, V.D., Yount, B.L., Jr., Debbink, K., Agnihothram, S., Gralinski, L.E., Plante, J.A., Graham, R.L., Scobey, T., Ge, X.Y., Donaldson, E.F., et al. (2015). A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat. Med. *21*, 1508–1513.

Menachery, V.D., Yount, B.L., Jr., Sims, A.C., Debbink, K., Agnihothram, S.S., Gralinski, L.E., Graham, R.L., Scobey, T., Plante, J.A., Royal, S.R., et al. (2016). SARS-like WIV1-CoV poised for human emergence. Proc. Natl. Acad. Sci. USA *113*, 3048–3053.

Mercado, N.B., Zahn, R., Wegmann, F., Loos, C., Chandrashekar, A., Yu, J., Liu, J., Peter, L., McMahan, K., Tostanoski, L.H., et al. (2020). Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature *586*, 583–588.

Millet, J.K., and Whittaker, G.R. (2016). Murine Leukemia Virus (MLV)-based Coronavirus Spike-pseudotyped Particle Production and Infection. Biol. Protoc. *6*, e2035.

Otwinowski, J., McCandlish, D.M., and Plotkin, J.B. (2018). Inferring the shape of global epistasis. Proc. Natl. Acad. Sci. USA *115*, E7550–E7558.

Pallesen, J., Wang, N., Corbett, K.S., Wrapp, D., Kirchdoerfer, R.N., Turner, H.L., Cottrell, C.A., Becker, M.M., Wang, L., Shi, W., et al. (2017). Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. Proc. Natl. Acad. Sci. USA *114*, E7348–E7357.

Piccoli, L., Park, Y.J., Tortorici, M.A., Czudnochowski, N., Walls, A.C., Beltramello, M., Silacci-Fregni, C., Pinto, D., Rosen, L.E., Bowen, J.E., et al. (2020). Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. Cell *183*, 1024–1042.e21.

Pinto, D., Park, Y.J., Beltramello, M., Walls, A.C., Tortorici, M.A., Bianchi, S., Jaconi, S., Culap, K., Zatta, F., De Marco, A., et al. (2020). Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. Nature *583*, 290–295.

Pinto, D., Sauer, M.M., Czudnochowski, N., Low, J.S., Alejandra Tortorici, M., Housley, M.P., Noack, J., Walls, A.C., Bowen, J.E., Guarino, B., et al. (2021). Broad betacoronavirus neutralization by a stem helix-specific human antibody. Science *373*, 1109–1116.

Plante, J.A., Liu, Y., Liu, J., Xia, H., Johnson, B.A., Lokugamage, K.G., Zhang, X., Muruato, A.E., Zou, J., Fontes-Garfias, C.R., et al. (2020). Spike mutation D614G alters SARS-CoV-2 fitness. Nature *592*, 116–121.

Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J.L., Pérez Marc, G., Moreira, E.D., Zerbini, C., et al.; C4591001 Clinical Trial Group (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N. Engl. J. Med. *383*, 2603–2615.

Rappazzo, C.G., Tse, L.V., Kaku, C.I., Wrapp, D., Sakharkar, M., Huang, D., Deveau, L.M., Yockachonis, T.J., Herbert, A.S., Battles, M.B., et al. (2021). Broad and potent activity against SARS-like viruses by an engineered human monoclonal antibody. Science *371*, 823–829.

Roberts, A., Deming, D., Paddock, C.D., Cheng, A., Yount, B., Vogel, L., Herman, B.D., Sheahan, T., Heise, M., Genrich, G.L., et al. (2007). A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. *3*, e5.

Rockx, B., Corti, D., Donaldson, E., Sheahan, T., Stadler, K., Lanzavecchia, A., and Baric, R. (2008). Structural basis for potent cross-neutralizing human monoclonal antibody protection against lethal human and zoonotic severe acute respiratory syndrome coronavirus challenge. J. Virol. *82*, 3220–3235.

Rogers, T.F., Zhao, F., Huang, D., Beutler, N., Burns, A., He, W.T., Limbo, O., Smith, C., Song, G., Woehl, J., et al. (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science *369*, 956–963.

Sauer, M.M., Tortorici, M.A., Park, Y.-J., Walls, A.C., Homad, L., Acton, O.J., Bowen, J.E., Wang, C., Xiong, X., de van der Schueren, W., et al. (2021). Structural basis for broad coronavirus neutralization. Nat. Struct. Mol. Biol. *28*, 478–486.

Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., Geng, Q., Auerbach, A., and Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. Nature *581*, 221–224.

Stamatatos, L., Czartoski, J., Wan, Y.-H., Homad, L.J., Rubin, V., Glantz, H., Neradilek, M., Seydoux, E., Jennewein, M.F., MacCamy, A.J., et al. (2021). mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science, eabg9175. Published online March 25, 2021.

Starr, T.N., Greaney, A.J., Hilton, S.K., Ellis, D., Crawford, K.H.D., Dingens, A.S., Navarro, M.J., Bowen, J.E., Tortorici, M.A., Walls, A.C., et al. (2020a). Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. Cell *182*, 1295–1310.e20.

Starr, T.N., Greaney, A.J., Addetia, A., Hannon, W.W., Choudhary, M.C., Dingens, A.S., Li, J.Z., and Bloom, J.D. (2020b). Prospective mapping of viral mutations that escape antibodies used to treat COVID-19. bioRxiv.

Starr, T.N., Czudnochowski, N., Liu, Z., Zatta, F., Park, Y.-J., Addetia, A., Pinto, D., Beltramello, M., Hernandez, P., Greaney, A.J., et al. (2021). SARS-CoV-2 RBD antibodies that maximize breadth and resistance to escape. Nature *597*, 97–102.

Suryadevara, N., Shrihari, S., Gilchuk, P., VanBlargan, L.A., Binshtein, E., Zost, S.J., Nargi, R.S., Sutton, R.E., Winkler, E.S., Chen, E.C., et al. (2021). Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. Cell *184*, 2316–2331.e15.

Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., Doolabh, D., Pillay, S., San, E.J., Msomi, N., et al. (2020). Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. medRxiv. https://doi.org/10.1101/2020.12.21.20248640.

ter Meulen, J., van den Brin, E.N., Poon, L.L.M., Marissen, W.E., Leung, C.S.W., Cox, F., Cheung, C..y., Bakker, A.Q., Bogaards, J.A., Deventer, E.V., et al. (2006). Human Monoclonal Antibody Combination against SARS Coronavirus: Synergy and Coverage of Escape Mutants. Plos Medicine *3*, e237.

Thomson, E.C., Rosen, L.E., Shepherd, J.G., Spreafico, R., da Silva Filipe, A., Wojcechowskyj, J.A., Davis, C., Piccoli, L., Pascall, D.J., Dillen, J., et al.; ISARIC4C Investigators; COVID-19 Genomics UK (COG-UK) Consortium (2021). Circulating SARS-CoV-2 spike N439K variants maintain fitness while evading antibody-mediated immunity. Cell *184*, 1171–1187.e20.

Tortorici, M.A., and Veesler, D. (2019). Structural insights into coronavirus entry. Adv. Virus Res. *105*, 93–116.

Tortorici, M.A., Beltramello, M., Lempp, F.A., Pinto, D., Dang, H.V., Rosen, L.E., McCallum, M., Bowen, J., Minola, A., Jaconi, S., et al. (2020). Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. Science *370*, 950–957.

Tortorici, M.A., Czudnochowski, N., Starr, T.N., Marzi, R., Walls, A.C., Zatta, F., Bowen, J.E., Jaconi, S., Di Iulio, J., Wang, Z., et al. (2021). Broad sarbecovirus neutralization by a human monoclonal antibody. Nature *597*, 103–108.

Tostanoski, L.H., Wegmann, F., Martinot, A.J., Loos, C., McMahan, K., Mercado, N.B., Yu, J., Chan, C.N., Bondoc, S., Starke, C.E., et al. (2020). Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters. Nat. Med. *26*, 1694–1700.

Walls, A.C., Tortorici, M.A., Bosch, B.J., Frenz, B., Rottier, P.J.M., DiMaio, F., Rey, F.A., and Veesler, D. (2016). Cryo-electron microscopy structure of a coronavirus spike glycoprotein trimer. Nature *531*, 114–117.

Walls, A.C., Xiong, X., Park, Y.J., Tortorici, M.A., Snijder, J., Quispe, J., Cameroni, E., Gopal, R., Dai, M., Lanzavecchia, A., et al. (2019). Unexpected Receptor Functional Mimicry Elucidates Activation of Coronavirus Fusion. Cell *176*, 1026–1039.e15.

Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T., and Veesler, D. (2020a). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell *181*, 281–292.e6.

Walls, A.C., Fiala, B., Schäfer, A., Wrenn, S., Pham, M.N., Murphy, M., Tse, L.V., Shehata, L., O'Connor, M.A., Chen, C., et al. (2020b). Elicitation of Potent Neutralizing Antibody Responses by Designed Protein Nanoparticle Vaccines for SARS-CoV-2. Cell *183*, 1367–1382.e17.

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K.Y., et al. (2020). Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell *181*, 894–904.e9.

Wang, P., Nair, M.S., Liu, L., Iketani, S., Luo, Y., Guo, Y., Wang, M., Yu, J., Zhang, B., Kwong, P.D., et al. (2021a). Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature *593*, 130–135.

Wang, P., Liu, L., Iketani, S., Luo, Y., Guo, Y., Wang, M., Yu, J., Zhang, B., Kwong, P.D., Graham, B.S., et al. (2021b). Increased resistance of SARS-CoV-2 variants B.I.35I and B.I.I.7. to antibody neutralization. bioRxiv. https://doi.org/10.1101/2021.01.25.428137.

Wang, Z., Schmidt, F., Weisblum, Y., Muecksch, F., Barnes, C.O., Finkin, S., Schaefer-Babajew, D., Cipolla, M., Gaebler, C., Lieberman, J.A., et al. (2021c). mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. Nature *592*, 616–622.

Wec, A.Z., Wrapp, D., Herbert, A.S., Maurer, D.P., Haslwanter, D., Sakharkar, M., Jangra, R.K., Dieterle, M.E., Lilov, A., Huang, D., et al. (2020). Broad neutralization of SARS-related viruses by human monoclonal antibodies. Science *369*, 731–736.

Weisblum, Y., Schmidt, F., Zhang, F., DaSilva, J., Poston, D., Lorenzi, J.C.C., Muecksch, F., Rutkowska, M., Hoffmann, H.-H., Michailidis, E., et al. (2020). Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. eLife *9*, e61312.

Wibmer, C.K., Ayres, F., Hermanus, T., Madzivhandila, M., Kgagudi, P., Oosthuysen, B., Lambson, B.E., de Oliveira, T., Vermeulen, M., van der Berg, K., et al. (2021). SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. Nat. Med. *27*, 622–625.

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S., and McLellan, J.S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science *367*, 1260–1263.

Wu, K., Werner, A.P., Koch, M., Choi, A., Narayanan, E., Stewart-Jones, G.B.E., Colpitts, T., Bennett, H., Boyoglu-Barnum, S., Shi, W., et al. (2021). Serum Neutralizing Activity Elicited by mRNA-1273 Vaccine - Preliminary Report. N. Engl. J. Med. *384*, 1468–1470.

Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., and Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science *367*, 1444–1448.

Yu, J., Tostanoski, L.H., Peter, L., Mercado, N.B., McMahan, K., Mahrokhian, S.H., Nkolola, J.P., Liu, J., Li, Z., Chandrashekar, A., et al. (2020). DNA vaccine protection against SARS-CoV-2 in rhesus macaques. Science *369*, 806–811.

Yuan, M., Wu, N.C., Zhu, X., Lee, C.D., So, R.T.Y., Lv, H., Mok, C.K.P., and Wilson, I.A. (2020). A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science *368*, 630–633.

Yurkovetskiy, L., Wang, X., Pascal, K.E., Tomkins-Tinch, C., Nyalile, T.P., Wang, Y., Baum, A., Diehl, W.E., Dauphin, A., Carbone, C., et al. (2020). Structural and Functional Analysis of the D614G SARS-CoV-2 Spike Protein Variant. Cell *183*, 739–751.e8.

Zhou, H., Chen, X., Hu, T., Li, J., Song, H., Liu, Y., Wang, P., Liu, D., Yang, J., Holmes, E.C., et al. (2020a). A Novel Bat Coronavirus Closely Related to SARS-CoV-2 Contains Natural Insertions at the S1/S2 Cleavage Site of the Spike Protein. Curr. Biol. *30*, 2196–2203.e3.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., et al. (2020b). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature *579*, 270–273.

Zost, S.J., Gilchuk, P., Case, J.B., Binshtein, E., Chen, R.E., Nkolola, J.P., Schäfer, A., Reidy, J.X., Trivette, A., Nargi, R.S., et al. (2020). Potently neutralizing and protective human antibodies against SARS-CoV-2. Nature *584*, 443–449.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CR3022	ter Meulen et al., 2006	N/A
S309	Pinto et al., 2020	N/A
S2X259	Tortorici et al., 2021	N/A
S2H14	Piccoli et al., 2020	N/A
S230	Rockx et al., 2008	N/A
Goat anti-mouse HRP	Invitrogen	Cat #626520; Lot #TG275230; AB_2533947
Goat anti-human HRP	Invitrogen	Cat #A18817; Lot #65-180-071919; AB_2535594
Streptavidin-HRP	Thermo Scientific	Cat #N100
CD45/LCA-A700-C61	BD Biosciences	Cat#5605010
CD11c-BV501	Biolegend	Cat#117338; AB_2562016
SiglecF-BV650	BD Bioseceinces	Cat#740557; AB_2740258
I1-Hybridoma	ATCC	CRL-2700
Biological samples		
BALB/c mice	Jackson Laboratory	Cat#000651
Pigtail macaques (Macaca nemestrina)	WANPRC	
Rhesus macaques (Macaca mulatta)	NIRC	
20/130 COVID-19 plasma	NIBSC	Sample#20/130
VSV (G*ΔG-luciferase)	Kaname et al., 2010	N/A
Chemicals, peptides, and recombinant proteins		
AddaVax adjuvant	InvivoGen	Cat# vac-adx-10
AS03	GSK	N/A
O/W	VFI	N/A
TMB	SeraCare	Cat# 5120-0083
Thrombin	Sigma	Cat# T9326-150UN
Immobilized Papain	ThermoScientific	Cat# 20341
LysC-endoproteinase	NEB	Cat# P8109S
EZ-Link Sulfo-NHS-LC Biotinylation Kit	Thermo Fisher Scientific	Cat#21435
Experimental models: Cell lines		
Expi 293F	ThermoFisher	Cat #A14527
HEK-ACE2 adherent	BEI (Gift from Bloom lab)	Sample#NR-52511
HEK293T/17 Adherent	ATCC	Cat# CRL-11268
Recombinant DNA		
HexaPro S	Hsieh et al., 2020	N/A
RBD-16GS-50A	GenScript Walls et al., 2020b	N/A
RBD-12GS-50A	GenScript Walls et al., 2020b	N/A
SARS-COV-RBD-16GS-50A	GenScript	N/A
WIV1-RBD-16GS-50A	GenScript	N/A
RaTG13-RBD-16GS-50A	GenScript	N/A
SARS-CoV-2 S-2P trimer	GenScript Walls et al., 2020a	Vector# BEI NR-52421
SARS-CoV S-2P trimer	GeneArt Walls et al., 2019	N/A
SARS-CoV-2 RBD	GenScript Walls et al., 2020a	Vector# BEI NR-52422
SARS-CoV RBD	GenScript	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
WIV1 RBD	GenScript	N/A
RaTG13 RBD	GenScript	N/A/
SARS-CoV-2 S full-length D614G (YP 009724390.1)	Crawford et al., 2020	Vector# BEI NR-52514
SHC014 S full-length (AGZ48806.1)	GenScript	N/A
SARS-CoV S full-length (YP 009825051.1)	GeneArt (Walls et al., 2020)	N/A
RaTG13 S full-length (QHR63300.2)	GenScript	N/A
WIV1 full-length S (AGZ48831.1)	GenScript	N/A
PangolinGD full-length S (QLR06867.1)	GenScript	N/A
Murine leukemia virus gag-pol	Millet and Whittaker 2016	N/A
pTG-Luciferase	Millet and Whittaker 2016	N/A
HIV Hgpm2	Crawford et al., 2020	Vector# BEI NR-52517
HIV luciferase	Crawford et al., 2020	Vector# BEI NR-52516
HIV tat1b	Crawford et al., 2020	Vector# BEI NR-52518
HIV Rev1b	Crawford et al., 2020	Vector# BEI NR-52519
Software and algorithms		
UCSF ChimeraX	Goddard et al., 2018	https://www.rbvi.ucsf.edu/chimerax/
Prism	Graphpad	https://www.graphpad.com/scientific-software/prism/
FlowJo v10	FlowJo	https://www.flowjo.com
Dms-variants package	Greaney et al., 2020	https://jbloomlab.github.io/dms_variants/dms_variants.globalepistasis.html
Other		
Octet Biosensors: protein A	Sartorius (FortéBio)	Cat# 18-5010
Octet Biosensors: ARG2	Sartorius (FortéBio)	Cat# 18-5092
EM supplies 300 mesh grids	Ted Pella	Cat# 01843-F
Filter paper	Cytiva	Cat# 1004047
Uranyl formate	SPI Chem	Cat# 02545-AA
Unis Capillary Cassettes	Unchained Labs	Cat# 201-1010
PrismA Protein A resin	Cytiva	Cat# 17549802
Superdex 200 Increase SEC column	Cytiva	Cat# 28-9909-44
Superose 6 Increase SEC column	Cytiva	Cat# 29091596
Talon resin	TaKaRa	Cat# 635652
Excel resin	Cytiva	Cat# 17371203
Patterson Veterinary, Isoflurane, USP	Patterson	Cat# 07-893-1389
EndoSafe LAL Test Cartridges	Charles River Labs	Cat # PTS20005F
Lemo21(DE3)	New England BioLabs	Cat#C2528J
Isopropyl-B-D-thiogalactoside (IPTG)	Sigma Aldrich	Cat#I6758
Kanamycin Sulfate	Sigma-Aldrich	Cat#K1876
HiLoad S200 pg	Cytiva	Cat#28989336
Ni Sepharose 6 FF	Cytiva	Cat#17531808
HisTrap FF	Cytiva	Cat#17525501

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, David Veesler (dveesler@uw.edu).

Materials availability

All reagents will be made available on request after completion of a Materials Transfer Agreement.

Data and code availability

All data supporting the findings of this study are found within the paper and its Supplemental information, and are available from the Lead Contact author upon request. Additional supplemental items are available from Mendeley Data at https://doi.org/10.17632/5k989kb8t7.1.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines

Expi293F (derived from 293 cells which are female) cells are derived from the HEK293F cell line (Life Technologies). Expi293F cells were grown in Expi293 Expression Medium (Life Technologies), cultured at 36.5°C with 8% CO_2 and shaking at 150 rpm. HEK293T/17 is a female human embryonic kidney cell line (ATCC). The HEK-ACE2 (derived from HEK293T cells which are female) adherent cell line was obtained through BEI Resources, NIAID, NIH: Human Embryonic Kidney Cells (HEK293T) Expressing Human Angiotensin-Converting Enzyme 2, HEK293T-hACE2 Cell Line, NR-52511. All adherent cells were cultured at 37°C with 8% CO_2 in flasks with DMEM + 10% FBS (Hyclone) + 1% penicillin-streptomycin. Cell lines other than Expi293F were not tested for mycoplasma contamination nor authenticated.

Mice

Female BALB/c mice (Stock # 000651, BALB/c cByJ mice) four weeks old were obtained from Jackson Laboratory, Bar Harbor, Maine, and maintained at the Comparative Medicine Facility at the University of Washington, Seattle, WA, accredited by the American Association for the Accreditation of Laboratory Animal Care International (AAALAC). Animal procedures were performed under the approvals of the Institutional Animal Care and Use Committee (IACUC) of University of Washington, Seattle, WA, and University of North Carolina, Chapel Hill, NC.

Pigtail macaques

Two adult male Pigtail macaques (*Macaca nemestrina*) were immunized in this study. All animals were housed at the Washington National Primate Research Center (WaNPRC), an AAALAC International accredited institution. All experiments were approved by The University of Washington's IACUC. Animals were singly housed in comfortable, clean, adequately-sized cages with ambient temperatures between 72–82°F. Animals received environmental enrichment for the duration of the study including grooming contact, perches, toys, foraging experiences and access to additional environment enrichment devices. Water was available through automatic watering devices and animals were fed a commercial monkey chow, supplemented daily with fruits and vegetables. Throughout the study, animals were checked twice daily by husbandry staff.

Rhesus macaques

Male Rhesus macaques (Macaca mulatta) of Indian origin, aged 3–7 years were assigned to the study (Arunachalam et al., 2021). Animals were distributed between the groups such that the age and weight distribution were comparable across the groups. Animals were housed and maintained at the New Iberia Research Center (NIRC) of the University of Louisiana at Lafayette, an AAALAC International accredited institution, in accordance with the rules and regulations of the Guide for the Care and Use of Laboratory Animal Resources. The entire study (protocol 2020-8808-15) was reviewed and approved by the University of Louisiana at Lafayette IACUC. All animals were negative for SIV, simian T cell leukemia virus, and simian retrovirus.

Convalescent human sera

Samples collected between 1–60 days post infection from individuals who tested positive for SARS-CoV-2 by PCR were profiled for anti-SARS-CoV-2 S antibody responses and those with anti-S Ab responses were maintained in the cohort (Walls et al., 2020b). Individuals were enrolled as part of the HAARVI study at the University of Washington in Seattle, WA. Baseline sociodemographic and clinical data for these individuals are summarized in Table S1. This study was approved by the University of Washington Human Subjects Division Institutional Review Board (STUDY00000959 and STUDY00003376). All experiments were performed in at least two replicates. One sample is the 20/130 COVID-19 plasma from NIBSC (https://www.nibsc.org/documents/ifu/20-130.pdf).

Pfizer vaccinated human sera

Blood samples were collected from participants who had received both doses of the Pfizer mRNA vaccine and were 7–30 days post second vaccine dose. Clinical data for these individuals are summarized in Table S1. Individuals were enrolled in the UWARN: COVID-19 in WA study at the University of Washington in Seattle, WA. This study was approved by the University of Washington Human Subjects Division Institutional Review Board (STUDY00010350).

METHOD DETAILS

Plasmid construction

The SARS-CoV-2-RBD-Avi construct was synthesized by GenScript into pcDNA3.1- with an N-terminal mu-phosphatase signal peptide and a C-terminal octa-histidine tag, flexible linker, and avi tag (GHHHHHHHHGGSSGLNDIFEAQKIEWHE). The boundaries of the construct are N-$_{328}$RFPN$_{331}$ and $_{528}$KKST$_{531}$-C (Walls et al., 2020a). The GD-Pangolin (326-527), WIV1 (316-518), RaTG13 (359-562), RmYN02 (307-492), and ZXC21 (323-507) were synthesized by GenScript into vector pcDNA3.1- or CMVR with a preceding mu-phosphatase signal peptide and a C-terminal octahistidine tag. SARS-CoV-1 (306-575) was subcloned from a GenArt synthesized SARS-CoV-1 Spike ectodomain. The SARS-CoV S2P ((Pallesen et al., 2017)) was synthesized by GeneArt and placed into a modified pOPING vector with its original N-terminal mu-phosphatase signal peptide, and an engineered C-terminal extension: SG-RENLYFQG (TEV protease site), GGGSG-YIPEAPRDGQAYVRKDGEWVLLSTFL (foldon trimerization motif), G-HHHHHH (hexa-histidine tag), just upstream of the predicted transmembrane region (YIK). The SARS-CoV-2 S2P ectodomain trimer (GenBank: YP_009724390.1, BEI NR-52420) was synthesized by GenScript into pCMV with an N-terminal mu-phosphatase signal peptide and a C-terminal TEV cleavage site (GSGRENLYPQG), T4 fibritin foldon (GGGSGYIPEAPRDGQAYVRKDGEWVLLSTPL), and octa-histidine tag (GHHHHHHHH) (Walls et al., 2020a). The construct contains the 2P mutations (proline substitutions at residues 986 and 987; and an $_{682}$SGAG$_{685}$ substitution at the furin cleavage site. The SARS-CoV-2 RBD was genetically fused to the N terminus of the trimeric I53-50A nanoparticle component using 12 or 16 glycine and serine residues. RBD-12GS-I53-50A fusions were synthesized and cloned by Genscript into pCMV. The RBD-16GS-I53-50A fusion was cloned into pCMV/R using the Xba1 and AvrII restriction sites and Gibson assembly (Gibson et al., 2009). All RBD-bearing components contained an N-terminal mu-phosphatase signal peptide and a C-terminal octa-histidine tag. Human ACE2 ectodomain was genetically fused to a sequence encoding a thrombin cleavage site and a human Fc fragment at the C-terminal end. hACE2-Fc was synthesized and cloned by GenScript with a BM40 signal peptide. Genes encoding CR3022 heavy and light chains were ordered from GenScript and cloned into pCMV/R. S309 construct as previously described (Pinto et al., 2020). SARS-CoV-2 HexaPro construct is as previously described (Hsieh et al., 2020) and placed into CMVR with an octa-his tag.

Transient transfection

Proteins were produced using endotoxin free DNA in Expi293F cells grown in suspension using Expi293F expression medium (Life Technologies) at 33°C, 70% humidity, 8% CO_2 rotating at 150 rpm. The cultures were transfected using PEI-MAX (Polyscience) with cells grown to a density of 3.0 million cells per mL and cultivated for 3 days. Supernatants were clarified by centrifugation (5 min at 4000 *rcf.*), addition of PDADMAC solution to a final concentration of 0.0375% (Sigma Aldrich, #409014), and a second spin (5 min at 4000 *rcf.*).

Microbial protein expression and purification

The I53-50A and I53-50B.4.PT1 proteins (Bale et al., 2016) were expressed in Lemo21(DE3) (NEB) in LB (10 g Tryptone, 5 g Yeast Extract, 10 g NaCl) grown in 2 L baffled shake flasks or a 10 L BioFlo 320 Fermenter (Eppendorf). Cells were grown at 37°C to an OD600 ~0.8, and then induced with 1 mM IPTG. Expression temperature was reduced to 18°C and the cells shaken for ~16 h. The cells were harvested and lysed by microfluidization using a Microfluidics M110P at 18,000 psi in 50 mM Tris, 500 mM NaCl, 30 mM imidazole, 1 mM PMSF, 0.75% CHAPS. Lysates were clarified by centrifugation at 24,000 g for 30 min and applied to a 2.6 × 10 cm Ni Sepharose 6 FF column (Cytiva) for purification by IMAC on an AKTA Avant150 FPLC system (Cytiva). Protein of interest was eluted over a linear gradient of 30 mM to 500 mM imidazole in a background of 50 mM Tris pH 8, 500 mM NaCl, 0.75% CHAPS buffer. Peak fractions were pooled, concentrated in 10K MWCO centrifugal filters (Millipore), sterile filtered (0.22 μm) and applied to either a Superdex 200 Increase 10/300, or HiLoad S200 pg GL SEC column (Cytiva) using 50 mM Tris pH 8, 500 mM NaCl, 0.75% CHAPS buffer. I53-50A elutes at ~0.6 column volume (CV). I53-50B.4PT1 elutes at ~0.45 CV. After sizing, bacterial-derived components were tested to confirm low levels of endotoxin before using for nanoparticle assembly.

Protein purification

Proteins containing His tags were purified from clarified supernatants via a batch bind method where each clarified supernatant was supplemented with 1 M Tris-HCl pH 8.0 to a final concentration of 45 mM and 5 M NaCl to a final concentration of ~310 mM. Talon cobalt affinity resin (Takara) was added to the treated supernatants and allowed to incubate for 15 min with gentle shaking. Resin was collected using vacuum filtration with a 0.2 μm filter and transferred to a gravity column. The resin was washed with 20 mM Tris pH 8.0, 300 mM NaCl, and the protein was eluted with 3 column volumes of 20 mM Tris pH 8.0, 300 mM NaCl, 300 mM imidazole. The batch bind process was then repeated and the first and second elutions combined. SDS-PAGE was used to assess purity. RBD-I53-50A fusion protein IMAC elutions were concentrated to > 1 mg/mL and subjected to three rounds of dialysis into 50 mM Tris pH 7.4, 185 mM NaCl, 100 mM Arginine, 4.5% glycerol, and 0.75% w/v 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) in a hydrated 10K molecular weight cutoff dialysis cassette (Thermo Scientific). S2P and HexaPro IMAC elution fractions were concentrated to ~1 mg/mL and dialyzed three times into 50 mM Tris pH 8, 150 mM NaCl, 0.25% L-Histidine in a hydrated 10K molecular weight cutoff dialysis cassette (Thermo Scientific).

Clarified supernatants of cells expressing monoclonal antibodies and human ACE2-Fc were purified using a MabSelect PrismA 2.6 × 5 cm column (Cytiva) on an AKTA Avant150 FPLC (Cytiva). Bound antibodies were washed with five column volumes of 20 mM $NaPO_4$, 150 mM NaCl pH 7.2, then five column volumes of 20 mM $NaPO_4$, 1 M NaCl pH 7.4 and eluted with three column volumes of 100 mM glycine at pH 3.0. The eluate was neutralized with 2 M Trizma base to 50 mM final concentration. SDS-PAGE was used to assess purity.

Recombinant S309 was expressed as a Fab in expiCHO cells transiently co-transfected with plasmids expressing the heavy and light chain, as described above (see Transient transfection) (Pinto et al., 2020). The protein was affinity-purified using a HiTrap Protein A Mab select Xtra column (Cytiva) followed by desalting against 20 mM $NaPO_4$, 150 mM NaCl pH 7.2 using a HiTrap Fast desalting column (Cytiva). The protein was sterilized with a 0.22 μm filter and stored at 4°C until use.

In vitro nanoparticle assembly and purification

Total protein concentration of purified individual nanoparticle components was determined by measuring absorbance at 280 nm using a UV/vis spectrophotometer (Agilent Cary 8454) and calculated extinction coefficients. The assembly steps were performed at room temperature with addition in the following order: RBD-I53-50A trimeric fusion protein, followed by additional buffer (50 mM Tris pH 7.4, 185 mM NaCl, 100 mM Arginine, 4.5% glycerol, and 0.75% w/v CHAPS) as needed to achieve desired final concentration, and finally I53-50B.4PT1 pentameric component (in 50 mM Tris pH 8, 500 mM NaCl, 0.75% w/v CHAPS), with a molar ratio of RBD-I53-50A:I53-50B.4PT1 of 1.1:1. All RBD-I53-50 *in vitro* assemblies were incubated at 2-8°C with gentle rocking for at least 30 min before subsequent purification by SEC in order to remove residual unassembled component. Different columns were utilized depending on purpose: Superose 6 Increase 10/300 GL column was used analytically for nanoparticle size estimation, a Superdex 200 Increase 10/300 GL column used for small-scale pilot assemblies, and a HiLoad 26/600 Superdex 200 pg column used for nanoparticle production. Assembled particles were purified in 50 mM Tris pH 7.4, 185 mM NaCl, 100 mM Arginine, 4.5% glycerol, and 0.75% w/v CHAPS, and elute at ~11 mL on the Superose 6 column and in the void volume of Superdex 200 columns. Assembled nanoparticles were sterile filtered (0.22 μm) immediately prior to column application and following pooling of fractions.

Endotoxin measurements

Endotoxin levels in protein samples were measured using the EndoSafe Nexgen-MCS System (Charles River). Samples were diluted 1:50 or 1:100 in Endotoxin-free LAL reagent water, and applied into wells of an EndoSafe LAL reagent cartridge. Charles River Endo-Scan-V software was used to analyze endotoxin content, automatically back-calculating for the dilution factor. Endotoxin values were reported as EU/mL which were then converted to EU/mg based on UV/vis measurements. Our threshold for samples suitable for immunization was < 50 EU/mg.

Pigtail macaque immunization

Two Pigtail macaques were immunized with 250 μg of RBD-12GS-I53-50 nanoparticle (88 μg RBD antigen) with AddaVax at day 0, day 28, and O/W at day 168. Blood was collected every 14 days post-prime. Blood was collected in serum collection tubes and allowed to clot at room temperature. Serum was isolated after a 15 min spin at 1455 x g for 15 min and stored at −80C until use. Prior to vaccination or blood collection, animals were sedated with an intramuscular injection (10 mg/kg) of ketamine (Ketaset®; Henry Schein). Prior to inoculation, immunogen suspensions were gently mixed 1:1 vol/vol with AddaVax adjuvant for immunizations 1 and 2 and O/W for immunization 3 (VFI) to reach a final concentration of 0.250 mg/mL antigen. The vaccine was delivered intramuscularly into both quadriceps muscles with 1 mL per injection site on days 0, 28, and 168. All injection sites were shaved prior to injection. Animals were observed daily for general health (activity and appetite, urine/feces output) and for evidence of reactogenicity at the vaccine inoculation site (swelling, erythema, and pruritus) for up to 1 week following vaccination. They also received physical exams including temperature and weight measurements at each study time point. None of the animals became severely ill during the course of the study nor required euthanasia.

Rhesus macaque immunization

Adapted from Arunachalam et al. (2021). AS03 was kindly provided by GSK Vaccines. AS03 is an oil-in-water emulsion that contains 11.86 mg a-tocopherol, 10.69 mg squalene, and 4.86 mg polysorbate 80 (Tween-80) in PBS. For each dose, RBD-NP was diluted to 50 μg/ml (RBD component) in 250 μL of Tris-buffered saline (TBS) and mixed with an equal volume of AS03.The dose of AS03 was 50% v/v (equivalent of one human dose). Soluble HexaPro was diluted to 50 μg/ml in 250 μL of Tris-buffered saline (TBS) and mixed with an equal volume of AS03. All immunizations were administered via the intramuscular route in right forelimbs. The volume of each dose was 0.5 ml.

Deep mutational scanning

All mutations that escape serum antibody binding were mapped via a deep mutational scanning approach (Greaney et al., 2020, 2021b). We used previously described yeast-display RBD mutant libraries (Greaney et al., 2020; Starr et al., 2020a). Briefly, duplicate mutant libraries were constructed in the spike receptor binding domain (RBD) from SARS-CoV-2 (isolate Wuhan-Hu-1, GenBank: MN908947, residues N331-T531) and contain 3,804 of the 3,819 possible amino-acid mutations, with > 95% present as single mutants. Each RBD variant was linked to a unique 16-nucleotide barcode sequence to facilitate downstream sequencing. As previously described, libraries were sorted for RBD expression and ACE2 binding to eliminate RBD variants that are completely misfolded or non-functional (i.e., lacking modest ACE2 binding affinity) (Greaney et al., 2020).

Antibody escape mapping experiments were performed in biological duplicate using two independent mutant RBD libraries, with minor modifications from Greaney et al. (2020), and exactly as described in Greaney et al. (2021b). The antibody escape mapping for the vaccinated NHP serum was performed in this study; the antibody escape mapping from convalescent human plasma was performed in Greaney et al. (2021b). Briefly, mutant yeast libraries induced to express RBD were washed and incubated with plasma or serum at a range of dilutions for 1 h at room temperature with gentle agitation. For each sample, we chose a sub-saturating dilution such that the amount of fluorescent signal due to plasma antibody binding to RBD was approximately equal across samples. A 1:1000 dilution was used for the vaccinated NHP serum, and the exact dilutions of human convalescent plasma are reported in Greaney et al. (2021b). After the antibody incubations, the libraries were secondarily labeled with 1:100 FITC-conjugated anti-MYC antibody (Immunology Consultants Lab, CYMC-45F) to label for RBD expression and and 1:200 Alexa-647-conjugated goat anti-human-IgA+IgG+IgM (Jackson ImmunoResearch 109-605-064) to label for bound serum or plasma antibodies. A flow cytometric selection gate was drawn to capture approximately 5% of the RBD mutant libraries with the lowest amount of plasma binding for their degree of RBD expression. For each sample, approximately 10 million RBD+ cells were processed on the cytometer. Antibody-escaped cells were grown overnight in SD-CAA (6.7g/L Yeast Nitrogen Base, 5.0g/L Casamino acids, 1.065 g/L MES acid, and 2% w/v dextrose) to expand cells prior to plasmid extraction.

Plasmid samples were prepared from pre-selection and overnight cultures of antibody-escaped cells (Zymoprep Yeast Plasmid Miniprep II) as previously described (Greaney et al., 2020). The 16-nucleotide barcode sequences identifying each RBD variant were amplified by PCR and sequenced on an Illumina HiSeq 2500 with 50 bp single-end reads as described in Greaney et al. (2020 and Starr et al. (2020a).

Escape fractions were computed as described in Greaney et al. (2020), and exactly as described in Greaney et al. (2021b). We used the dms_variants package (https://jbloomlab.github.io/dms_variants/, version 0.8.2) to process Illumina sequences into counts of each barcoded RBD variant in each pre-sort and antibody-escape population using the barcode/RBD look-up table from Starr et al. (2021).

For each serum selection, we computed the "escape fraction" for each barcoded variant using the deep sequencing counts for each variant in the original and serum-escape populations and the total fraction of the library that escaped antibody binding via the formula provided in Greaney et al. (2020). These escape fractions represent the estimated fraction of cells expressing that specific variant that fall in the antibody escape bin, such that a value of 0 means the variant is always bound by serum and a value of 1 means that it always escapes serum binding. We then applied a computational filter to remove variants with low sequencing counts or highly deleterious mutations that might cause antibody escape simply by leading to poor expression of properly folded RBD on the yeast cell surface (Greaney et al., 2020; Starr et al., 2020a). Specifically, we removed variants that had (or contained mutations with) ACE2 binding scores < -2.35 or expression scores < -1, using the variant- and mutation-level deep mutational scanning scores from Starr et al. (2020a). Note that these filtering criteria are slightly more stringent than those used in Greaney et al. (2020) but are identical to those used in Greaney et al. (2021b) and Starr et al. (2020b).

We next deconvolved variant-level escape scores into escape fraction estimates for single mutations using global epistasis models (Otwinowski et al., 2018) implemented in the dms_variants package, as detailed at (https://jbloomlab.github.io/dms_variants/dms_variants.globalepistasis.html) and described in Greaney et al. (2020). The reported escape fractions throughout the paper are the average across the libraries (correlations shown in Figure S2); these scores are also in Table S1 and at https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_RBD-nano-vax-NHP1/blob/main/results/supp_data/NHP_HCS_raw_data.csv. Sites of strong escape from each antibody were determined heuristically as sites whose summed mutational escape scores were at least 10 times the median sitewise sum of selection, and within 10-fold of the sitewise sum of the most strongly selected site. Sites shown in Figures 2 and S2A are the sites of strong escape for any of the three human convalescent plasma, plus sites 417, 452, and 501 due to their prevalence in circulating SARS-CoV-2 variants. For each plasma, the y axis is scaled to be the greatest of (a) the maximum site-wise escape metric observed for that plasma, (b) 20x the median site-wise escape fraction observed across all sites for that plasma, or (c) an absolute value of 1.0 (to appropriately scale plasma that are not "noisy" but for which no mutation has a strong effect on plasma binding). Full documentation of the computational analysis is at https://github.com/jbloomlab/SARS-CoV-2-RBD_MAP_RBD-nano-vax-NHP1. These results are also available in a zoomable, interactive form at https://jbloomlab.github.io/SARS-CoV-2-RBD_MAP_RBD-nano-vax-NHP1/.

ELISA

For anti-S2P ELISA, 25 μL of 2 μg/mL S2P was plated onto 384-well Nunc Maxisorp (ThermoFisher) plates in PBS and sealed overnight at RT. The next day plates were washed 4 × in Tris Buffered Saline Tween (TBST) using a plate washer (BioTek) and blocked with SuperBlock (ThermoFisher) for 1 h at 37°C. Plates were washed 4 × in TBST and 1:5 serial dilutions of mouse, NHP, or human sera were made in 25 μL TBST and incubated at 37°C for 1 h. Plates were washed 4 × in TBST, then anti-mouse (Invitrogen), anti-NHP (AlphaDiagnostics), or anti-human (Invitrogen) horseradish peroxidase-conjugated antibodies were diluted 1:5,000 and 25 μL added to each well and incubated at 37°C for 1 h. Plates were washed 4 × in TBST and 25 μL of TMB (SeraCare) was added to every well for

~5 min at room temperature. The reaction was quenched with the addition of 25 μL of 1 N HCl. Plates were immediately read at 450 nm on a BioTek plate reader and data plotted and fit in Prism (GraphPad) using nonlinear regression sigmoidal, 4PL, X is log(concentration) to determine EC_{50} values from curve fits.

Competition ELISA of NHP sera with hACE2, CR3022 IgG, and S309 IgG for immobilized SARS-CoV-2 S2P

384-well Maxisorp plates (Thermo Fisher) were coated overnight at room temperature with 3 μg/mL of SARS-CoV-2 S2P (Pallesen et al., 2017) in 20mM Tris pH 8 and 150mM NaCl. Plates were slapped dry and blocked with Blocker Casein in TBS (Thermo Fisher) for one hour at 37°C. Plates were slapped dry and NHP sera was serially diluted 1:4 in TBST with an initial dilution of 1:4 for hACE2 competition or 1:2 for antibody competition. Random primary amine biotinylated (Thermo Fisher) hACE2-Fc, CR3022 (Yuan et al., 2020), or S309 (Pinto et al., 2020) were added, bringing the concentration of each well to the EC_{50} values of 0.2nM, 2nm, and 0.01nM, respectively. Plates were left for one hour at 37°C, then washed 4x with TBST using a 405 TS Microplate Washer (BioTek) followed by addition of 1:500 streptavidin-HRP (Thermo Fisher) for one hour at 37°C. Plates were washed 4x and TMB Microwell Peroxidase (Seracare) was added. The reaction was quenched after 1-2 minutes with 1 N HCl and the A450 of each well was read using a BioTek plate reader (BioTek). Data plotted and fit in Prism (GraphPad) using nonlinear regression sigmoidal, 4PL, X is log(concentration) to determine EC_{50} values from curve fits with upper and lower constraints determined by uncompeted ELISA per antigen.

Competition ELISA of mouse sera and immobilized hACE2 or mAbs with SARS-CoV-2 or SARS-CoV S2P

384-well Maxisorp plates (Thermo Fisher) were coated overnight at room temperature with 3 μg/mL of hACE2-Fc, CR3022 (Yuan et al., 2020), or S309 (Pinto et al., 2020) in 20mM Tris pH 8 and 150mM NaCl. Plates were slapped dry and blocked with Blocker Casein in TBS (Thermo Fisher) for one hour at 37°C. Plates were slapped dry and a 30-minute pre-incubated 1:5 serial dilution of mouse sera in TBST, with in initial dilution of 1:50 for hACE2-Fc competition or 1:10 for antibody competition, and a constant concentration of biotinylated (Avidity) SARS-CoV-2 S2P or SARS-CoV 2P at their EC_{50} values were added. Spike concentrations were 0.63nM, 5.98nM, and 0.22nM of SARS-CoV-2 S2P or 4.11nM, 2.89nM, and 0.19nM of SARS-CoV S2P for immobilized hACE2, CR3022, and S309, respectively. Plates were left for one hour at 37°C, then washed 4x with TBST using a 405 TS Microplate Washer (BioTek) followed by addition of 1:500 streptavidin-HRP (Thermo Fisher) for one hour at 37°C. Plates were washed 4x and TMB Microwell Peroxidase (Seracare) was added. The reaction was quenched after 1-2 minutes with 1 N HCl and the A450 of each well was read using a BioTek plate reader (BioTek). Data plotted and fit in Prism (GraphPad) using nonlinear regression sigmoidal, 4PL, X is log(concentration) to determine EC_{50} values from curve fits.

Pseudovirus production

MLV-based D614G SARS-CoV-2 S and SARS-CoV S pseudotypes were prepared as previously described (Millet and Whittaker, 2016; Walls et al., 2020a, 2020b). Briefly, HEK293T cells were co-transfected using Lipofectamine 2000 (Life Technologies) with an S-encoding plasmid, an MLV Gag-Pol packaging construct, and the MLV transfer vector encoding a luciferase reporter according to the manufacturer's instructions. Cells were washed 3 × with Opti-MEM and incubated for 5 h at 37°C with transfection medium. DMEM containing 10% FBS was added for 60 h. The supernatants were harvested by spinning at 2,500 g, filtered through a 0.45 μm filter, concentrated with a 100 kDa membrane for 10 min at 2,500 g and then aliquoted and stored at −80°C.

HIV-based pseudotypes were prepared as previously described (Crawford et al., 2020). Briefly, HEK293T cells were cotransfected using Lipofectamine 2000 (Life Technologies) with an S-encoding plasmid (D614G SARS-CoV-2 S (YP 009724390.1), Pangolin-Guangdong S (QLR06867.1), SARS-CoV S (YP 009825051.1), P1, B.1.351 S, B.1.1.7, and B.1.1.7+E484K S) an HIV Gag-Pol, Tat, Rev1B packaging construct, and the HIV transfer vector encoding a luciferase reporter according to the manufacturer's instructions. Cells were washed 3 × with Opti-MEM and incubated for 5 h at 37°C with transfection medium. DMEM containing 10% FBS was added for 60 h. The supernatants were harvested by spinning at 2,500 g, filtered through a 0.45 μm filter, concentrated with a 100 kDa membrane for 10 min at 2,500 g and then aliquoted and stored at −80°C.

D614G SARS-CoV-2 S (YP 009724390.1), D614G SARS-CoV-2 point mutants, SHC014 (AGZ48806.1), RaTG13 S (QHR63300.2), Pangolin-Guangdong S (QLR06867.1), SARS-CoV S (YP 009825051.1), WIV1 S (AGZ48831.1), B.1.351 S, P1, and B.1.1.7 S pseudotyped VSV viruses were prepared as described previously (McCallum et al., 2021a; Sauer et al., 2021). Briefly, 293T cells in DMEM supplemented with 10% FBS, 1% PenStrep seeded in 10-cm dishes were transfected with the plasmid encoding for the corresponding S glycoprotein using lipofectamine 2000 (Life Technologies) following manufacturer's indications. One day post-transfection, cells were infected with VSV(G*ΔG-luciferase) and after 2 h were washed five times with DMEM before adding medium supplemented with anti-VSV-G antibody (I1- mouse hybridoma supernatant, CRL- 2700, ATCC). Virus pseudotypes were harvested 18-24 h post-inoculation, clarified by centrifugation at 2,500 x g for 5 min, filtered through a 0.45 μm cut off membrane, concentrated 10 times with a 30 kDa cut off membrane, aliquoted and stored at −80°C.

Pseudovirus neutralization

HEK293-hACE2 cells (Crawford et al., 2020) were cultured in DMEM with 10% FBS (Hyclone) and 1% PenStrep with 8% CO_2 in a 37°C incubator (ThermoFisher). One day prior to infection, 40 μL of poly-lysine (Sigma) was placed into 96-well plates and incubated with rotation for 5 min. Poly-lysine was removed, plates were dried for 5 min then washed 1 × with water prior to plating with 40,000 cells. The following day, cells were checked to be at 80% confluence. In an empty half-area 96-well plate a 1:3 serial dilution of sera was made in DMEM and diluted pseudovirus was then added to the serial dilution and incubated at room temperature for 30-60 min. After incubation, the sera-virus mixture was added to the cells at 37°C and 2 hours post-infection, 40 μL 20% FBS-2% PenStrep DMEM was added. After 17-20 hours (VSV) or 48 hours (HIV, MLV) 40 μL/well of One-Glo-EX substrate (Promega) was added to the cells and incubated in the dark for 5-10 min prior reading on a BioTek plate reader. Measurements were done in at least duplicate. Relative luciferase units were plotted and normalized in Prism (GraphPad). Nonlinear regression of log(inhibitor) versus normalized response was used to determine IC_{50} values from curve fits. Kruskal Wallis tests were used to compare two groups to determine whether they were statistically different.

Sarbecovirus biolayer interferometry binding analysis

Purification of Fabs from NHP serum was adapted from Boyoglu-Barnum et al. (2021). Briefly, 1 mL of day 70 serum was diluted to 10 mL with PBS and incubated with 1 mL of 3 × PBS-washed protein A beads (GenScript) with agitation overnight at 37°C. The next day beads were thoroughly washed with PBS using a gravity flow column and bound Abs were eluted with 0.1 M glycine pH 3.5 into 1M Tris-HCl (pH 8.0) to a final concentration of 100 mM. Serum and early washes that flowed through were re-bound to beads overnight again for a second, repeat elution. IgGs were concentrated (Amicon 30 kDa) and buffer exchanged into PBS. 2 × digestion buffer (40 mM sodium phosphate pH 6.5, 20 mM EDTA, 40 mM cysteine) was added to concentrated and pooled IgGs. 500 μL of resuspended immobilized papain resin (ThermoFisher Scientific) freshly washed in 1 × digestion buffer (20 mM sodium phosphate, 10 mM EDTA, 20 mM cysteine, pH 6.5) was added to purified IgGs in 2 × digestion buffer and samples were agitated for 5 h at 37°C. The supernatant was separated from resin and resin washes were collected and pooled with the resin flow through. Pooled supernatants were sterile-filtered at 0.22 μm and applied 6 × to PBS-washed protein A beads in a gravity flow column. The column was eluted as described above and the papain procedure repeated overnight with undigested IgGs to increase yield. The protein A flow throughs were pooled, concentrated (using an Amicon 10 kDa), and buffer exchanged into PBS. Purity was checked by SDS-PAGE.

Assays were performed and analyzed using biolayer interferometry on an Octet Red 96 System (Pall Forte Bio/Sartorius) at ambient temperature with shaking at 1000 rpm. Different Sarbeco RBDs were purified like in Walls et al., 2020 and were diluted with different acetate buffers and applied to a black 96-well Greiner Bio-one microplate at 200ul per well. GD-Pangolin RBD was diluted in pH 6 buffer to 5 μg/mL, RmNY02 were diluted in pH 5 to 25 μg/mL, WIV16 was diluted in pH 5 to 10 μg/mL, SARS-CoV-2 was diluted in pH 6 to 5 μg/mL, RaTG13 was diluted in pH 6 to 10 μg/mL, RaTG13 was diluted in pH 6 to 10 μg/mL, SARS-CoV was diluted in pH 6 to 50 μg/mL, and finally ZXC21 was diluted in pH 6 to 10μg/mL. AR2G biosensors (ForteBio/Sartorius) following 600 s hydration were normalized in water for 180 s. Then tips were NHS-EDC activated for 300 s and the different sarbecovirus RBDs were loaded up to a 1.50 nm threshold for up to 600 s. Immobilized RBDs on the tips were quenched for 300 s in ethanolamine and dipped into kinetics buffer for a 60 s baseline. The association step was performed by dipping the mobilized RBDs into diluted purified polyclonal pigtail macaque IgGs for 600 s. Dissociation was measured by inserting the biosensors in kinetics buffer for 600 s. The data were baseline subtracted and the plots fitted using the Pall ForteBio/Sartorius analysis software (version 12.0).

Cocktail and mosaic bio-layer interferometry (antigenicity)

Binding of hACE2-Fc to monovalent RBD-I53-50 nanoparticles, mosaic-RBD-I53-50 nanoparticles, and cocktail of RBD-nanoparticles was analyzed for antigenicity experiments and real-time stability studies using an Octet Red 96 System (Pall FortéBio/Sartorius) at ambient temperature with shaking at 1000 rpm. Protein samples were diluted to 100 nM in Kinetics buffer (1 × HEPES-EP+ (Pall Forté Bio), 0.05% nonfat milk, and 0.02% sodium azide). Buffer, receptor, and analyte were then applied to a black 96-well Greiner Bio-one microplate at 200 μL per well. Protein A biosensors (FortéBio/Sartorius) were first hydrated for 10 minutes in Kinetics buffer, then dipped into hACE2-Fc diluted to 10 μg/mL in Kinetics buffer in the immobilization step. After 150 s, the tips were transferred to kinetics buffer for 60 s to reach a baseline. The association step was performed by dipping the loaded biosensors into the immunogens for 300 s, and subsequent dissociation was performed by dipping the biosensors back into Kinetics buffer for an additional 300 s. The data were baseline subtracted prior for plotting using the FortéBio analysis software (version 12.0). Plots in Figure S3 show the 600 s of association and dissociation.

Sandwich bio-layer interferometry (mosaic display antigenicity)

Binding of hACE2-Fc or S2H14 mAb and S230 Fab to WIV1-RBD-I53-50, RaTG13-RBD-I53-50, SARS-CoV-SARS-CoV2-RBD-I53-50, SARS-CoV2-I53-50, and mosaic-RBD-I53-50 nanoparticles were analyzed for co-display of RBDs using an Octet Red 96 System (Pall FortéBio/Sartorius) at ambient temperature with shaking at 1000 rpm. Nanoparticles were diluted to 100 nM in Kinetics buffer. Kinetics buffer, mAb, nanoparticles and Fab were then applied to a black 96-well Greiner Bio-one microplate at 200 μL per well. Protein A biosensors (FortéBio/Sartorius) were first hydrated for 10 minutes in Kinetics buffer, then dipped into hACE2-Fc or S2H14 mAb diluted to 10 μg/mL in Kinetics buffer in the immobilization step. After 150 s, the tips were transferred to Kinetics buffer for 60 s to reach a baseline. The receptor or mAb was then loaded with nanoparticle by dipping the loaded biosensors into the immunogens for 300 s, and subsequent baseline was performed by dipping the biosensors back into the Kinetics buffer for an additional 60 s. Association of S230 Fab diluted to 100 nM in Kinetics buffer was then measured for 300 s and subsequent dissociation in Kinetics buffer of S230 Fab for 300 s. The data were baseline subtracted prior for plotting using the FortéBio analysis software (version 12.0). Plots in Figure S3 exclude the initial mAb loading and the first baseline.

Cocktail and mosaic negative stain electron microscopy

Monovalent RBD-I53-50 nanoparticles, mosaic-RBD-I53-50 nanoparticles, and cocktail of RBD-nanoparticles were first diluted to 75 μg/mL in 50 mM Tris pH 7.4, 185 mM NaCl, 100 mM Arginine, 4.5% v/v Glycerol, 0.75% w/v CHAPS prior to application of 3 μL of sample onto freshly glow-discharged 300-mesh copper grids. Sample was incubated on the grid for 1 minute before the grid was dipped in a 50 μL droplet of water and excess liquid blotted away with filter paper (Whatman). The grids were then dipped into 6 μL of 0.75% w/v uranyl formate stain. Stain was blotted off with filter paper, then the grids were dipped into another 6 μL of stain and incubated for ~70 s. Finally, the stain was blotted away and the grids were allowed to dry for 1 minute. Prepared grids were imaged in a Talos model L120C electron microscope at 57,000 × (nanoparticles).

Cocktail and mosaic dynamic light scattering

Dynamic Light Scattering (DLS) was used to measure hydrodynamic diameter (Dh) and % Polydispersity (%Pd) of monovalent RBD-I53-50 nanoparticles, mosaic-RBD-I53-50 nanoparticles, and cocktail of RBD-nanoparticles on an UNcle Nano-DSF (UNchained Laboratories). Sample was applied to a 8.8 μL quartz capillary cassette (UNi, UNchained Laboratories) and measured with 10 acquisitions of 5 s each, using auto-attenuation of the laser. Increased viscosity due to 4.5% v/v glycerol in the RBD nanoparticle buffer was accounted for by the UNcle Client software in Dh measurements.

Mouse immunizations and challenge

At six weeks of age, 8 female BALB/c mice per dosing group were vaccinated with a prime immunization, and three weeks later mice were boosted with a second vaccination (IACUC protocol 4470.01). Prior to inoculation, immunogen suspensions were gently mixed 1:1 vol/vol with AddaVax adjuvant (Invivogen, San Diego, CA) to reach a final concentration of 0.01 mg/mL antigen. Mice were injected intramuscularly into the gastrocnemius muscle of each hind leg using a 27-gauge needle (BD, San Diego, CA) with 50 μL per injection site (100 μL total) of immunogen under isoflurane anesthesia. To obtain sera all mice were bled two weeks after prime and boost immunizations. Blood was collected via submental venous puncture and rested in 1.5 mL plastic Eppendorf tubes at room temperature for 30 min to allow for coagulation. Serum was separated from red blood cells via centrifugation at 2,000 g for 10 min. Complement factors and pathogens in isolated serum were heat-inactivated via incubation at 56°C for 60 min. Serum was stored at 4°C or −80°C until use. The study was repeated twice. Five weeks post-boost, mice (aged 14 weeks) were exported from Comparative Medicine Facility at the University of Washington, Seattle, WA to an AAALAC accredited Animal Biosafety Level 3 (ABSL3) Laboratory at the University of North Carolina, Chapel Hill. After a 7-day acclimation time, mice were anesthetized with a mixture of ketamine/xylazine and challenged intranasally with 10^5 plaque-forming units (pfu) of mouse-adapted SARS-CoV-2 MA10 or SARS-CoV MA15 strain for the evaluation of vaccine efficacy (IACUC protocol 20-114.0). After infection, body weight and congestion score were monitored daily until the termination of the study two days post-infection, when lung and nasal turbinate tissues were harvested to evaluate viral load by plaque assay.

Histopathology of post challenge mouse lungs

For each mouse the left lung was incubated in formalin at 4°C for at least 7 days to fix tissue and inactivate virus. The fixed tissue was processed and embedded in paraffin. 5 μm sections were cut and stained with either Congo red or hematoxylin and eosin (H&E). Eosinophils were enumerated by counting the Congo red positive leukocytes in 10 high power fields (400X final magnification) per mouse lung. Representative images were minimally and similarly adjusted with Adobe Photoshop 2020 to enhance contrast. Airway pathology was assessed in H&E stained sections to assess bronchial epithelial cell death (score: 0 = no dead cells, 1 = 1-5 dead cells, 2 = 6-10 dead cells, 3 = 11-20 dead cells and 4 = > 20 dead cells; scored for 10 400X fields per mouse lung), peribronchial inflammation (score 0 = none, 1 = 0%–25% circumference with > 1 leukocyte cell layer, 2 = 26%–50% circumference with > 1 leukocyte cell layer, and 3 = 50%–100% circumference with > 1 leukocyte cell layer; scored for 10 400X fields per mouse lung), and interstitial pneumonitis (score = percentage of pulmonary alveolar parenchyma with septae expanded by leukocyte; scored for 10 100X fields).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical details of experiments can be found in the figure legends. For NHP experiments, 2-6 sera samples were used and experiments were done in at least duplicate unless mentioned. For mouse ELISAs, neutralization, and challenge experiments, sera from 6, 8, or 10 BALB/c animals were used and experiments were completed in at least duplicate unless mentioned. Geometric mean titers were calculated. Kruskal Wallis tests were performed to compare two groups to determine whether they were statistically different for ELISA and neutralization experiments. Significance is indicated with stars: *, $p < 0.05$; **** $p < 0.0001$ and non significant groups are not shown.

Supplemental figures

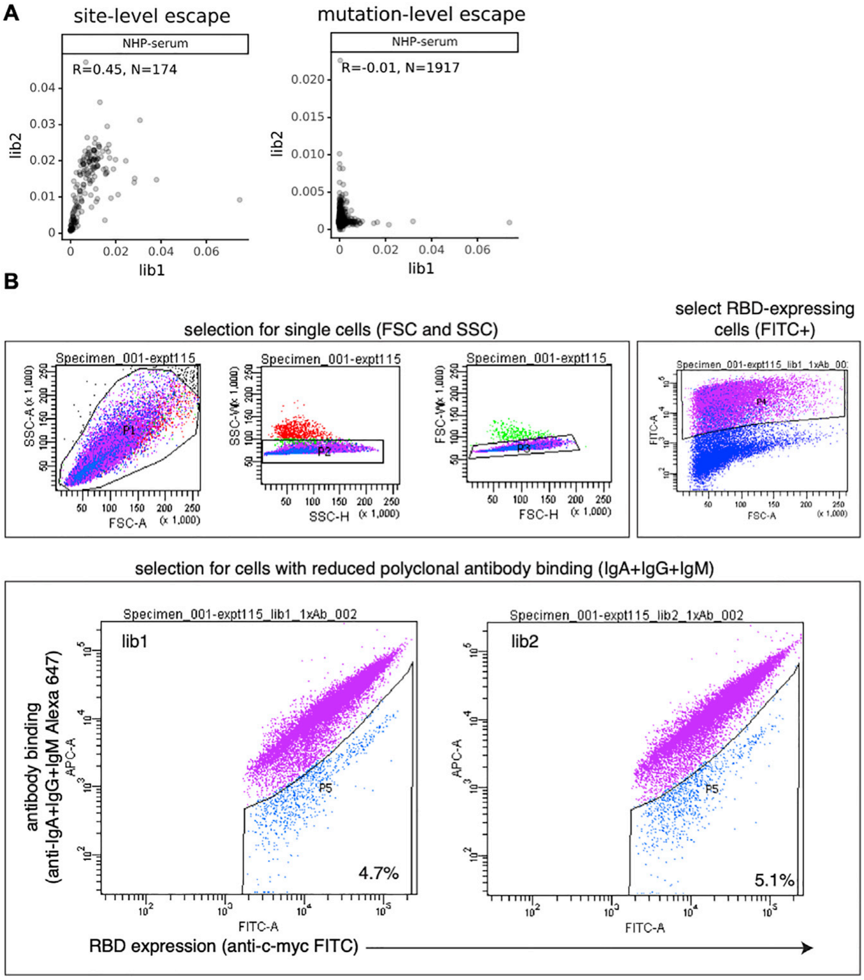

Figure S1. Effects of mutations on binding of HCP Abs to RBD and FACS gating strategy, related to Figure 2

(A) Correlation plots of site- and mutation-level escape for each of the two independent RBD mutant libraries for the Ab-escape map shown in Figure 2B. Site-level escape is the sum of the escape fractions for each mutation at a site. (B) Hierarchical FACS gating strategy used for selecting yeast cells expressing Ab-escape RBD variants. First, gates are selected to enrich for single cells (SSC-A versus FSC-A, and FSC-W versus FSC-H) that also express RBD (FITC-A versus FSC-A, cells in pink). Second, cells expressing RBD mutants with reduced polyclonal Ab binding, detected with an anti-IgA+IgG+IgM secondary Ab, were selected with a gate that captured the ~5% of cells with the lowest Ab binding (cells in blue).

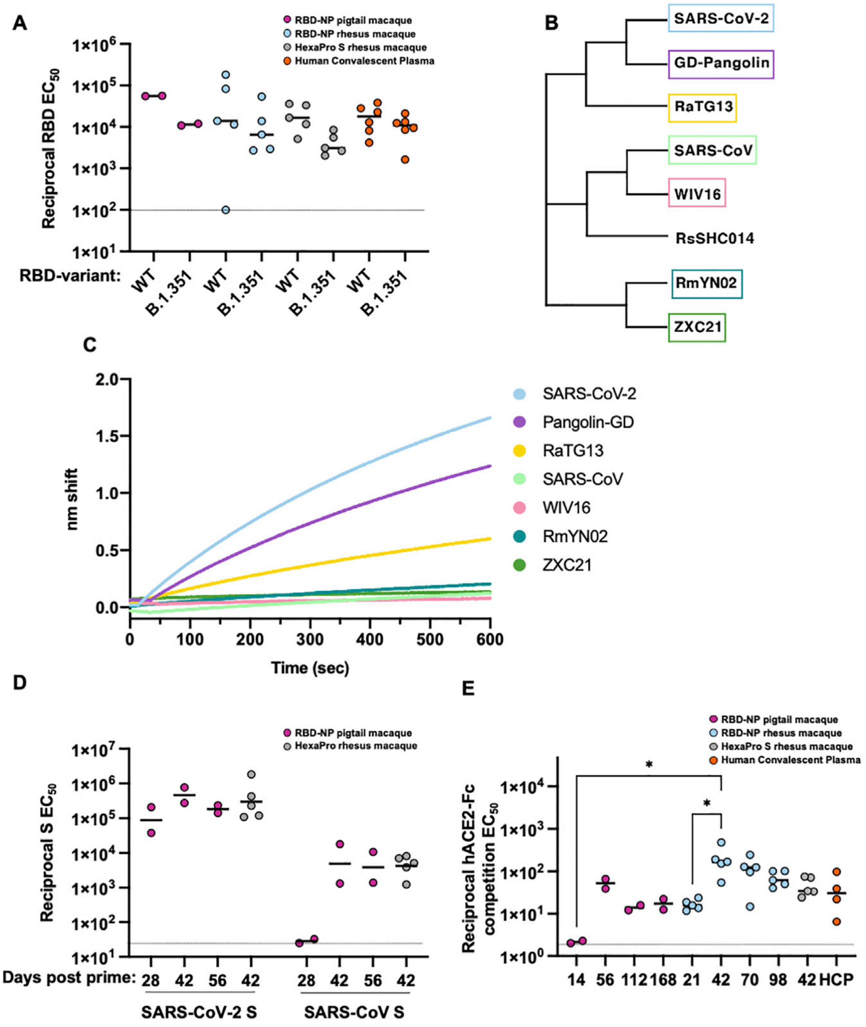

Figure S2. Evaluation of vaccine-elicited binding and neutralizing Ab titers against SARS-CoV-2 variants and sarbecoviruses, related to Figure 3

(A) Wild-type (Wuhan-Hu-1) and B.1.351 SARS-CoV-2 RBD-specific Ab binding titers of RBD-NP-elicited sera in pigtail macaques (magenta, n = 2) and rhesus macaques (blue, n = 5), HexaPro S-elicited sera in rhesus macaques (gray, n = 5), or HCP (orange, n = 6, Table S1) analyzed by ELISA with an LOD of 1×10^2. (B) Cladogram based on sarbecovirus RBD amino acid sequences. (C) Biolayer interferometry analysis of binding of 1 μM purified polyclonal pigtail macaque IgGs (obtained 70 days post prime) to sarbecovirus RBDs immobilized at the surface of biosensors. (D) SARS-CoV-2 S2P (left) or SARS-CoV S2P (right) Ab binding titers of RBD-NP-elicited sera in pigtail macaques (magenta) or HexaPro S-elicited sera in rhesus macaques (gray) analyzed by ELISA with an LOD of 2.5×10^1. (E) Competition ELISA between 0.13 nM human ACE2-Fc and RBD-NP-elicited sera in pigtail macaques (magenta) and rhesus macaques (blue), or HexaPro S-elicited sera in rhesus macaques (gray) at various time points following vaccination, benchmarked against COVID-19 HCP with an LOD of 4 × 10°, showing the magnitude of inhibition of ACE2 binding to immobilized SARS-CoV S2P expressed as reciprocal serum dilution blocking 50% of the maximum binding response. Statistical significance was determined by Kruskal-Wallis test and shown when significant. **, $p < 0.01$. All data repeated twice. LODs are shown as gray horizontal dotted lines. Raw data curves shown in Data S1.

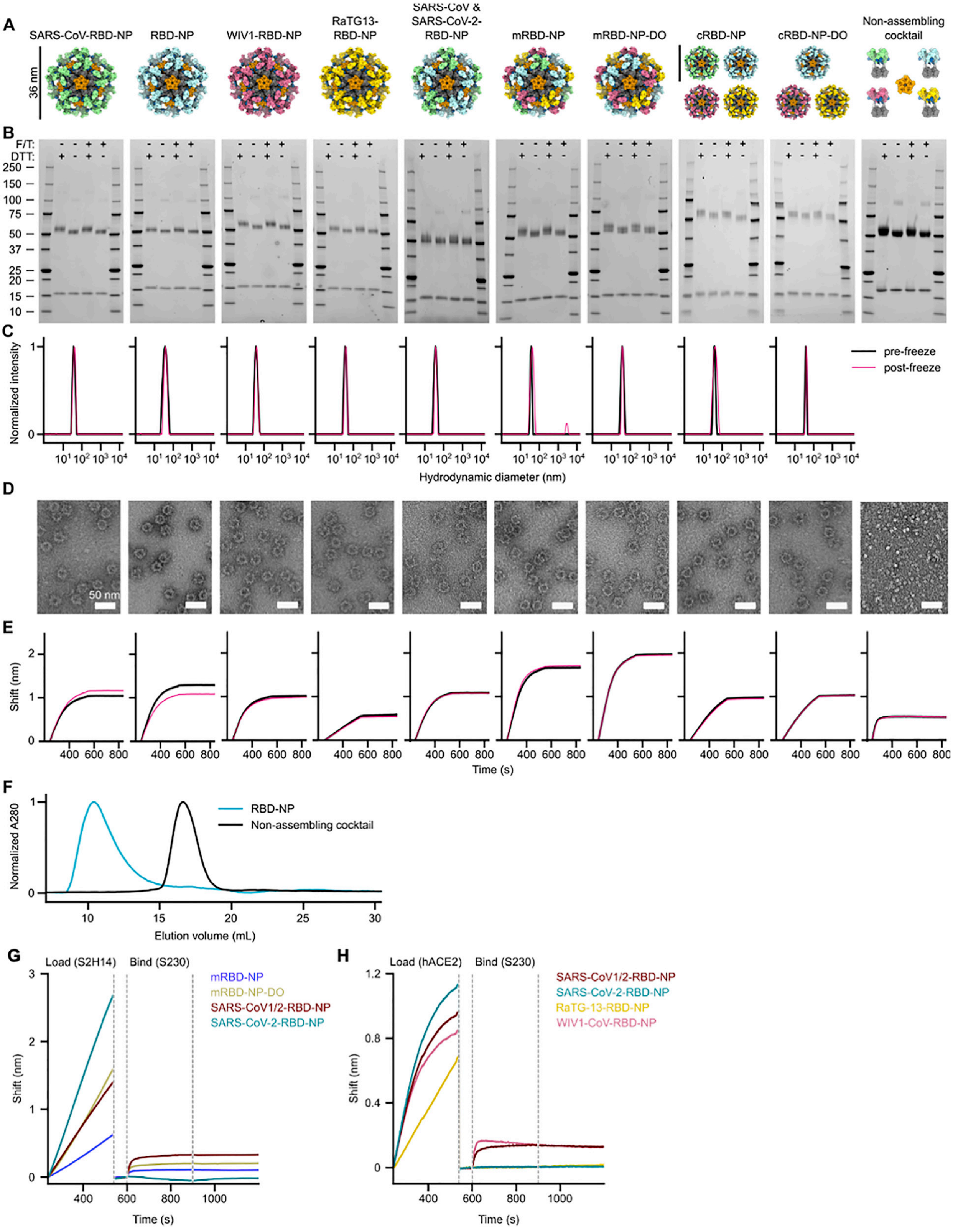

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Figure S3. ***In vitro*** **characterization and confirmation of co-display of sarbecovirus RBD-NP immunogens, related to Figure 4**

(A) Design models of the various vaccine candidates evaluated. Scale bars, 36 nm. (B) SDS-PAGE analysis of purified nanoparticles. DTT, dithiothreitol; F/T, freeze/thaw. (C) Dynamic light scattering. (D) Electron micrographs of negatively stained samples. Scale bars, 50 nm. (E) Binding of 100 nM SEC-purified nanoparticle immunogens and the non-assembling cocktail immunogen (which was not purified with SEC) to immobilized hACE2-Fc. (F) SEC chromatogram overlay of purified RBD-NP and non-assembling cocktail. (G-H) Sandwich biolayer interferometry. The SARS-CoV-2 S-specific mAb S2H14 immobilized on protein A biosensors was used to capture various nanoparticle immunogens from 300-480 s. The captured nanoparticles were subsequently exposed to a Fab derived from the SARS-CoV S-specific mAb S230 from 600-900 s (G). hACE2-Fc immobilized on protein A biosensors was used to capture various nanoparticle immunogens from 300-480 s. The captured nanoparticles were subsequently exposed to a Fab derived from the SARS-CoV S-specific mAb S230 from 600-900 s (H).

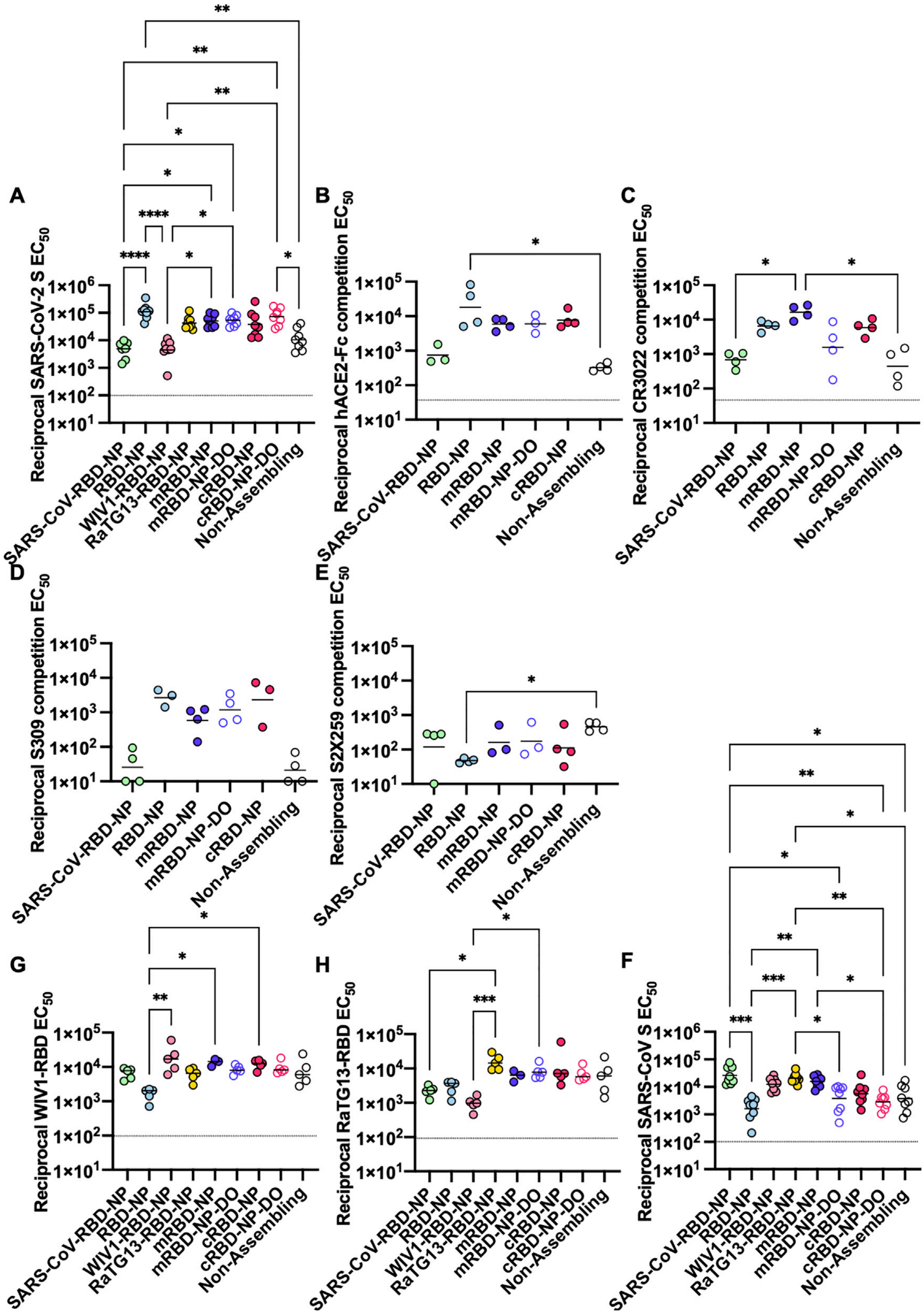

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Figure S4. Serum Ab binding titers elicited by mosaic and cocktail RBD-NPs, related to Figure 5

(A) Ab binding titers to SARS-CoV-2 S2P at five weeks post prime analyzed by ELISA with an LOD of 1x10^{2}. (B–E) Titers of SARS-CoV-2 S-specific Abs competing with ACE2-Fc with an LOD of 5x10^{1} (B), CR3022 with an LOD of 5x10^{1} (C), S309 with an LOD of 1x10^{1} (D), and S2X259 with an LOD of 1x10^{1} (E) in immunized mouse sera analyzed by competition ELISA. (F) Ab binding titers to SARS-CoV S2P at week 5 analyzed by ELISA. (G–H) Ab binding titers to the WIV1 (G), and RaTG13 (H) RBDs analyzed by ELISA with an LOD of 1x10^{2}. Statistical significance was determined by Kruskal Wallis test and shown when significant. **$p < 0.01$. LODs are shown as gray horizontal dotted lines. Raw data curves shown in Data S1.

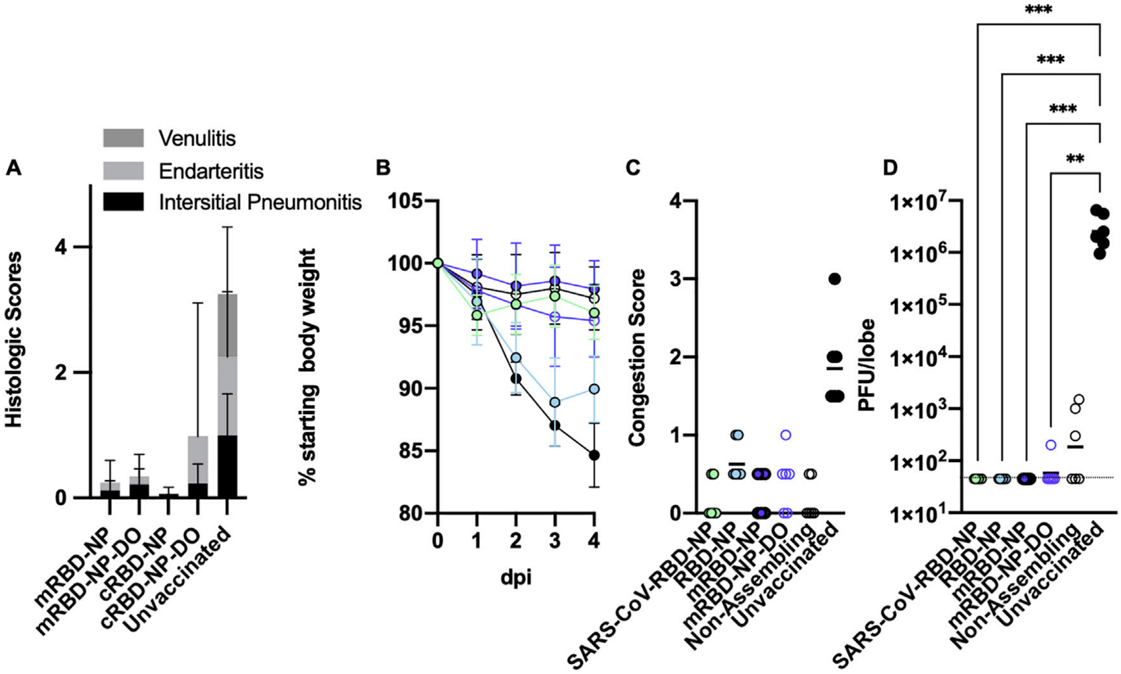

Figure S5. Monovalent, mosaic, and cocktail RBD-NPs protect against heterotypic SARS-CoV-MA15 challenge in 15-week-old BALB/c cByJ mice, related to Figure 6

(A) Normalized active inflammation following SARS-CoV MA 15 challenge shown in Figure 6 with venulitis, endarteritis, and interstitial pneumonitis shown as stacked bar graphs in dark gray, light gray, and black respectively. (B) Weight loss following SARS-CoV MA15 challenge (N = 6). Unvaccinated animals are shown as black circles. (C) Congestion score following SARS-CoV MA15 challenge with a score of 0 indicating unchanged lung color and 4 indicating a darkened and diseased lung (N = 6). (D) Viral titers in mice lungs (expressed in plaque forming units per lobe) following challenge (N = 8) with an LOD of $9x10^{1}$. Statistical significance was determined by Kruskal Wallis test and shown when significant and $^{**}p < 0.01$. LOD is shown as a gray horizontal dotted line.

From: Broder, Christopher on behalf of Broder, Christopher <christopher.broder@usuhs.edu>
To: Falo Jr, Louis D; Stefan Hamm; Victor Leyva-Grado; Andrianov; Korkmaz, Emrullah; twgeisbe@UTMB.EDU; Cross, Robert W.; Antony Dimitrov; Gambotto, Andrea; Moushimi Amaya; Boxley, Kimberly
Subject: NIAID funding new RFA
Date: Thursday, March 23, 2023 4:08:46 PM
Attachments: RFA-AI-23-020 RandD Vacs-mabs for Pandemic Prep-Bunya, Paramyxo and Picorna-U19.pdf
Veesler-SARS-nanoparticle-2021.pdf

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hi all,

thanks for taking the time for the call today on the sG Nipah/Hendra
patch/PCEP vaccine project.
It is looking really terrific.

Attached is the RFA from NIAID on Pandemic Preparedness
that our patch vaccine approaches can be integrated into.

I envision, continuation with NiV/HeV patch,
but the new area is doing Langya virus sG and/or Mojiang virus (both are the other henipaviruses
with different features. Perhaps also Ghana bat virus. All three have human infections, and/or evidence of human infections.
In parallel, we can propose reverse genetics rescue of these viruses for experimental pathogenesis studies.

The other vaccine method to continue, would be with nanoparticles which can also be explored with the patch. (attached)
David Veesler would potentially also be a structural bio Core for the submission. We are now testing
a pilot exp with the nanoparticle method now with David's group.

Bunyavirus (focus on Hantaan virus) can be integrated with either vaccine approach. We have a
pending submission for nanobody generation against these related viruses in collaboration with Tom and Bob at UTMB
with recombinant proteins produced by Genovac. Pete Leland at Genovac, has been able to make Langya Henipavirus soluble
F and G viral glycoproteins which we just received to take a look at. Genovac is also state of the art for
producing human and animal mAbs.

Looking forward to keep our collaborations going.

Chris

--
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BILL & MELINDA GATES foundation

INVESTMENT DOCUMENT – GRANT

Cover Page & Document Guidance

Welcome! The Investment Document is both your proposal shaping document, as well as your reporting document should your project receive funding. Before beginning, discuss the requirements for this document with your foundation contact for guidance and expectations on content, length, and level of detail.

Proposal Sections	Reporting Sections
Section A constitutes the grant proposal narrative. Typically, this section and the budget template (separate Excel document) are considered your proposal package.	**Section B** contains progress narrative questions to be completed for each progress reporting cycle defined in your grant agreement. **Section C** contains final narrative questions to be completed at the end of your grant.

Important Guidance:

- This document is not a commitment by the foundation to fund the work. If a grant agreement is executed, the grant proposal narrative (Section A) and budget will be incorporated therein by reference.
- To better understand the restrictions on foundation funding, please review the foundation's standard grant terms and conditions. This is not an exhaustive list and is subject to change. This list is provided for informational purposes only and does not imply an award, agreement, or offer to contract.
- This document must be submitted in English and as a Microsoft Word file. **PDFs and other file types will not be accepted**.
- **Privacy and Non-Confidentiality Notice:** The Internal Revenue Service (IRS) administers U.S. federal tax laws and requires that the foundation publish a list of its grants. We may also provide a general description of our grants and contracts on our web sites, in press releases, and in other marketing materials. Subject to the foundation's Privacy & Cookies Notice, the foundation may also share information you provide to us (either orally or in writing) with third parties, including external reviewers, key partners and co-funders. This document is subject to the foundation's Terms of Use.

If you have any questions or need support, please reach out to your foundation contact at the email listed on the next page.

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Use the hyperlinked sections below to navigate the document:

SECTION A – GRANT PROPOSAL

Investment Name	HTS for small molecule drug development for henipaviruses		
Investment ID	INV-048917		
Investment Owner	Rob Jordan	**Investment Owner Email**	robert.jordan@gatesfoundation.org
Investment Coordinator	Lisa Galaites	**Investment Coordinator Email**	lisa.galaites@gatesfoundation.org

INVESTMENT DETAILS

Estimated Grant Start Date	01 October 2022	**Grant End Date**	30 September 2025
Requested Grant Amount (USD)	$2,954,167	**Total Project Cost (USD)**	$2,954,167
Organization Legal Name[1]	The Board of Trustees of the University of Illinois		
Organization Doing Business As/Trade Name[2]			
Fiscal Agent[3] (if applicable)		**Fiscal Agent Contact Name** (if applicable)	
Tax Status (if known and applicable) Refer to Tax Status Definitions	509(a)(1) Public Charity	**U.S. Employer Identification Number (EIN)** (if applicable)	37-6000511
Mailing Address		**Primary Contact Name**	Karen McCormack
Street Address 1	MB 502, M/C 551	**Primary Contact Title**	Executive Director, Office of Sponsored Programs
Street Address 2	809 S. Marshfield Avenue	**Primary Contact Email**	Awards@uic.edu
Street Address 3		**Primary Contact Phone**	312-996-2862
City	Chicago	**Additional Contact**	
State / Province	Illinois	**Additional Contact Title**	
Zip / Postal Code	60612-4305	**Additional Contact Email**	

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[1] Legal Name will be used in the agreement and should match the name on the bank account that receives the grant funds (assuming fully executed agreement).
[2] Trade Name or d/b/a ("doing business as") only required if different from Legal Name.
[3] A fiscal agent is a third-party organization that is not the grantee. The fiscal agent, although not the grantee, signs the grant agreement in addition to the grantee and will receive the grant payments.

Country	U.S.A.	**Authorized Signer Name**	Paul N. Ellinger
Website (if applicable)	https://www.uic.edu	**Authorized Signer Title**	Interim Comptroller
		Authorized Signer Email	Awards@uic.edu
Organization's Fiscal Year End	06/30	**Organization's Total Revenue for Most Recent Fiscal Year (USD)**	$
Proposal Submitted By (name)	Lijun Rong	**Proposal Submitted Date**	24/07/2022

Charitable Purpose - completed by the foundation

❖ The following describes the charitable purpose of this project. It is written in a standard format by your foundation contact for publication on the foundation's public website should your project receive funding.

to identify novel agents targeting henipavirus replication for pandemic antiviral preparedness

Strategic Fit - completed by the foundation

❖ The following includes the foundation's 3-5-year strategic goal(s) towards which this project contributes most directly.

The goal of this proposal is to develop fast-acting, orally active small molecule therapeutic agents with an effective mechanism of action that could be stockpiled for any future pandemic. This program will use high throughput screening technologies to identify inhibitor compounds of cedar virus replication and counter screen against other paramyxoviruses to select compounds with broad spectrum antiviral activity. This project is one of several screening programs designed to identify new chemical matter for paramyxovirus antiviral development and is part of the portfolio being developed by the Pandemic Antiviral Discovery (PAD) Initiative to identify antiviral candidates targeting paramyxoviruses that can be deployed in an outbreak setting for pandemic preparedness.

Project Description

Note: this section may be pre-populated with your foundation contact's initial draft, please expand upon and/or continue to refine.

In this section, please describe the proposed work, including a clear outline of the project plan, intended results, time frame, funding considerations and how you and your organization will work to further educational, scientific, or other charitable purposes and impacts for the intended beneficiaries. Please be sure that your project description is sufficiently detailed and includes responses to the below questions:

- What are the primary results (outcomes and outputs) this project will achieve or significantly contribute to? How will you know when the results have been achieved? How will the results be measured?
- How will your management of the project and the intended outcomes align with the Charitable Purpose, Strategic Fit, and the foundation's Global Access requirements?
- Describe potential risks/challenges to the success of this project and how you plan to address them. Include any external factors or critical relationships with other partners/projects that may influence the success of this project (including any anticipated agreements to be entered into for purposes of the project).
- Describe any changes or improvements you plan to make to your organization's capacity to achieve the results of the project.
- Beyond this project's time frame and funding, what factors (financial/economic, organizational, behavioral, etc.) are required to sustain or build on the project's results?

Nipah virus is highly pathogenic human paramyxovirus that is considered a major pandemic threat pathogen. The virus is transmitted to humans through contact with infected bats or domestic animals that serve as amplifying hosts and infection results in both respiratory and neurological symptoms resulting in high mortality. There is documented evidence of person-to-person transmission and long term asymptomatic viral persistence that can lead to lethal disease in those who survive primary infection. Related paramyxoviruses (RSV, PIV, HMPV, measles) cause seasonal infections with low mortality but are highly transmissible via the respiratory route. Evolution of henipavirus to acquire the ability for efficient human-to-human transmission poses a major concern for pandemic preparedness.

A high throughput phenotypic pilot screen of 10K compounds identified two hit series that will be further optimized for potency and drug-like properties. Optimization of hits that target the viral polymerase will be supported by cryoEM studies designed to solve the polymerase-inhibitor complex structure. In addition, an extended HTS of new compound libraries will be conducted using a reporter Cedar virus expressing the luciferase gene (CedV-Luc) to identify additional hit compounds for optimization. Promising hit compounds will undergo a thorough characterization process including initial chemical exploration and antiviral validation in dose response titrations using NiV-B and HeV assays to quantify potency and determine the magnitude of the antiviral effect.

Specific Aims:

Aim 1A: Identify additional chemotypes that inhibit henipavirus replication by high throughput screening. A high throughput screen of a compound library will be conducted using the CedV-Luc construct. Active compounds will be evaluated in dose response experiments and virus yield assays in multiple cell types to determine the potency and magnitude of the antiviral effect.

Aim 1B: Optimize furopyrimidine and 4-aminoquinazoline scaffolds to improve druglike properties. Medicinal chemistry optimization of two series identified from a pilot high throughput screen of 10K compounds will be conducted. Optimization will be facilitated by solving the structure of the henipavirus polymerase in complex with key inhibitors by cryoEM techniques (Aim 2).

Aim 2: Investigate the mechanism of action (MOA) of lead inhibitors. We will use resistance selection assays and an SPR binding assay for target identification. High-resolution structures of the NiV RdRp complex with key inhibitors will be obtained by cryoEM. These structures will provide necessary structural information to facilitate lead optimization.

Aim 3: *In vivo* analysis of lead compounds in animal models of nipah virus replication in BSL-4 containment. Promising candidates with appropriate ADME and PK properties will be evaluated in animal models of Nipah virus replication conducted in BSL-4 containment at UTMB.

Milestones:

1. Complete HTS to identify antivirals active against Cedar virus and validate them against authentic NiV and HeV. (Year 1)
2. Optimize 4-aminoquinazoline and furopyrimidine series to late lead stage (Year 3)
3. Solve structure of henipaivirus polymerase complexes with key inhibitors by cryoEM (Year 2)
4. Evaluate at least nine lead compounds in a hamster model of nipah virus infection (Year 2)
5. Evaluate at least two advanced lead compounds in an African Green monkey model of nipah virus infection (Year 3)

Deliverable:

1. 1-2 small molecule compound series active across the henipavirus subfamily that can be optimized through medicinal chemistry.
2. 1-2 late lead compounds optimized from the 4-aminoquinazoline and furopyrimidine series active in animal models of nipah virus replication advanced for IND-enabling studies.
3. Structure of the henipavirus polymerase complex with key inhibitors.

Risks and Risk Mitigation

1. There is a possibility that the HTS campaign will not yield viable hits that can be advanced into hit-to-lead optimization. While adjustments to the screening assay may yield more hits, the investigators should consider screening additional libraries. (Multiple ready-to-use chemical libraries to facilitate drug discovery and drug target identification are curated at the UIC Research Resource Center (RRC). They are comprised of > 150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional “Antiviral Library” from ChemDiv, composed of 65,937 compounds total, will be purchased by the Rong Lab for this project).

2. Lead optimization of the 4-aminoquinazoline and furopyrimidine may uncover terminal liabilities that can not be fixed by medicinal chemistry. While there is always inherent risk with drug discovery, conducting additional screens for new chemical matter can help mitigate this risk.
   (If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy using a different reaction sequence. Should we encounter a scaffold-related problem with our furopyrimidine scaffold, we will switch to our backup quinazolin-4-one scaffold or other HTS hits identified using the expanded libraries of compounds. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc., and Pharmaron, Inc. and supervised by Dr. Peet. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim. We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable advanced lead compounds, which will be advanced to *in vivo* studies and will allow for the future

selection of a preclinical candidate.)

3. Optimized compounds do not inhibit nipah virus replication in animal models. While not ideal, a negative result can help inform the field and guide medicinal chemists to improve molecules for this indication.
(A critical aspect of antiviral discovery that is often overlooked is the early-stage testing of lead compounds in well-studied and well-validated in vivo animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster and AGM. *In vivo* studies in hamsters will be initiated in Year 1)

4. PI and collaborators are AViDD grant recipients so clear delineation of their roles in these projects is essential to prevent duplication of effort.

Drs. Rong and Gaisina are Co-Is on the National Institutes of Health (U19 AI171954) grant titled: “Midwest Antiviral Drug Discovery Center”(PIs: Reuben Harris and Fang Li); and their specific roles on the Project 1 (“Small molecule viral entry inhibitors”) are to develop small molecule entry inhibitors against Ebolaviruses. The lead series of compounds are not related to the henipavirus lead compounds proposed here. No overlap.

Team: Dr. Lijun Rong (PI) is a Professor in the Department of Microbiology and Immunology at UIC, with expertise in mechanisms of viral entry and antiviral drug discovery. Dr. Rong will be responsible for the overall project and coordinate the assembled team for the proposed experiments. Dr. Irina Gaisina (Co-I) is a Research Associate Professor in the Department of Pharmaceutical Sciences and UICentre at UIC. She has extensive experience in hit-to-lead and lead optimization medicinal chemistry and preclinical drug development. She will assume overall responsibility for the medicinal chemistry work carried out at UIC. Dr. Thomas Geisbert (Co-I), a Professor of Microbiology and Immunology at UTMB, with expertise in highly pathogenic viruses and his group will perform the in vitro and in vivo studies using infectious henipaviruses in a BSL-4 facility. Dr. David Veesler (Co-I), an Associate Professor of Biochemistry at UW and HHMI investigator, is a world leader in structural studies of viral glycoproteins and will carry out cryoEM and X-ray crystallographic studies of the most potent inhibitors in complex with the NiV or HeV polymerase. Dr. Christopher Broder (Collaborator) is a professor and Chairperson of the Department of Microbiology and Immunology, USU, and he is one of the leading experts in the development of therapeutic agents for Nipah virus and Hendra virus. Dr. Norton P. Peet (Consultant), Chief Scientific Officer for Chicago BioSolutions, Inc., is a medicinal chemist who has over 30 years of experience with preclinical development of small molecules as therapeutic agents and their advancement to a clinical setting, working with both multinational pharmaceutical and biotechnology companies. He will oversee the proposed ADME/Tox and PK studies.

A. BACKGROUND AND SIGNIFICANCE

For several decades, the major agents of emerged or newly identified infectious diseases in humans have been viruses of zoonotic origins.[10-12] Among these, two paramyxoviruses of importance are Nipah virus (NiV) and Hendra virus (HeV); the prototype species of the genus *Henipavirus* and are single-stranded, negative sense, enveloped RNA viruses.[13] HeV appeared in 1994, in an outbreak of severe respiratory disease in horses in Australia, with the death of 14 horses and their trainer.[14,15] NiV (Malaysia strain, NiV-M) appeared in an outbreak of encephalitis in pig farmers in Malaysia in 1998.[16,17] NiV outbreaks have since been seen in Bangladesh and India by a related strain NiV-Bangladesh (NiV-B).[18] Pteropid fruit bats are the major reservoir hosts for henipaviruses.[6,19] NiV and HeV have broad host tropism, infecting at least 18 species across 6 orders of mammals. Among these, NiV and HeV will cause a systemic and often fatal respiratory and/or neurological disease in at least 11 species including humans, with human fatality rates ranging from 40-100%.[2,20] A third henipavirus isolate, Cedar virus (CedV), was discovered in 2010.[21] However, CedV is not known to be zoonotic and has been shown to be nonpathogenic in HeV and NiV animal models.[21-23] Two additional henipaviruses are also known, Ghana bat virus and Mojiang virus, but only from sequence data.[24-26] Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and 3 outbreaks in India.[27] In 2014, an outbreak of NiV-M occurred in the Philippines with 9 fatalities of 11 human cases, with horse-to-human and human-to-human transmission.[28] The combined NiV case fatality rate is ~60% across a large region of Asia in five countries.[3] The recent 2018 NiV outbreak in Kerala, India was significant, occurring in a new geographic region, with a case fatality rate of 91% and 22 of 23 cases the result of from human-to-human transmission. Most recently, a fatal case of NiV-B in Kerala occurred in September of 2021 reigniting the outbreak concerns associated with this deadly zoonotic viral agent, underscoring both the transmissibility and the lethal nature of this virus.[4] In the latter and most recent case, an eleven-year-old boy was infected with Nipah virus and he succumbed, along with three health care workers who were in close contact.

Human NiV/HeV infection is generally accepted to occur via the oronasal route with an incubation period of 1 to 2 weeks.[4,29] Acute NiV/HeV infection in people is systemic and likely via hematogenous viral spread from the respiratory system.[30] Disease onset is characterized by fever, myalgia, shortness of breath, and cough.[15,31] Human HeV infections have resulted in both fatal respiratory or encephalitic disease, and also recovery.[29,32] The predominant clinical feature in the Malaysia outbreak was encephalitis, but respiratory symptoms were also common.[31,33] The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease and in the 2018 NiV-B outbreak in Kerala, 83% of cases had acute respiratory distress syndrome (ARDS).[4,34] The key findings of human NiV/HeV infection are wide-spread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected.[35,36] Human NiV/HeV infection can also be prolonged following apparent recovery, and some patients can experience a late-onset or relapsed encephalitis.[32,37] Relapsed encephalitis caused by NiV appears to be a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later.[38] NiV and HeV are biosafety level-4 (BSL-4) viruses and classified as Category C priority pathogens and transboundary agents by several US agencies. Also, both NiV-M and NiV-B can cause lethal infection in nonhuman primates (NHPs), specifically the African green monkey (AGM), when delivered by small particle aerosol.[39-41] NiV and henipaviral diseases (HeV) are included on the WHO’s Blueprint List of Priority Pathogens.[42] NiV is a potential pandemic threat because of its high case-fatality rates, capacity for human-to-human transmission and potential to mutate, and a human-adapted strain in a region of high population density could lead to rapid spread.[43] *There are presently no approved vaccines or therapeutic modalities for NiV/HeV infections in people.*

The viral RNA-dependent-RNA polymerases of paramyxoviruses are attractive targets for antiviral compound discovery, but antiviral drug discovery for NiV and HeV has been hampered by requirements of BSL-4 containment. We recently developed and optimized an authentic henipavirus replication cell-based system using a recombinant CedV (rCedV) encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications at the BSL-2 containment level (**Figure 1**) and identified several inhibitory compounds that were further validated with authentic NiV-B and HeV.[7] Those leads provide the rationale for the current application. Our ability to employ rCedV provides an opportunity for anti-henipavirus drug discovery not previously available, that also allows for discovery across the whole replication cycle, not restricted to only the viral polymerase. Another well-exploited stage of virus infection is the attachment and entry process.[44] NiV/HeV possess two membrane anchored glycoproteins required for infection; one for attachment, the G glycoprotein, and the other for membrane fusion of the F glycoprotein.[45] The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and all vaccine strategies.[27]

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of the investigators in this proposal. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may well have activity across related members of the henipavirus genus. *Second,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification. *Third*, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity among small molecules.

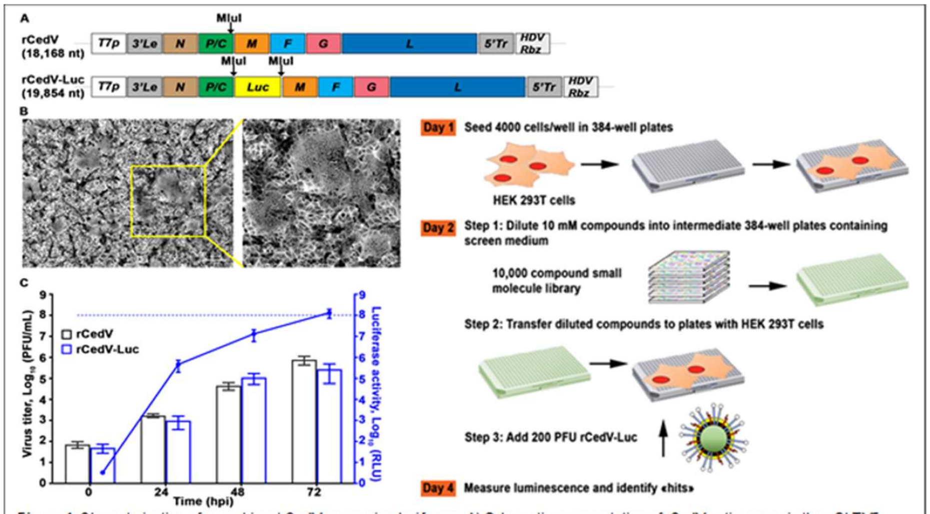


Figure 1. Characterization of recombinant CedV expressing luciferase. A) Schematic representation of rCedV antigenome in the pOLTV5 vector. The firefly luciferase (*Luc*) gene was inserted at the unique MluI restriction site between the *P* and *M* genes to produce rCedV-Luc. To preserve the "rule of six", an additional start codon was added to the 5' terminus of the *Luc* gene. B) At 48 hpi Vero E6 cells infected with rCedV-Luc were fixed with methanol and stained with 0.5% crystal violet solution (80% methanol). Images were captured with a Zeiss Axio Observer A1 inverted microscope using a 5X objective. Yellow box indicates giant cells, and scale bar represents 50 µm. Inset indicates zoomed-in region within the yellow box. C) Vero E6 cells in a 96-well plate were infected with either rCedV or rCedV-Luc at an MOI of 0.01. At 0, 24, 48 and 72 hpi supernatants were collected and viral titers determined by plaque assay and calculated as PFU/ml. Concurrently all infected cells were lysed and relative light units (RLU) were measured and calculated by subtracting the signal of rCedV infected cells from the signal of the rCedV-Luc infected cells and are represented on the right Y-axis in blue. The data represent mean ± standard error from two independent experiments. The blue dashed line represents the upper limit of detection of the luminometer. Viral titers and luciferase activity levels at 0 hpi indicate the lower limit of detection for the plaque assay and the luminometer, respectively. D) HTS workflow. Compounds diluted in medium were added to pre-seeded HEK 293T cells in a 384-well plate, followed by addition of rCedV-Luc (MOI: 0.05). Luminescence was measured 48 h later.

C. PRELIMINARY RESULTS

Identification of small molecule inhibitors of henipaviruses.

C.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2), cell-based HTS platform to identify small molecule antiviral agents targeting henipavirus replication without the requirement for BSL-4 containment (**Figure 1**).[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with thoset of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera using guidelines and procedures at the BSL-2 level were reviewed and approved by the Institutional Biosafety Committee (IBC) in accordance with NIH guidelines.

C.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from a ChemDiv library by UIC medicinal chemists and called "SMART Set") was used for a pilot HTS campaign to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative compounds for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 µM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 µM, and 47 hits showed only minimal cytotoxicity at 33 µM. Importantly, several small molecule inhibitors (**Figure 2**) have been validated *in vitro* using infectious HeV and NiV in the BSL-4 facility, demonstrating the proof-of-concept feasibility of using this rCedV-based surrogate HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for the current drug discovery project. Particularly, our quinazoline-based hit **Z1** and furopyrimidine-based hit **Z2** were found to have EC_{50} values of 3.3 and 0.5 µM, respectively, against NiV-B (**Table 1**). Note that both HEK293T and Vero cell lines were employed in these assays, and that CC_{50} values were recorded for these two cell lines. Thus, <u>we concluded that our HTS approach was a valid method for defining NiV inhibitors</u>. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5. These hits showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins (0% inhibition at 12.5 µM). In addition to the high potency and selectivity, **Z2** displays excellent *in vitro* metabolic stability. The percentage of compound remaining after 60 minutes of incubation in both human plasma and liver microsomes is significant (91 and 68%, respectively), indicating good stability in both media. Stability in hamster plasma and liver microsomes will be evaluated by Pharmaron Inc.

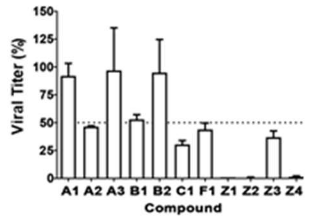
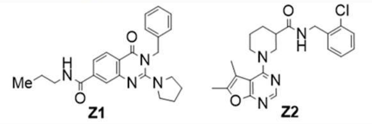


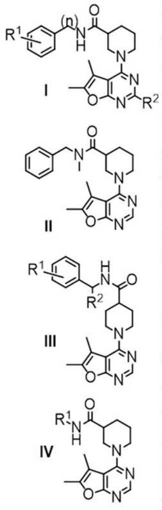
Figure 2. Inhibition of NiV-B replication in Vero 76 cells measured by virus titer reduction assay (% infection relative to the DMSO control).

Table 1. Validation of rCedV-based HTS assay hits for inhibition of NiV_B.



Hit	CedV (293T)		CedV (Vero)		NiV_B (Vero)
	IC_{50} (µM)	CC_{50} (µM)	IC_{50} (µM)	CC_{50} (µM)	IC_{50} (µM)
Z1	0.70	100	2.1	100	3.3
Z2	0.78	37	13.2	100	0.5

Table 2. Antiviral activity of furopyrimidines in the CedV-luc reporter virus assay in Vero 76 cells



	Core	R1	R2	n	EC_{50} (µM)*	CC_{50} (µM)
1	I	2-Cl	H	1	1.52 ± 0.66	>100
2	I	3-OMe	H	1	5.73 ± 1.95	>100
3	I	4-F	H	1	3.38 ± 0.23	>100
4	I	4-SMe	H	1	7.27 ± 2.14	>100
5	I	3,4,5-*tris*-OEt	H	1	10.03 ± 2.09	>50
6	I	2-Cl	H	2	1.87 ± 0.05	>100
7	I	3-Cl	H	0	1.73 ± 0.35	>100
8	I	$3-CF_3$	H	0	1.66 ± 0.08	>100
9	I	2-Me, 4-F	H	0	0.87 ± 0.001	>100
10	I	2-Br, 4-Me	H	0	6.24 ± 1.53	>100
11	I	3,5-*di*-OMe	H	0	1.66 ± 0.08	>100
12	I	3,4-methylene dioxy	H	1	0.94 ± 0.22	>100
13	I	2-Cl	Me	0	14.11 ± 2.08	>100
14	II	-	-	-	1.18 ± 0.001	>100
15	III	2-Cl	H	1	15.01 ± 2.51	>100
16	III	3-Cl	H	1	9.22 ± 2.34	>100
17	III	H	Me	-	3.32 ± 0.94	>100
18	IV	*c*-hexyl	-	-	7.89 ± 0.06	>50
19	IV	*i*-Pr	-	-	13.21 ± 2.49	>50

* Compounds were 3-fold serially diluted in cell culture medium containing 2% DMSO from 200 µM to 0.09 µM. Compounds (50 µL) were added to pre-seeded Vero 76 cells followed by rCedV-Luc (MOI: 0.01) (50 µL) and incubated for 48 hours at 37 °C. Luminescence was measured using the Steady Glo Assay Reagent

In **Table 2** are shown additional inhibitors in the furopyrimidine series that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). We do not yet fully understand the emerging SAR around substituents included on the phenyl ring, the tether length, and substituents on the tether and the pyrimidine ring, but we can make some comments. We do see activity with all three of the tether lengths studied, where n=0, 1 or 2. Replacement of the tether and the phenyl group with large hydrophobes, such as isopropyl or cyclohexyl, is not productive. Comparison of compounds **13** and **6** indicates that a methyl group on the pyrimidine ring is detrimental to activity. A methyl group on the sidechain in the benzyl subset of compounds appears to be tolerated; however, this inclusion adds an asymmetric center to the molecule. Disubstituted compounds **9** (n=0), **11** (n=0) and **12** (n=1) represent a subset of compounds that are of higher affinity, which suggests that we should explore more compounds with two diverse phenyl substituents with all tether lengths.

In the Research Plan we discuss methods for the preparation of new compounds in each series as well as specific plans for developing the SARs. Our recombinant CedV HTS system will be used to support Aim 1.

D. RESEARCH DESIGN AND METHODS

<u>D.1. Aim 1: Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays.</u>

D.1.1. Synthesize diverse analogs of the furopyrimidine and quinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 µM) and low cytotoxicity (CC_{50} >100 µM).

Chemical optimization and SAR studies. In the initial stages, we will keep the heterocyclic core of the molecules of interest constant while making well-defined changes in substituents. We will initially probe steric and electronic factors of the substituents, but more substantial changes in the scaffold will be investigated as the structure is optimized. By an iterative process of design, synthesis, and assay, we will improve the structure, and ultimately produce a fully optimized structure. We will also assess the cytotoxicity and construct Structure-Toxicity Relationships (STRs). For potent, specific inhibitors, the SAR and the STR will diverge at critical points, and we will synthesize novel inhibitors with maximized activity and minimized cytotoxicity. The SAR plan for optimization of the HTS hit **Z2** is exemplified in **Figure 3**. Substituent changes on the core fused scaffold will involve variation of the alkyl groups on the furan ring, as well as adding alkyl groups at the 2-position of the pyrimidine ring, to explore the optimum overall size for the core. Note that we will be able to use these substituents as “ADME handles” to control the overall hydrophobicity of the molecules and the cLogP values. We will replace the oxygen atom in the furan with a sulfur atom to compare the effect of changing the furan ring to the larger thiophene ring. Importantly, we will explore the biological activities of the *R*- and *S*-stereoisomers of this racemic molecule to determine whether there is differential recognition of these antipodes at the site of interaction in the inhibition assays. In addition to evaluating a variety of mono- and disubstituted aromatic rings, we will also explore length of the tether connecting these aromatic units to the amide nitrogen atom, knowing that both benzyl groups and phenyl groups give rise to active compounds.

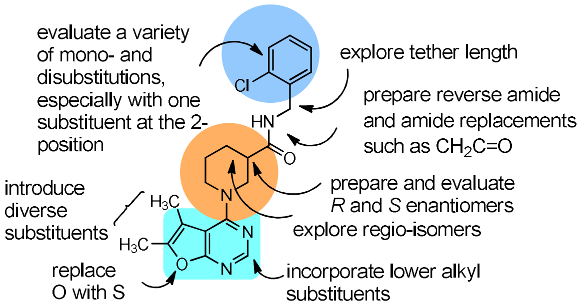
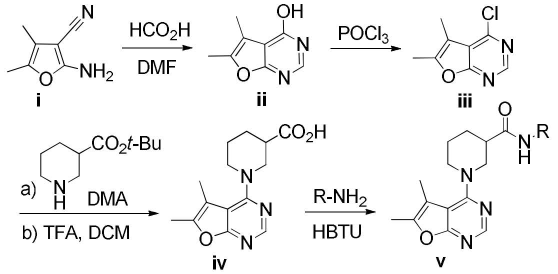


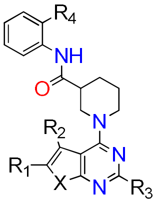
Figure 3. SAR development plan for furopyrimidine **Z2**.

Scheme 1. Preparation of analogs of furopyrimidine **Z2** with optimized amide *N*-substituent.



The synthetic route that we envision for the preparation of the furopyrimidines is shown in **Scheme 1**, as illustrated for the optimization of the amide *N*-substituent for furopyrimidine analogs of compound **Z2**. Starting with the commercially available 2-amino-4,5-dimethyl-3-furancarbonitrile (**i**), treatment with formic acid in dimethylformamide will produce the 5,6-dimethylfuro[2,3-*d*]pyrimidine (**ii**). Conversion of **ii** to the corresponding chloro compound **iii** is accomplished by treatment with phosphoryl chloride. Displacement of the chloro group with piperidine-3-carboxylic acid *t*-butyl ester, followed by removal of the *t*-butyl ester with trifluoroacetic acid, gives key intermediate **iv**, which can then be elaborated to amides **v**.[46] In **Table 3** is shown a set of proposed compounds related to **Z2** (compound **A**) for which we show parameters calculated using Actelion OSIRIS software[47]. All of the proposed compounds (**B** through **H**) show improved druglikeness and drug scores with respect to **Z2**, and therefore represent promising new targets for synthesis and evaluation.

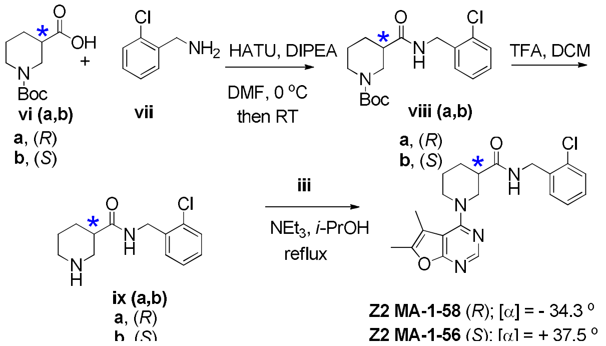
Table 3. Actelion OSIRIS calculated parameters for **Z2** (compound A) and proposed analogs.



ID	R_1	R_2	R_3	R_4	X	cLogP	Sol	MW	Druglikeness	Drug Score
A	Me	Me	H	Cl	O	4.0	-6.7	384	-0.95	0.23
B	Me	Me	H	Cl	S	4.4	-6.8	400	2.01	0.32
C	H	H	H	Cl	O	3.3	-6.0	356	3.04	0.42
D	H	H	H	F	O	2.8	-5.6	340	1.47	0.55
E	H	H	H	Cl	S	3.6	-6.0	372	3.70	0.41
F	H	H	H	F	S	3.1	-5.5	356	2.14	0.54
G	H	H	Me	F	O	3.0	-5.2	354	2.82	0.61
H	H	H	Me	F	S	3.3	-5.2	370	3.48	0.60

We recently synthesized the *R* and *S* enantiomers of compound **Z2** (**MA-1-58** and **MA-1-56,** respectively) using the sequence shown in **Scheme 2**. Thus, starting with the optically active nipecotic acids **vi(a)** (*R*) and **vi(b)** (*S*), coupling with 3-chlorobenzylamine (**vii**) using a standard amide coupling procedure yielded amides **vii(a)** and **viii(b)**, which were then subjected to deprotection conditions to give the free piperidines **ix(a)** and **ix(b)**. Treatment of **ix(a)** and **ix(b)** with chloro compound **iii** in the presence of triethylamine in isopropanol at reflux produced the two final epimeric target compounds **Z2 MA-1-58** and **Z2 MA-1-56**, respectively. As expected, these two epimers rotated the plane of polarized light in opposite directions, to approximately the same degree, as shown in **Scheme 2.** Note that these two epimers have just been prepared and we have not yet tested these most interesting compounds to determine whether there is differentiated recognition of these epimers at their site of action.

Scheme 2. Synthesis of *R*- and *S*-enantiomers of **Z2**.



As cryo-EM structures of polymerase in complex with key inhibitors (i.e., **Z2** and additional derivatives) become available, we will use this model for computational chemistry/docking studies to assist in the design of additional analogs with improved binding properties.

In addition, we have plans to introduce a diversity of substituents in the furan ring, as shown in **Scheme 3**. Using a variety of alpha-bromoketones **x(a-e)** in a condensation rection with malononitrile (**xi**),[48] we will produce furans **xii(a-e)** and use them in our one-pot protocol for preparing furanopyrimidines **xiii(a-e)**. We will then elaborate compounds **xiii(a-e)** to the state of target compounds **xiv(a-e)** as previously described in **Schemes 1-2**. This chemistry will allow us to study the installation of a variety of substituents in the furan ring and to evaluate the consequences of these changes to the biological activity.

Also, the furo[2,3-*d*]pyrimidine moiety will be replaced with the heterocyclic cores showcased in **Figure 4**. Although we will not show the chemistries that we will employ for the preparation of these additional fused ring systems that are bioisosteric with our hit series of furopyrimidines in this document, we have researched methods for the synthesis of these additional cores and are quite confident that we will be able to prepare representatives of these ring systems. Importantly, we will use the knowledge we have gained in developing the SARs with our furopyrimidines, and we will prepare selected derivatives in the additional 5,6-fused systems, based on the biological activities shown with substitutions present on our most active furopyrimidines.

Figure 4. Additional core modifications.

In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. We have also researched and found additional synthetic methods available for the construction of both 2,3,7-trisubstituted quinazolin-4-ones and an additional backup scaffold of 4-aminoquinazolines (**Table 4**).

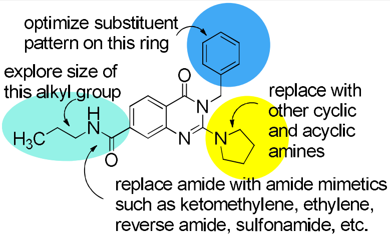
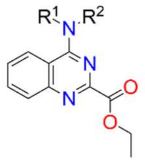


Figure 5. SAR development for **Z1**.

Table 4 shows eight additional inhibitors in the aminoquinazoline series, that we identified from a recent focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 4**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **24** (**Table 4**), bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **24** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the *n*-propyl compound **16** and *iso*-propyl-phenyl compound **18** are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, it may be worth exploring with a larger set of derivatives. In **Figure 6** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **27**. Substituent changes on the core fused scaffold will involve exploration of mono- and di-substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we will optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. We will also optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 4** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **xv** is heated with diethyl oxalate in a solution of dimethylformamide to produce 2-carboethoxyquinazoline-4-one (**xvi**) in a condensation/cyclization process. Treatment of **xvi** with

Table 4. Antiviral activity of 4-amino-quinazoline-2-carboxylates **20-27** in the rCedV luciferase reporter virus assay.



N	R1	R2	% inhib at 10 μM
20	H	3-chloro-4-methoxyphenyl	81
21	H	2,4-difluorophenyl	18
22	H	2-chloro-4-fluorophenyl	58
23	H	3-methoxyphenyl	46
24	Me	cyclohexyl	93
25	H	4-isopropylphenyl	85
26	*n*-propyl	4-fluorophenyl	86
27	Me	2-methylphenyl	94

Compounds (50 μL, 10 μM final concentration) were added to pre-seeded Vero 76 cells followed by rCedV-Luc (MOI: 0.01) (50 μL) and incubated for 48 hours at 37 °C. Luminescence was measured using the Steady Glo Assay Reagent phosphorous oxychloride next provides a reactive intermediate, namely, 2-carboethoxy-4-chloroquinazoline-4-one (**xvii**), which affords the specific inhibitor compound **27** after a displacement reaction with 2-methyl-*N*-methylaniline. A variety of alternate methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[49-50] as well as other methods.[51,52] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible. In **Figure 7** is shown a set of proposed compounds related to compound **27** for which we show parameters calculated using Actelion OSIRIS software[47]. The proposed compounds (**I** through **M**) generally show improved druglikeness and drug scores (and other parameters) with respect to compound **27**, and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester with an amide by substituting NH for O, to provide proposed new compound **I**.

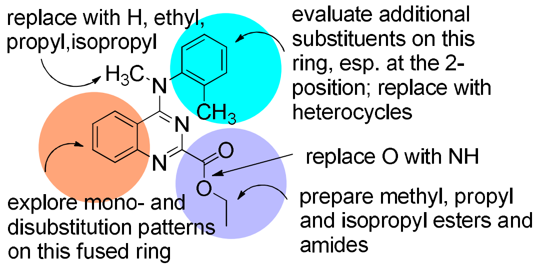
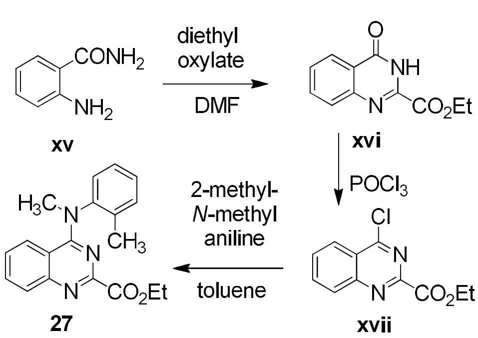


Figure 6. SAR development for 4-aminoquinazolines

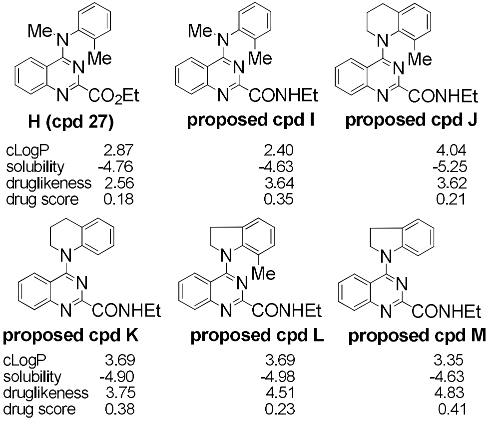
In progressing through the constrained compounds **J** through **M**, improvement of parameters culminates with the indoline derivative **M**, whose druglikeness and druglike scores have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **J** and **K** and indolines **L** and **M**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding.

D.1.2. Conduct HTS assays and identify additional hit compounds. *Screening Libraries.* The HTS will be performed in the **Rong Lab**. The libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of >150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including an Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be acquired in Year 1.

Scheme 4. Synthesis of compound **27**.



Hit Selection Algorithm. ***Validation:*** A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used as a specificity control. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (nonpotent) will not be actively pursued, while the more potent compounds (EC_{50} values less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. ***Cytotoxicity Assessment.*** We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). ***Ranking***: The results from these screening assays will be analyzed and the compounds ranked according to antiviral potency with consideration of SI values.



	H (cpd 27)	proposed cpd I	proposed cpd J
cLogP	2.87	2.40	4.04
solubility	-4.76	-4.63	-5.25
druglikeness	2.56	3.64	3.62
drug score	0.18	0.35	0.21

	proposed cpd K	proposed cpd L	proposed cpd M
cLogP	3.69	3.69	3.35
solubility	-4.90	-4.98	-4.63
druglikeness	3.75	4.51	4.83
drug score	0.38	0.23	0.41

Figure 7. Actelion OSIRIS predicted parameters for the proposed quinazoline-based inhibitors.

Hit Quality Control and Identity Assurance. All hits will be analyzed for chemical purity (HPLC), and we will confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds found to be <80% pure will be purified and retested. Compounds with incorrect structures assigned will be corrected. We will eliminate compounds that are known PAINS[53] (e.g., rhodanines, aralkyl pyrroles, hydroxyphenylhydrazones), or that have reactive functional groups (e.g., Michael acceptors, alkylating agents).

Synthesis. Lead candidate optimization and SAR development based on established drug design principles will be started as soon as affirmative results from the advanced validation assays are available. Appropriate drug design principles and *in silico* ADMET calculations will be used to direct new compound synthesis, so that analogs will have optimal druglike characteristics while minimizing side effects/toxicity. We will synthesize analogs with altered structures to test the limits of steric, electronic, and configurational factors in the activity and selectivity within the chemotypes. For synthesizing new analogs, we will use our considerable collective knowledge, synthetic search engines and state-of-the-art preparative procedures to prepare specific derivatives, e.g., conformationally constrained versions of the best inhibitors, which will provide the maximum amount of SAR information. When undesirable PK features are present in a hit molecule, we will address structural changes to eliminate these features to improve the molecule. For instance, if the molecule is too flexible due to a high number of rotatable bonds, we will impose conformational restraints that will reduce the number of rotatable bonds and freeze a conformation that might reproduce the conformation required for binding of the inhibitor. Thus, in this process, we may be able to also reduce the energy required to produce this conformation from the unconstrained molecule, and thus reduce the entropic penalty of binding and produce a more potent inhibitor. Moreover, we will use Actelion OSIRIS software to design new derivatives of hit compounds to maximize druglike features of the new compounds and overall drug score. This software allows us to quantify PK parameters and use these numbers as comparators to define improved molecules for synthesis. We expect that we will be able to synthesize between 20 to 50 analogs of each hit compound to ascertain preliminary SARs and structure-toxicity relationships (STRs) for further optimization. From the "Expanded Screening" library screening campaigns, we will ultimately choose a primary scaffold and a backup scaffold for performing extensive lead optimization studies and will produce optimal candidates for further development. The advantage of defining two quality scaffolds is that should a liability arise that is scaffold-associated, e.g., relating to toxicity, we can switch to the alternate scaffold and proceed with optimizing development candidates.

D.1.3. Evaluate efficacy of various derivatives in the furopyrimidine, quinazolin-4-one, and 4-aminoquinazoline and series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5x10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at a 3-fold serial dilution ranging from 30 nM to 200 µM for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in both HEK293T and Vero 76 cells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with GraphPad Prism. These assays will be conducted in the **Rong Lab.**

D.1.4. Evaluation of antiviral activity of advanced candidates. Inhibitors will be tested against authentic NiV and HeV as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2 × 10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 µM to 0.01 µM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to parallel control cultures of virus and DMSO. This work will be performed in the BSL-4 facility at UTMB (**Geisbert Lab)**.

D.1.5. Evaluation of *in vitro* ADME properties and prioritization of the most potent compounds exhibiting optimal profile for whole animal testing. Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. These *in vitro* ADME-like predictors will not be used as exclusive criteria to select or eliminate inhibitors, but selection will be based upon the combination of all properties determined (e.g., potency, plasma stability, liver microsomal stability, etc.). Up to twelve potent inhibitors with good *in vitro* ADME properties will be chosen for pharmacokinetic/toxicokinetic evaluation in mice.

Cytotoxicity. We will measure the direct toxicity of compounds in human cell lines to assess the overall cytotoxicity of potent inhibitors. A variety of human cell lines, including PTEC, Jurkat, HepG2, Huh-7 and Hep3B, will be examined utilizing the CellTiter 96 aqueous nonradioactive cell proliferation assay (Promega, Madison, WI) as previously described[54] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI).

Rat/Human Liver Microsome Stability: Rat and human liver microsome preparations will be used to evaluate the metabolic stability of inhibitors using the method described by Kuhnz and Geischen[55]. Inhibitors with >60% recovery (1h) will be considered for further advancement. **CYP450 inhibition:** The CYP450 inhibition assays will be carried out using CYP3A4 and CYP2C9 Human Cytochrome P450 High Throughput Inhibitor Screening Kits (BD Gentest Corp., Woburn, MA). Inhibitors with IC_{50} values ≥30 µM for CYP isoforms will be prioritized for further advancement. **hERG channel blockade:** The ability of inhibitors to block the hERG channel will be assessed in a [^{3}H] dofetilide-binding assay as described by Finlayson *et al*[56] using SHSY5Y cells. Inhibitors with <50% inhibition [^{3}H]dofetilide binding at 5 µM concentration will advance. **Plasma protein binding (PPB):** because PPB affects drug metabolism and PK properties, we will measure the PPB of selected analogs. **Cellular permeability:** We will use the Caco-2 method as described by Gres*et al.*[57] to evaluate the potential for oral bioavailability of the analogs. Inhibitors exhibiting a Caco-2 permeability value (P_{app}) greater than 1 × 10^{-6} cm/sec will be prioritized.

Advanced Lead candidates will be tested in **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels and any reverse mutations will be determined. They will also be tested in the Tox-Genotoxicity panel assays, which includes cytotoxicity testing on primary human hepatocytes, cytotoxicity with primarily cultured neuronal model, and nephrotoxicity with HK2 cells (by Pharmaron, Inc.).

Pitfalls and alternative strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Gaisina and Peet.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy using a different reaction sequence. Should we encounter a scaffold-related problem with our furopyrimidine scaffold, we will switch to our backup quinazolin-4-one scaffold or other HTS hits identified using the expanded libraries of compounds. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc., and Pharmaron, Inc. and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim. We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds (Figure 9)**, which will be advanced to *in vivo* studies (**Aim 3**) and will allow for the future selection of a preclinical candidate.

D.2. Aim 2. Investigate the mechanism of action (MOA) of lead inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed:

D.2.1. Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with medium containing either DMSO or selected inhibitors at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by western blot techniques or viral RNA levels by q-RT-PCR. CedV specific antibodies against G F N and P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene and performed using the ABI 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time.

D.2.2. Determine the stage(s) of inhibition of furopyrimidines and quinazolin-4-ones in the viral lifecycle. For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed, and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting.

D.2.3. Analyze escape mutants against the advanced lead compounds with Cedar virus. ***Target identification for small molecules.*** The rCedV tools used in **Aim 1** will also be used for antiviral target identification. *In vitro* selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutants. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[58,59] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a "drug resistant" virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any "drug resistant" virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inhibitors using authentic NiV-B and HeV in BSL-4 by the UTMB BSL-4 Core.

D.2.4. Assess the binding properties by surface plasmon resonance (SPR). The NiV polymerase (L protein and a phosphoprotein P, or RdRp complex) will be prepared using a previously published protocol[60]. Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, and kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[61] RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (–.1 - 50 μM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab.[62]**

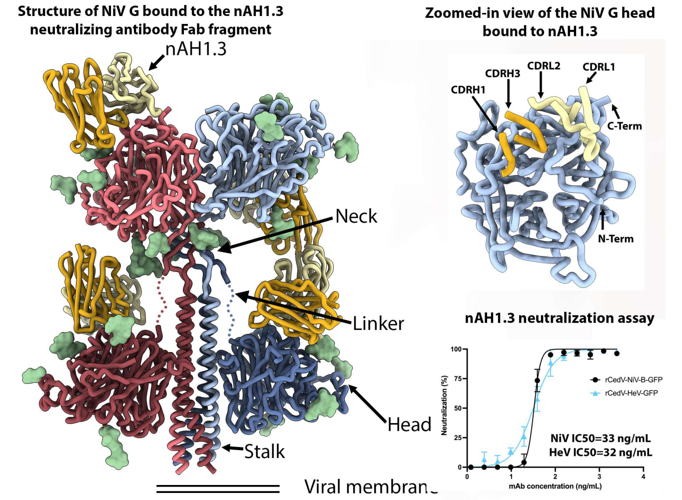


Figure 8. NiV G bound to the nAH1.3 neutralizing antibody Fab fragment. **A**) Ribbon diagram of the NiV G ectodomain bound to the broadly neutralizing nAH1.3 Fab fragment in two orthogonal orientations. Each of the four NiV G protomers is colored distinctly and resolved N-linked glycans are rendered as green surfaces. The nAH1.3 heavy and light chains are colored gold and yellow, respectively, and only the variable domains were modeled in density. The linkers connecting the neck to the two proximal head domains are shown as dashed lines due to weaker density in the cryoEM reconstruction. (**B**) Ribbon diagram of a NiV G head domain (blue) with the interacting nAH1.3 heavy and light chains CDRs rendered in gold and yellow. (**C**) nAH1.3-mediated neutralization of rCedV-NiV-B-GFP (black) and rCedV-HeV-GFP (blue).

D.2.5. Structure determination of recombinant proteins with bound inhibitors by CryoEM. The **Veesler lab** is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with the NiV RdRp to visualize recognition at the atomic level and inform further optimization of target binding affinity. The **Veesler lab** has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software, which runs 24 hours per day and enables automated targeting and image acquisition, while keeping a relational database of all micrographs to expedite subsequent processing.[63] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[64,65] Complexes of small molecule inhibitors with the NiV RdRp will be vitrified and imaged using cryoEM to visualize directly the molecular basis of binding. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[66-70] mechanical stage tilting with gold grids[71,72] or detergents to modify surface tension[73]. To overcome possible conformational heterogeneity, we will use workflows developed in-house specifically for that purpose for previously characterized small molecules, miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[71,74-80] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[81-83]

As cryo-EM structures of polymerase in complex with key inhibitors (i.e., **Z2** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[84-88], to explore the conformational landscape[89] of ligand binding events, and to capture conformational variability of intermediate states[90,91], which can be crucial to gain mechanistic knowledge of biological function.

The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the NiV G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3) (**Figure 8**), which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[92-94] and high-resolution noise substitution prior to Fourier shell correlation calculations[95] to avoid overfitting. Atomic models will be assessed using Molprobity,[96] EMringer[97] and Privateer.[98] The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the inhibitors and the RdRp which will enable iterative optimization of binding affinity and the design of improved inhibitors.

Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory furopyrimidines or quinazoline-4-ones. In the unlikely event we encounter protein complexes that cannot be vitrified using the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which is available at the UW cryoEM center (**Dr. Veesler** as the co-director), and specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). Alternatively, we will crystallize the RdRp in complex with inhibitors to determine X-ray diffraction structures, as routinely done in the **Veesler lab**.[99,100]

D.3. AIM 3: Evaluation of lead compounds *in vivo*.

A critical aspect of antiviral discovery that is often overlooked is the early-stage testing of lead compounds in well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster and AGM.[2,101] PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs will be used in NiV-B and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV-B and HeV infected AGM models.

D.3.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC_{90} in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of henipavirus disease. Lead compounds with good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will help to select doses and dosing regimens for the subsequent protection studies in AGMs. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

D.3.2. *In vivo* testing of lead compounds in NiV/HeV infected Golden Syrian hamsters. The *in vivo* activity of the most potent compounds from Aim1 will be evaluated in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 PFU of NiV-B or HeV.[102-105] Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. The candidates showing the most protection will be selected for further analysis below.

D.3.3. Define breakthrough conditions for anti-henipavirus agents in hamsters. This study will employ the most promising drugs from Aim 1 that are shown to completely protect hamsters against lethal henipavirus infection. The goal is to determine the time points of treatment initiation when the antiviral agents fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5×10^6 PFU of NiV or HeV.[102-105] Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.3.4. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the LMA mucosal atomization device (MAD) as previously described[40] or by small particle aerosol as previously described[41,106] with a uniformly lethal challenge dose (~ 20,000 PFU of NiV or HeV). Importantly, the MAD system delivers atomized particles that range in size from 30 to 100 µm, which is highly consistent with the size of droplets exhaled by humans when coughing. Previous studies with AGMs infected with NiV-B using the MAD system resulted in lethality in 9/9 animals and a disease course that is highly consistent with human infection (T.W. Geisbert, unpublished data). The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion oral gavage up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival. Based on the results, the study will be repeated to further define the therapeutic window, e.g., if all five animals treated with the test compound survive the follow up study will begin treatment at day 5 after NiV infection. Conversely, if any of the drug treated AGMs succumb the follow up study will begin treatment at day 3 after NiV infection. A third study will be done to assess the protective efficacy of the drug for treating HeV-infected AGMs; test conditions for the HeV study will be the same as the test conditions showing the best protection against NiV-B. Defining the therapeutic window is not only important in terms of translating preclinical animal data to humans but also in regard to combination treatment studies where it is important to establish breakthrough for each individual drug to be used in combination approaches in order to be able to show a benefit of combining drugs.

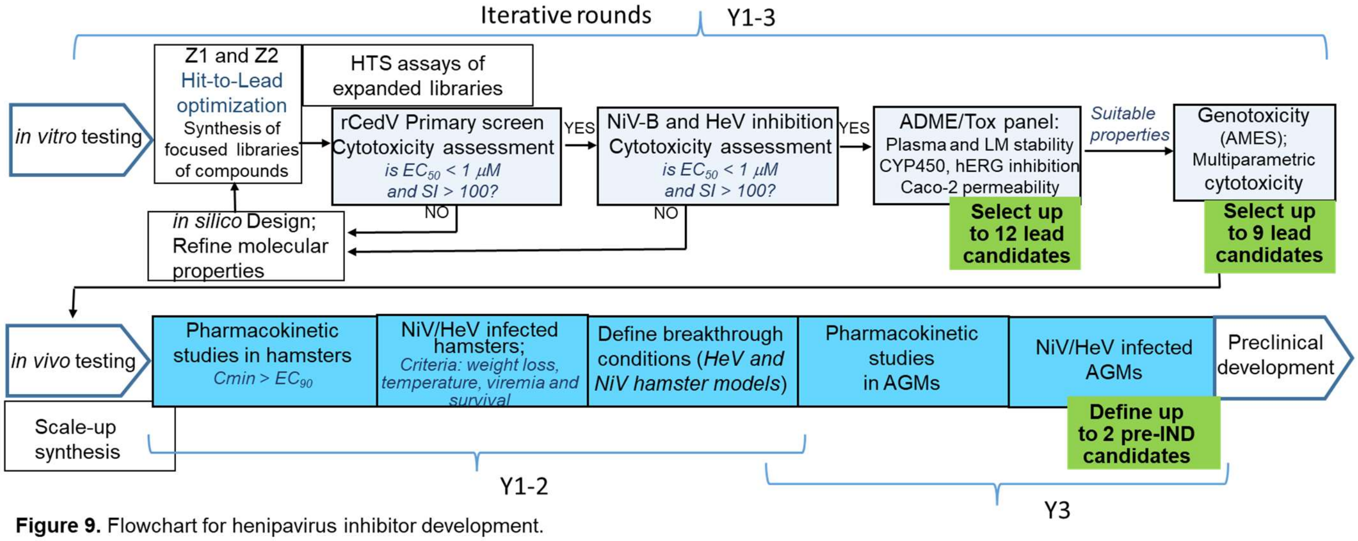


Figure 9. Flowchart for henipavirus inhibitor development.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

Here, we have established rigorous milestones to ensure the production of suitable advanced lead small molecule inhibitors, which will allow for the selection of preclinical candidates (**Figure 9**).

Product Development Plan. Our team will meet quarterly over zoom to discuss the project progress and prioritize promising lead inhibitors generated from each iterative round of synthetic medicinal chemistry-biological testing, from those with desired PK, good safety profile and *in vivo* efficacy, for further advancement.

In summary, the continued outbreaks of NiV across a large global region requires urgent translational countermeasure research to establish a readiness for the next NiV or related pathogenic henipavirus outbreak. We have discovered antiviral leads that show promise against NiV and HeV, and lay out a detailed plan to optimize, develop, and test these drugs. Together with the expertise, resources and experience within this proposal, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).
2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* **359**, 153-177, doi:10.1007/82_2012_208 (2012).
3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *N Engl J Med* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses and public health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Taylor, L. H., Latham, S. M. & Woolhouse, M. E. Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **356**, 983-989, doi:10.1098/rstb.2001.0888 (2001).

11 Desselberger, U. Emerging and re-emerging infectious diseases. *J Infect* **40**, 3-15 (2000).

12 van Doorn, H. R. The epidemiology of emerging infectious diseases and pandemics. *Medicine* **49**, 659-662, doi:10.1016/j.mpmed.2021.07.011 (2021).

13 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. *Henipavirus.*, Vol. 1 (Lippincott Williams & Wilkins 2013).

14 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

15 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *Med J Aust* **162**, 642-645 (1995).

16 Chua, K. B. *et al.* Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**, 1432-1435, doi:10.1126/science.288.5470.1432 (2000).

17 Chua, K. B. *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**, 1257-1259, doi:10.1016/S0140-6736(99)04299-3 (1999).

18 Harcourt, B. H. *et al.* Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis* **11**, 1594-1597, doi:10.3201/eid1110.050513 (2005).

19 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

20 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

21 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

22 Schountz, T. *et al.* Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **11**, doi:10.3390/v11030291 (2019).

23 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

24 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

25 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

26 Maes, P. *et al.* Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. *Archives of virology* **163**, 2295-2310, doi:10.1007/s00705-018-3843-5 (2018).

27 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annual review of virology* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

28 Ching, P. K. *et al.* Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis* **21**, 328-331, doi:10.3201/eid2102.141433 (2015).

29 Playford, E. G. *et al.* Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis* **16**, 219-223, doi:10.3201/eid1602.090552 (2010).

30 Wong, K. T., Shieh, W. J., Zaki, S. R. & Tan, C. T. Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer seminars in immunopathology* **24**, 215-228, doi:10.1007/s00281-002-0106-y (2002).

31 Goh, K. J. *et al.* Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *The New England journal of medicine* **342**, 1229-1235, doi:10.1056/NEJM200004273421701 (2000).

32 O'Sullivan, J. D. *et al.* Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* **349**, 93-95, doi:10.1016/s0140-6736(96)06162-4 (1997).

33 Paton, N. I. *et al.* Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* **354**, 1253-1256, doi:10.1016/S0140-6736(99)04379-2 (1999).

34 Hossain, M. J. *et al.* Clinical presentation of nipah virus infection in Bangladesh. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **46**, 977-984, doi:10.1086/529147 (2008).

35 Wong, K. T. *et al.* Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *The American journal of pathology* **161**, 2153-2167, doi:10.1016/S0002-9440(10)64493-8 (2002).

36 Wong, K. T. *et al.* Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathology and applied neurobiology* **35**, 296-305, doi:10.1111/j.1365-2990.2008.00991.x (2009).

37 Tan, C. T. *et al.* Relapsed and late-onset Nipah encephalitis. *Annals of neurology* **51**, 703-708, doi:10.1002/ana.10212 (2002).

38 Abdullah, S., Chang, L. Y., Rahmat, K., Goh, K. T. & Tan, C. T. Late-onset Nipah virus encephalitis 11 years after

the initial outbreak: A case report. *Neurology Asia* **17**, 71-74 (2012).

39 Hammoud, D. A. *et al.* Aerosol exposure to intermediate size Nipah virus particles induces neurological disease in African green monkeys. *PLoS neglected tropical diseases* **12**, e0006978, doi:10.1371/journal.pntd.0006978 (2018).

40 Geisbert, J. B. *et al.* An Intranasal Exposure Model of Lethal Nipah Virus Infection in African Green Monkeys. *The Journal of infectious diseases* **221**, S414-S418, doi:10.1093/infdis/jiz391 (2020).

41 Prasad, A. N. *et al.* A Lethal Aerosol Exposure Model of Nipah Virus Strain Bangladesh in African Green Monkeys. *The Journal of infectious diseases* **221**, S431-S435, doi:10.1093/infdis/jiz469 (2020).

42 Mehand, M. S., Al-Shorbaji, F., Millett, P. & Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research* **159**, 63-67, doi:10.1016/j.antiviral.2018.09.009 (2018).

43 Luby, S. P. The pandemic potential of Nipah virus. *Antiviral research* **100**, 38-43, doi:10.1016/j.antiviral.2013.07.011 (2013).

44 Broder, C. C. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. *Current opinion in virology* **2**, 176-187, doi:10.1016/j.coviro.2012.02.016 (2012).

45 Azarm, K. D. & Lee, B. Differential Features of Fusion Activation within the Paramyxoviridae. *Viruses* **12**, doi:10.3390/v12020161 (2020).

46 Aziz, M. A. *et al.* Discovery of Potent VEGFR-2 Inhibitors based on Furopyrimidine and Thienopyrimidne Scaffolds as Cancer Targeting Agents. *Scientific reports* **6**, 24460, doi:10.1038/srep24460 (2016).

47 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

48 Han, J. *et al.* Chiral 6-aryl-furo[2,3-d]pyrimidin-4-amines as EGFR inhibitors. *Eur J Med Chem* **119**, 278-299, doi:10.1016/j.ejmech.2016.04.054 (2016).

49 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

50 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

51 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

52 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

53 Baell, J. B. & Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J Med Chem* **53**, 2719-2740, doi:10.1021/jm901137j (2010).

54 Basu, A. *et al.* Stellate cell apoptosis by a soluble mediator from immortalized human hepatocytes. *Apoptosis* **11**, 1391-1400, doi:10.1007/s10495-006-8312-z (2006).

55 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

56 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

57 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

58 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

59 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

60 Jordan, P. C. *et al.* Initiation, extension, and termination of RNA synthesis by a paramyxovirus polymerase. *PLoS Pathog* **14**, e1006889, doi:10.1371/journal.ppat.1006889 (2018).

61 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Anal Biochem* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

62 Schafer, A. *et al.* Evidence for distinct mechanisms of small molecule inhibitors of filovirus entry. *Plos Pathog* **17**, e1009312, doi:10.1371/journal.ppat.1009312 (2021).

63 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

64 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

65 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *Journal of structural biology* **166**, 95-102 (2009).
66 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).
67 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).
68 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).
69 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).
70 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).
71 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).
72 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).
73 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).
74 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).
75 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).
76 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).
77 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).
78 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).
79 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-489, doi:10.1038/s41594-019-0233-y (2019).
80 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-1157, doi:10.1038/s41594-019-0334-7 (2019).
81 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).
82 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).
83 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).
84 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).
85 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).
86 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).
87 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).
88 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).
89 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).
90 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).
91 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).
92 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).
93 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

94 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).
95 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).
96 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).
97 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).
98 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).
99 Tortorici, M. A. *et al.* Structure, receptor recognition, and antigenicity of the human coronavirus CCoV-HuPn-2018 spike glycoprotein. *Cell* **185**, 2279-2291 e2217, doi:10.1016/j.cell.2022.05.019 (2022).
100 McCallum, M. *et al.* Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. *Science* **375**, 864-868, doi:10.1126/science.abn8652 (2022).
101 de Wit, E. & Munster, V. J. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *The Journal of pathology* **235**, 196-205, doi:10.1002/path.4444 (2015).
102 Baseler, L. *et al.* Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. *PLoS neglected tropical diseases* **10**, e0005120, doi:10.1371/journal.pntd.0005120 (2016).
103 Wong, K. T. *et al.* A golden hamster model for human acute Nipah virus infection. *The American journal of pathology* **163**, 2127-2137, doi:10.1016/S0002-9440(10)63569-9 (2003).
104 Rockx, B. *et al.* Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *Journal of virology* **85**, 7658-7671, doi:10.1128/JVI.00473-11 (2011).
105 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).
106 Cong, Y. *et al.* Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS neglected tropical diseases* **11**, e0005532, doi:10.1371/journal.pntd.0005532 (2017).

Investment Results - Outcomes and Outputs

In this section, please provide specific details on the results this project will achieve.

"Outcome" is the ultimate or overall change(s) in systems, populations, or behaviors that a project seeks to achieve within the time frame of the grant.

❖ Describe what success looks like for the project by stating the intended outcome(s) of the project and how they relate to the activities funded by the grant.

Definition three advanced lead compounds that are inhibitory against NiV and HeV, and identification of a potential clinical candidate for further development.

"Output" or "**Funded Development**" means the products, services, processes, technologies, materials, software, data, other innovations, and intellectual property (IP) resulting from the project (including modifications, improvements, and further developments to Background Technology).

❖ Please populate the table below with the intended outputs of the project. Add more rows as needed.

Number	**Output/Funded Development Description**	**Target Completion Date**	**Is a Third-Party agreement required? If yes, by when?**	**Will any IP rights be filed/generated?***
1	Preparation of furopyrimidine compound library	[30/09/24]	No	Yes
2	Preparation of quinazolinone compound library	[30/09/24]	No	Yes
3	Define three advanced lead compounds from the above compound libraries	[30/09/24]	No	Yes
4	Identify a potential clinical candidate compound for further development	[30/09/25]	No	Yes

**Note: In future progress report(s) you will be required to report on these outputs, and to disclose and update intellectual property (IP) and include any links to IP applications, filings, or registrations, as applicable.*

❖ If necessary, please add further explanations below:

Global Access – Charitable Impact for Beneficiaries

To ensure a positive impact for the intended beneficiaries, the foundation requires that all projects and outputs be managed to ensure Global Access.

"Global Access" is a foundation policy requiring that: (a) the knowledge and information gained from the project be promptly and broadly disseminated; and (b) the Funded Developments be made available and accessible at an affordable price (i) to people most in need within developing countries, or (ii) in support of the U.S. educational system and public libraries, as applicable to the project. For more information and resources on Global Access, see the foundation's Global Access Statement and Global Access webpage.

❖ How will you disseminate the knowledge and information gained from the project? For additional information for peer-reviewed publications, see the foundation's Open Access policy.

Data generated from this project will be publish in Open Access journals and presented at the International Society for Antiviral Research conferences.

"Background Technology" means all products, services, processes, technologies, materials, software, data, or other innovations, and intellectual property created by you or a third party prior to or outside of the project and used as part of the project.

Please list any Background Technology that will be used in the project. Commonly available, off-the-shelf products (such as Microsoft Excel, Adobe, etc.) do not need to be disclosed. Add more rows as needed.

Note: Background Technology previously funded by the foundation will be considered a Funded Development for purposes of Global Access and any license to the foundation.

List each Background Technology to be used in the project	Is this Background Technology owned, controlled, or developed by you or sublicensable by you?	Do you need permission or a license from any third party to use this Background Technology to achieve Global Access?	If any permission/license is needed, please detail below, and describe your plan and timeline to obtain such permission/license or submit a copy of the agreement.	If this Background Technology is subject to Intellectual Property rights, please identify and include any links to applications, filings, or registrations, as applicable.
Provisional patent application NOVEL INHIBITORS OF HENIPAVIRUSES	Yes	No		application serial no. is 63/391,893
	No/Yes	No/Yes		
	No/Yes	No/Yes		

❖ How will you ensure affordable and meaningful access to the Funded Developments and Background Technology, if any?

Publication in Open Access Journals; Patent sub-license.

❖ Do you foresee any obstacles to achieving Global Access (e.g., third-party rights, restrictions on Background Technology, time frame, affordability)?

__X_No___Yes (please explain and describe the specific steps that you will take to address the obstacles)

❖ For Global Access purposes, please confirm that you will make available the funded developments and any Background Technology that is (a) owned, controlled, or developed by you, or in-licensed with the right to sublicense; and (b) either incorporated into a funded development or reasonably required to use the funded development. For more information, see the Global Access terms located in the foundation's standard grant terms and conditions.

_X__Confirmed___Not confirmed (please explain)

Data Access

It is anticipated that the activities of this project will generate or collect datasets that may be useful to the foundation, project collaborators, third-party researchers, and/or the public. Please answer the questions below with a level of detail appropriate to the project activities.

- What types of data and specific datasets will be generated from the project activities and/or collected from other sources?

  HTS dataset; *In vitro* inhibition of CedV, NiV-B and HeV; PK and ADME/Tox data for lead compounds; *In vivo* efficacy data for advanced lead compounds

- Do you plan to create a data management plan? If not, why not?

  Yes

- What data curation (e.g., organizing, describing, cleaning, enhancing, and preserving) activities are planned to enable reuse or secondary use of the datasets?

  We will create a database in which we will routinely organize and store chemical and biological information that can be accessed by our team. We will use ChemBioFinder, because this program has the capability of searching compounds by sub-structures or similarity, facilitating data analysis and information retrieval.

- Where and when will the datasets be deposited and preserved to enable reuse or secondary use and under what license?

  All data generated in the course of this project will be published in Open Access Journals.

- Please describe any informed consents, approvals, and/or agreements that may be required to enable use or reuse of the datasets by the foundation, project collaborators, and/or third-party researchers. What steps do you plan to take to obtain such consents, approvals, and agreements?

  Office of Technology Management at UIC. To process material transfer agreements (MTA).

BUDGET INFORMATION

The purpose of this section is to supplement the information provided in the Excel budget template by justifying how the budgeted items are necessary to implement project activities and accomplish project results. Please focus on key costs and risks and avoid repeating information contained in the budget template. Together, this narrative section and Excel budget template should provide a complete description that supports the proposed budget. For questions below that are not relevant to your project, answer "N/A".

For additional instructions and guidance on the budget template, see the Grant Budgeting & Financial Reporting Detailed Instructions.

Budget Summary

- Explain the major cost drivers and how costs relate to planned activities and target outcomes. Also, explain any potential risks in spending and any plans to mitigate those risks. In the Excel budget template, an *additional dimension* can be defined to break down costs beyond the standard expense categories (e.g., by geography, product candidate, projects within a portfolio, or other). If you are defining such an additional dimension, please use it as you explain the major cost drivers here.

  We request funding to purchase the "ChemDiv Antiviral Library" ($124,621 – quote RT10212021-UIC-A), which will be screened for new inhibitors by HTS rCedV-Luc assay.
  ADME/Tox and PK screening (Y1-$35,000; Y2-$60,000, and Y3-$50,000).

ADME panel	Price *	Species	Comments
Thermodynamic solubility in PBS	$80		Plan to test: up to 15 compounds in LM and plasma stability assays in Y1-2; up to 12 compounds in CYP inhibition assay; up to 10 compounds in Caco-2, TS in PBS and Stability in Hep assays; up to 10 compounds in PPB,
Thermodynamic solubility in FeSSIF, FaSSIF, FaSSGF, SIF or SGF	$100 /incubation ($400 total)		
Stability in Liver Microsomes 4 non-zero tps	$200 / species	Human, hamster, and monkey	
Stability in hepatocytes (1 non-	$220 / species	Human, hamster, and monkey	

zero tps)			thermodynamic solubility in FeSSIF, FaSSIF, FaSSGF, SIF
Plasma stability (4 non-zero tps)	$250 /species	Human, hamster, and monkey	
Caco-2 Bidirectional	$450		
Plasma protein binding	$280		
CYP inhibition (2C9, 2C19, 2D6, 3A4) 6 non-zero concs	$1,120		
Tox-Genotoxicity panel			Plan to test up to 10 compounds in Tox-Genotoxicity panel in Y1-2
hERG assay by manual patch-clamp (IC50, n = 2)	$450		
Nephrotoxicity (HK2 cells, IC50)	$300		
Cytotoxicity with primarily cultured neuronal model (IC50)	$600		
Cytotoxicity testing on primary human hepatocytes	$1,200		
Mini-Ames 5 strains	$1,350		
In vivo PK			
Screening PK in hamsters Plasma PK (IV and PO administration), n = 3, 8-9 time points	$3,000		Plan to test up to 9 compounds
Bio analysis of samples (LC/MS/MS/ method development)	$1,000		
Metabolite identification in biosamples	$1,000/sample (blood, brain) $2,000 total		
Monkey standard PK Plasma PK (IV and PO administration), n = 3, 8-9 time points	$9,700		Plan to test up to 3 compounds in Y2-3
Bio analysis of samples (LC/MS/MS/ method development)	$1,000		
Metabolite identification in biosamples (monkey)	$1,000/sample (blood)		
*Pharmaron *in vitro* ADME-Tox and *in vivo* PK Pricing (2022)			

Detailed Budget Information

In this section, please complete the sub-sections that apply to your project and associated budget, and answer “N/A” for those that do not apply.

Personnel and Benefits

- Provide a description of budgeted personnel (including staff to-be-hired) and their responsibilities as they relate to the project. For to-be-hired individuals, please make sure your budgeting assumptions factor in realistic timing for recruiting.

  Describe the components included in the benefits percentage (column R of the “Budget Details” sheet in the budget template). For example: pension, health insurance, expatriate costs, etc.

Dr. Lijun Rong, PI (0.13, 0.1, and 0.08 FTE), is a Professor and Director of High Throughput Screening (HTS) facility at the Department of

Microbiology and Immunology at the University of Illinois at Chicago (UIC). Dr. Rong has extensive research experience in studying the entry and replication mechanisms of several emerging viruses including SARS2, Ebola virus, influenza and henipaviruses, and in developing small molecule inhibitors against these viruses. As the PI, he will supervise and coordinate all aspects of the proposed experiments in this proposal He will be also responsible for communicating with the Co-Is and collaborators.
Dr. Irina N. Gaisina, Co-Investigator (2.88 Acad, 0.6 Sum. Y1; 2.16 Acad 0.6 Sum. Y2; 1.44 Acad, 0.6 Sum Y3), is a Research Associate Professor in the Department of Pharmaceutical Sciences, College of Pharmacy, UIC. She has extensive experience in hit-to-lead and lead optimization medicinal chemistry and preclinical drug development. Dr. Gaisina will assume overall responsibility for the medicinal chemistry work carried out at UIC, including coordinating the work of project personnel and writing reports associated with the project. Dr. Gaisina will directly supervise Dr. Argade, and TBN technician. She will work on structure-activity relationship development, including computational aspects, and lead optimization. As a key team member in the medicinal chemistry core at UIC's collaborative drug discovery center, UICentre, Dr. Gaisina has been leading multiple antiviral drug discovery projects. She has a well-established collaboration with Dr. Rong.
Dr. Malaika Argade, Research Scientist (8.25 Cal. Y1; 6.6 Cal. Y2; 3.3 Cal. Y3), a medicinal chemist at UICentre, will perform the proposed synthetic medicinal chemistry and optimize scaleup synthesis of lead compounds for the PK assessments and the proposed in vivo studies. Salary support plus applicable fringe benefits for effort are requested.
TBN, Technician (12 Cal. Y1-Y2; 9 Cal. Y3), with background in organic synthesis and/or medicinal chemistry will be recruited to join the research team. The technician will synthesize focused libraries of small molecule inhibitors of henipaviruses and characterize them using NMR spectroscopy and mass-spectrometry.
Ryan Bott, B.S. Research Specialist (0.63, 0.5, and 0.38), has started to work in Dr. Rong Lab as a research specialist since May 2021. Before that he worked in Rong Lab as an undergraduate lab aide for three years. He has been trained for basic lab techniques and will assist the proposed experiments in this proposal.
Dr. Laura Cooper, Postdoctoral Fellow (1.25, 1, and 0.75), was a PhD student in Dr, Rong lab and her PhD research focused on antiviral drug discovery and MOA studies of small molecule inhibitors. She has published more than 15 peer-reviewed papers since she joined Dr. Rong Lab. Her responsibilities include bioassay development and MOA studies in this application.

Salary support plus applicable fringe benefits for effort are requested. UIC's FY23 projected fringe rate is 37.17% for academic professionals.

Fringe benefits include pension, health, life and dental insurance, workers comp, term. vacation/sick, medicare (for those hired after 4/1/1986), OASDI (for certain appointment types).

Travel

- Describe the travel budgeted and the assumptions used to estimate the appropriate number of trips and associated costs.

$3,000 /year funding is requested for the PI and Co-I to attend antiviral research meetings, such as the International Society for Antiviral Research (ISAR) conferences and present the project. This travel budget covers airfare, lodging, and per diem associated costs.

Consultants

- Describe the work to be performed by consultants and any reimbursable expenses that have been budgeted in addition to consulting fees.

Dr. Norton P. Peet, Consultant, is Chief Scientific Officer for Chicago BioSolutions. Dr. Peet is a medicinal chemist who has over 30 years of experience with both multinational pharmaceutical and biotechnology companies. Dr. Peet has previous experience with the preclinical development of therapeutic agents and their advancement to a clinical setting. He will oversee the proposed ADME/Tox and PK studies (his consulting fee is $300/h).

Capital Equipment

- Describe any budgeted capital equipment items and explain why they are needed for the project. Only items with a unit cost of more than $5,000 (USD) and a "useful life" of more than one year should be included here, whereas items with a lower cost or shorter life should be budgeted under Other Direct Costs (see below).

none

Other Direct Costs

- Describe other items required for the project that fall under direct costs and are not part of any cost category above. For additional information on direct and indirect costs, please see the foundation's Indirect Cost policy.

Chemistry Lab:
1) Chemical and solvents including, but not limited to specialty chemicals, inorganic salts, ACS grade solvents for synthesis ($28,697 Y1;

$23,000 Y2; $19,000 Y3).
2) Chromatography supplies including, but not limited to tubes for fractionation, replacement HPLC columns, lamps for spectrophotometers, preloaded silica gel cartridges, LC-MS grade solvents for LC-MS and HPLC ($3,000/year).
3) Glassware and disposable plasticware supplies including, but not limited to flasks, vials, pipette tips, containers for sample storage ($3,000/year).
4) $17,000 per year is requested to cover instrumentation charges include routine NMR analysis and characterization of intermediates and products ($10/hr); charges for IT-TOF-MS at $52/hr; UIC Research Resources Center (RRC) services including mass spectrometry, high field NMR, surface plasmon resonance (SPR) to assess binding of small molecules to target protein, and bioinformatics.
Biology Lab:
1) HTS reagents and supplies
2) Tissue culture supplies
3) Reagents for DNA and protein work
4) Tissue culture supplies to characterize the lead inhibitors, and to study MOA of these inhibitors.
5) Assay kits and reagents (such as luciferase substrate, LiveBLAzer-FRET B/substrate).
6) Glassware, plasticware and other miscellaneous items.
Publication in Open Access Journals:
Funding is requested to cover the publication costs ($5,000/Year)

Subawards

Subawards are contracts or grants that you, as the prospective primary grantee, negotiate with other organizations who contribute to the completion of this project. A subaward is "under" the main grant award; as the primary grantee you will receive funds from the foundation and manage the disbursements to the subawardee. You are responsible for negotiating the subaward budget, overseeing the work of the subawardee, disbursing funds in accordance with the subaward agreement and ensuring all applicable policies are met. For additional information, see Exercising Expenditure Responsibility Over Subgrants.

If your project will include subawards, please include the following information:

❖ If you are an Indian organization/entity subject to India's Foreign Contribution Regulation Act (FCRA), then in compliance with FCRA, do you confirm that your proposed project will not include any subgrantees? This prohibition does not preclude subcontracts in the ordinary course of business.

_X__Not applicable ___Confirmed ___Not confirmed (please explain)

❖ In the Excel budget spreadsheet, please enter each subaward as a separate line item with the expected disbursement for each period broken out.

If your proposal includes any subawards greater than $1 million USD, please provide a separate budget spreadsheet for each subaward as an appendix to the proposal materials, using the foundations Excel budget template. Please reach out to your foundation contact if you need a blank budget template or have questions or concerns about using this format with any of your subawardees.

❖ In addition to the information included in the Excel budget template, please also list all subgrantees or subcontractors involved in this project in the table below. Add more rows as needed.

Type of Subaward (grant or contract)	**Organization Name**	**Organization Location** (Country)	**Website or Email** (please include email if subcontracting to an individual)
Contract	The University of Texas Medical Branch at Galveston	U.S.A.	
Contract	The University of Washington Seattle	U.S.A.	

❖ In the space below, provide the following additional information for each organization chosen to participate on this project as a subawardee:

Describe the work the subawardee is going to perform, what selection process was used, how the sub-budgets were negotiated, and what the key cost drivers are. If organizations are not yet known or "to-be-determined" (TBD), include the assumptions used to estimate the cost for the subaward, and the process and timeline you will use to select these organizations and negotiate budgets. Once subaward organizations are selected, you will be required to submit the subaward information and budget (as necessary) to the foundation in writing.

The University of Texas Medical Branch at Galveston: Evaluation of antiviral activity of advanced candidates against authentic NiV and HeV *in vitro* and i*n vivo* testing of lead compounds in NiV/HeV infected Golden Syrian hamster and AGM models.

Dr. Thomas W. Geisbert (**Co-I**), Professor, Department of Microbiology & Immunology at UTMB-Galveston and of the Galveston National Laboratory (GNL), will serve as the UTMB principal investigator for a subcontract for this project. He has expertise in nearly all technical aspects of this project. Dr. Geisbert has over 30 years' BSL-4 experience at USAMRIID and at the GNL. For these many years, his research interests have focused on the study of hemorrhagic fever viruses, and he has published extensively in this area. His group demonstrated complete protection of nonhuman primates against Ebola, Marburg, and Lassa viruses using a novel vaccine based on recombinant vesicular stomatitis virus. He will devote 0.6 calendar months in Periods 1-3 to project planning and performance and hands-on participation in these experiments. Dr. Geisbert's institutional base salary exceeds the current salary limitation for Executive Level II of the Federal Executive pay scale; therefore, the salary cap was used. Fringe rates are calculated using his actual institutional salary.

BSL-4 Research Scientist: Robert Cross, PhD, Assistant Professor, Department of Microbiology & Immunology at UTMB-Galveston and of the GNL will devote 0.8 calendar months per year in Periods 1-2 and 0.9 calendar months in Period 3 of the project assisting Dr. Geisbert with the conduct of this research. He will participate in the BSL-4 portions of the work including hands on procedures with the mice including necropsies. He will also assist in performing various assays including virology assays. Dr. Cross has seven years' BSL-4 experience.

BSL-4 Technical Director: Joan B. Geisbert has more than 35 years' hands-on BSL-4 laboratory experience and serves as the Associate Director for In Vivo Training at the GNL. Ms. Geisbert will serve as chief technical manager for all of the mouse challenge studies including laboratory set-up, supervision of the BSL-4 research technicians and graduate student, and hands-on participation in these experiments. She will devote 0.75 calendar months each year in all periods of the project. Ms. Geisbert will also perform animal challenges, treatments, clinical observations, and assist with necropsies. Ms. Geisbert will also oversee the select agent inventory and controlled substances inventory for this project.

BSL-4 Veterinary Pathologist: Karla Fenton, DVM, PhD, Senior Scientist, Department of Microbiology & Immunology at UTMB-Galveston and of the GNL will devote 0.8 calendar months each year in Periods 1-2 and 0.9 calendar months in Period 3 assisting Dr. Geisbert with the conduct of this research. Dr. Fenton is an ACVP board-certified veterinary pathologist and has been trained in BSL-4/ABSL-4 in the Geisbert laboratory. She will perform and oversee necropsies on the animals and will assist in animal challenges, treatments, clinical observations, and sample collection, and will perform gross and histological analysis of selected tissues and will prepare pathology reports. Dr. Fenton has six years' BSL4 experience.

BSL-4 Research Scientist: Abhishek Prasad, PhD, Department of Microbiology & Immunology at UTMB- Galveston and of the GNL, will devote 0.96 calendar months in Period 1-2 and 1.2 calendar months in Period 3 of the project assisting Dr. Geisbert with the conduct of this research. He will participate in the BSL-4 portions of the work, including hands on procedures with the mice and performing various assays including virology assays. Dr. Prasad has four years' BSL-4 experience.

BSL-4 Postdoctoral Fellow: Dylan Johnson, postdoctoral fellow in the Department of Microbiology & Immunology at UTMB- Galveston and of the GNL will devote 0.96 calendar months in all Periods of the project assisting Dr. Geisbert with the conduct of this research. He will participate in the BSL-4 portions of the work, including reverse genetics, hands-on procedures with the mice, and performing various assays including virology assays.

BSL-4 Postdoctoral Fellow: Alyssa Fears, postdoctoral fellow in the Department of Microbiology & Immunology at UTMB- Galveston and of the GNL will devote 0.96 calendar months in all Periods of the project assisting Dr. Geisbert with the conduct of this research. She will participate in the BSL-4 portions of the work, including reverse genetics, hands-on procedures with the mice, and performing various assays including virology assays.

BSL-4 Research Scientist: Courtney Woolsey, PhD, was a graduate student and postdoctoral fellow in Dr. Geisbert's laboratory and has since formerly assumed a role as a research scientist in the Department of Microbiology & Immunology at UTMB. Dr. Woolsey will devote 0.96 calendar months in Periods 1-2 and 1.2 calendar months in Period 3 assisting Dr. Geisbert with the conduct of this research. She will participate in the BSL-4 portions of the work, including performing various procedures including hematology, clinical chemistry, and virology assays.

BSL-4 Research Technician: Krystle Agans, BS, will devote 1.2 calendar months in Periods 1-2 and 1.5 calendar month in Period 3 of the project performing BSL-4 in vitro and animal studies. Ms. Agans will participate in the BSL-4 portions of the work including virology assays as well as the BSL-2 RT-PCR assays. She has over seven years' BSL-4 experience.

BSL-4 Research Technician: Daniel Deer, BS, will devote 1.2 calendar months in Periods 1-2 and 1.5 calendar month in Period 3 of the project performing BSL-4 in vitro and animal studies. Mr. Deer will participate in the BSL- 4 portions of the work including virology assays as well as clinical observations. He has over six years' BSL-4 experience.

BSL-4 Research Technician: Viktoriya Borisevich, MD, PhD will devote 1.2 calendar months in Periods 1-2 and 1.5 calendar month in Period 3 of the project performing BSL-4 in vitro and animal studies. Dr. Borisevich will participate in the BSL-4 portions of the work including sequencing and virology assays, as well as the BSL-2 RT-PCR assays. Dr. Borisevich has over eight years' BSL-4 experience.

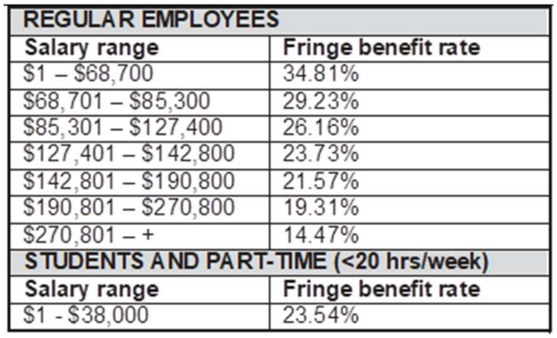
Research Technician: Brittany Franshaw will assist Dr. Borisevich in performing BSL-2 RT-PCR assays and will devote 1.2 calendar months each year in all Periods of the project. She is currently being trained in BSL-4 and will assist with various procedures including virology assays.

Research Technician: Rachel O'Toole will assist Dr. Borisevich in performing BSL-2 RT-PCR assays and will devote 1.2 calendar months each year in all Periods of the project. She is currently being trained in BSL-4 and will assist with various procedures including virology assays.

Histopathology Technician: Natalie Dobias, HT(ASCP), is a histologist with 11 years' experience (5 of those working with NHP tissue) who will be tasked with processing, embedding, sectioning, and staining mouse tissues as needed for the project. She will devote 0.8 calendar

months in Periods 1-2 and 0.96 calendar months in Period 3 of the project assisting Dr. Fenton with the histology studies.
Program Administrator: Sherry Haller, PhD, Associate Director, Center for Biodefense and Emerging Infectious Diseases, will oversee all administrative aspects of the project and will work with Dr. Geisbert to see that deliverables are being met. She will devote 0.2 calendar months per year in all Periods of the project.

The table below reflects staff effort to be devoted to this project.
Fringe Benefits: The UTMB-Galveston fringe benefit rate for FY22 is calculated by salary range as follows:



REGULAR EMPLOYEES	
Salary range	**Fringe benefit rate**
$1 – $68,700	34.81%
$68,701 – $85,300	29.23%
$85,301 – $127,400	26.16%
$127,401 – $142,800	23.73%
$142,801 – $190,800	21.57%
$190,801 – $270,800	19.31%
$270,801 – +	14.47%
STUDENTS AND PART-TIME (<20 hrs/week)	
Salary range	**Fringe benefit rate**
$1 - $38,000	23.54%

OTHER DIRECT COSTS
Materials and Supplies.

General lab supplies- Funds are requested for laboratory supplies to perform in vitro assays including costs for PPE (gloves, masks, etc.), cell culture material, plasticware, flasks, pipette tips and disinfectant as well as for in vivo work including costs to perform hematology, clinical chemistry, virology, animal procedures, and necropsies, based on historical usage of these items. We request $64,401 in Period 1, $29,321 in Period 2 and $32,562 in Period 3 for a total of $126,285.

Hamster purchase, shipping and per diems- Funds are requested for the purchase of 100 hamsters in Period 1 and 200 hamsters in Period 2. A total of $18,500 is requested in Period 1 and $37,000 in Period 2 to include the cost of purchasing the hamsters, shipping them to UTMB, and housing them in the UTMB Animal Resource Center for the duration of the studies.

NHP purchase, shipping and per diems- Funds are requested for the purchase of 12 African green monkeys in Period 3. African green monkeys currently cost $8,870 per animal plus shipping. A total of $152,000 is requested in Period 3 to include the cost of purchasing, testing and shipping the NHPs to UTMB. Also included are costs to cover per diem charges.

INDIRECT COSTS
According to the Pandemic Antiviral Discovery guidelines, the indirect costs for this work have been set at 10% of modified total direct costs for the project period.

The University of Washington Seattle: Structure determination of recombinant proteins with bound inhibitors by CryoEM.
Dr. David Veesler (**Co-I**), an Associate Professor of Biochemistry at UW and HHMI investigator (no salary requested), is a world leader in structural studies of viral glycoproteins and will carry out cryoEM and X-ray crystallographic studies of the most potent inhibitors in complex with the NiV or HeV polymerase.
Dr. Daniel Asarnow, Postdoctoral Fellow, (effort 69% FTE). Dr. Asarnow is an expert in biophysics and has a strong background in structural biology. Dr. Asarnow determined cryoEM and X-ray crystallographic structures of neutralizing coronavirus antibodies targeting the spike and will participate in structural determination efforts. Dr. Asarnow will be playing a major role in experimental design, data analysis and interpretation, writing manuscripts and will report directly to Dr. Veesler.
William Sharkey, Research Technician, (effort 75% FTE). Mr. Sharkey has extensive experience in recombinant glycoprotein production in mammalian cells, purification and biophysical characterization. His work will support cryoEM structural studies under the supervision of Dr. Veesler to whom he will directly report.
Zaneta Holman, Lab manager, (effort 7% FTE). Ms. Holman is in charge of ordering and stocking lab supplies and of interactions with vendors under the supervision of Dr. Veesler to whom she will directly report.

Mammalian cell culture, protein production and purification: Funds are requested to support the purchase of protein production materials and supplies including media, chromatography columns, gels, stains, ladders, purification reagents, concentrators, and buffer reagents. It will also include transfection reagents, cell culture media and plasticware for suspension cells to support structural biology efforts.

Microscope time. This will cover access to our Thermo-Fisher Titan Krios, Glacios and Tecnai T12 Spirit electron microscopes for screening and high-resolution data collection.

INDIRECT COSTS
According to the Pandemic Antiviral Discovery guidelines, the indirect costs for this work have been set at 10% of modified total direct costs for the project period.

Indirect Cost Rate

- Briefly explain the indirect cost rate being charged on this project and the rationale and assumptions behind it.

10% maximum amount per sponsor policy.

Currency Exchange

- What is the operating currency you will be using to manage project financials? If different from USD, please explain your organization's foreign exchange policy, including how and when U.S. Dollars are converted to local currency. (If no formal policy exists, explain the approach you plan to follow on this project.)

USD

- If the project is exposed to additional currencies, other than your operating currency and USD, please name these currencies and provide a rough estimate on how much will be spent in these currencies.

- Are there any measures you plan to take for reducing currency risk?

Other Sources of Support

- Beyond the requested funding from this grant, will the total cost of this project require contributions of funding from other sources (either from your organization or others)?

_X__No (skip questions below) ___Yes (please explain)

Please fill out the Total Project Cost and Funding Plan sections in the Excel Budget template and provide further context and explanations here, such as timing, uncertainties/dependencies and important restrictions that apply. If applicable, please also describe any expected in-kind contributions (e.g., drug donations, personnel time), and explain how dollar values were estimated.

- If any of the funding (other than this grant) is not yet secured, please describe what you will do if it does not become available.

Geography Served

- List all countries, sub-regions, and/or states that will benefit from this work and the associated dollar amounts for the full term of the grant. Please avoid listing "World," unless the work truly serves beneficiaries globally. If geographies include the United States, please indicate city and state. The total of "Foundation Funding" amounts should match the requested grant funding. Add more rows as needed.

For additional information, see the Geography and Location of Work – Frequently Asked Questions.

Geography Served	Foundation Funding Amount (USD)
World	$2,954,167
	$
	$

Location of Work

- List all countries, sub-regions, and/or states where this work would be performed, and associated dollar amounts for the full term of the grant. This includes your location and any locations where you plan to make payments to individuals or organizations with grant funds. If locations include the United States, please indicate city and state. The total of "Foundation Funding" amounts should match the requested grant funding. Add more rows as needed.

  For additional information, see the Geography and Location of Work – Frequently Asked Questions.

Location of Work	**Foundation Funding Amount (USD)**
United States of America, Illinois, Chicago	$1,655,106
United States of America, Michigan, Holland	$36,000
United States of America, Texas, Galveston	$780,000
United States of America, Washington, Seattle	$483,061

- Many countries, including India, Vietnam, Bangladesh, and Pakistan require organizations conducting activities funded with foreign funds to comply with local registrations or other requirements. These restrictions may apply to funds you disburse to sub-awardees under this project. Please confirm that your organization will ensure compliance with any such requirements, including taking reasonable steps to ensure that any subgrantees subject to India's Foreign Contribution Regulation Act (FCRA) are in compliance with this law and the regulations thereunder (e.g., depositing subgrant funds only into accounts maintained at the State Bank of India, New Delhi Main Branch and ensuring such subgrantees do not further subgrant funds).

  _X__Not applicable ___Confirmed ___Not confirmed (please explain)

Anti-Terrorism and Use of Funds

- Did you list any locations of work as "to be determined" (TBD) or geographic areas instead of specific countries (e.g., Sub-Saharan Africa or Middle East)?

  _X__No ___Yes

- Did you list any locations of work that are subject to U.S. government sanctions as identified below?

Comprehensive Sanctioned Countries	**List-based Sanctioned Countries**
___Cuba ___Ukraine's Crimea Region or so-called Luhansk or Donetsk People's Republics ___Iran ___North Korea ___Syria	___Countries subject to list-based sanctions* **Note: Countries subject to list-based sanctions can be found on the U.S. Department of Treasury website*

- If you selected 'yes' to TBD locations or locations that are not country-specific, and/or selected locations that are subject to U.S. government sanctions or believe that project work could reasonably occur in a location that is subject to U.S. government sanctions, please review the guidance in the Office of Foreign Assets Control (OFAC) Guidelines and confirm that: (a) you have discussed the location of work with your foundation contact, and (b) your organization will ensure compliance throughout the term of the project with the anti-terrorism clause in our grant agreement.

  ___Confirmed ___Not confirmed (please explain)

ROLES AND RESPONSIBILITIES

Conduct and Control

In answering the questions in this section, please consider all project activities, such as those involving: confidential or protected information (including personally identifiable information or protected health information); the inclusion of children or vulnerable populations; research involving human subjects; clinical trials; post-approval studies; field trials; experimental medicine; provision of medical services (diagnostic, prophylactic or treatment); product development; use of genetically modified organisms, human tissue, animals, radioactive isotopes, pathogenic organisms, recombinant nucleic acids, select agents or toxins, dual-use technology, or any substance, organism, or material that is toxic or hazardous; use of aircraft, unmanned vehicle systems, drones or satellites; and the import, export, transfer, approvals, consents, records, data, specimens, images, and materials related to any of the foregoing.

Please confirm that your organization:

- Will maintain the expertise necessary to conduct, control, manage, and monitor all aspects of the project in compliance with all applicable ethical, legal, regulatory, and safety requirements including applicable international, national, state, local, and institutional, school district or school network standards and policies and is responsible for determining and complying with these requirements and standards;
- Will not disclose any confidential or protected information to the foundation without obtaining prior written approval from the foundation and all necessary consents to disclose such information;
- Acknowledges that any activities by the foundation in reviewing documents, providing input or funding does not modify your organization’s responsibility for determining and complying with all applicable ethical, legal, regulatory, and safety requirements for the project in all places;
- Is a government agency, public institution or multilateral organization or will otherwise maintain insurance coverage sufficient to cover the activities, risks, and potential omissions of the project in accordance with generally-accepted standards and as required by law (for instance, general, professional, clinical trial, product liability, medical malpractice, workers' compensation, or otherwise); and
- Will not transfer any biological materials, chemicals, reagents, hazardous materials, or the like to the foundation.

_X__Confirmed ___Not confirmed (please explain)

❖ Does the project involve any of the following: clinical trial, other trial involving human subjects, post-approval study, experimental medicine, field trial of genetically modified organisms, or the provision of medical/health services?

_X__No ___Yes (please explain)

If yes, please list all approvals and consents required for each site and describe the time frame in which your organization will acquire the necessary approvals and consents.

❖ If yes, please identify the name of the entity that will be the sponsor/responsible party of any clinical trials, studies involving human subjects, experimental medicine studies, post-approval studies, products, or regulatory filings contemplated by the project.

Note: The foundation will not serve as the sponsor/responsible party nor accept delegation of any of these responsibilities. If the project will not involve such activities, please indicate not applicable or N/A below.

Advocacy and Lobbying

❖ While the foundation funds a broad range of advocacy activities, U.S. law prohibits foundation funds from being earmarked to support direct or grassroots lobbying communications. You should answer “Yes” to this question if this project will include engaging with the public or government partners to influence government policy decisions or actions. Does your project include advocacy activities?

_X__No ___Yes (please explain)

If “yes,” please describe how the project will be conducted to ensure foundation funds are not earmarked to support grassroots or direct lobbying, as summarized in the foundation’s Advocacy Guidelines, identifying any potential legislative actions related to your advocacy goals. Your response should demonstrate your understanding of the relevant restrictions and exceptions and explain how you will manage compliance during the term of the project. If relevant, also explain how you will comply with other pertinent lobbying rules or requirements (e.g., local, state, or federal disclosure rules).

SECTION B – PROGRESS NARRATIVE(S)

This section is for progress reporting on funded grants. For each required progress report, complete a version of this progress narrative section and submit the Investment Document to your foundation contact by the date indicated in your grant agreement or latest amendment. If you have any questions or need support, please reach out to your foundation contact. Please find contact information in Section A – Investment Details. The progress narrative is your opportunity to update the foundation on the following:

- Progress made toward achieving your project's stated outputs and outcomes
- Any adjustments to the project
- Financial update for the reporting period
- Feedback for the foundation

Reminder: This document must be submitted in English and as a Word file. PDFs and other file types will not be accepted.

IMPORTANT: For each subsequent progress report, do not overwrite existing progress narratives. Instead, copy the following questions and paste to the end of this section before Section C – Final Narrative.

PROGRESS REPORTING PERIOD 1

Progress Report Number	**Reporting Period Start Date**	**Reporting Period End Date**	**Progress Report Due Date**	**Scheduled Payment Amount** (if applicable)
	[DD Month YYYY]	[DD Month YYYY]	[DD Month YYYY]	$

Progress Details

❖ What progress have you made toward achieving the project's desired outcomes? Describe what you did in this reporting period and what work is planned or anticipated for the next period.

Outputs/Funded Developments and Background Technology Update

❖ Update the table below to reflect completed and/or adjusted outputs, and new outputs/Funded Developments not previously reported. See your original results table in Section A of your proposal for reference. Add more rows as needed.

Number	**Output/Funded Development Description**	**Newly Added Output/Funded Development?**	**Status** (e.g., planned, in progress, complete, canceled)	**Target Completion Date***	**Actual Completion Date**	**Third-Party agreement required? If yes, by when?**	**Will any IP rights be filed/ generated?**
1		No/Yes		[DD Month YYYY]	[DD Month YYYY]	No/Yes	No/Yes
2		No/Yes				No/Yes	No/Yes
3		No/Yes				No/Yes	No/Yes
4		No/Yes				No/Yes	No/Yes

**Note: For items that are complete or canceled, this should be the date that was stated in the most recent progress report or in the proposal if this is the first report. For items that are not yet complete, either restate the most recent target date or propose a revised target date (e.g., ~~30 Nov 2023~~ 31 Jan 2024).*

❖ If necessary, please add further explanation below.

❖ Are there any additional Background Technologies to report which were not described in any documents previously submitted to the foundation?

___No ___Yes (please explain)

Project Adjustments

- What adjustments to the project (scope, timeline, resources) have you made over the past period or are you proposing to make?

Global Access Update

- Are there any updates to your responses to the Global Access questions in Section A?

___No ___Yes (please explain)

Roles and Responsibilities Update

- Do you represent that the project activities were conducted in compliance with all applicable ethical, legal, regulatory and safety requirements?

___Yes ___No (please explain)

- Are all the project activities described in the documents previously submitted to the foundation?

___Yes ___No (please explain and describe any new project activities)

Financial Update

The purpose of this section is to help the foundation understand expenditures in relation to the work performed under this grant during the most recent reporting period and the upcoming one(s). Please provide actual expenditures for past periods and projected expenditures for future periods by updating the Excel budget template and use this narrative document to share additional explanations. Together, this narrative section and Excel budget should provide a complete quantitative and qualitative description of the current financial state of the project.

For additional instructions, see the Grant Budgeting & Financial Reporting Detailed Instructions.

Latest Period Variance

"Latest period variance" compares expenditures that occurred in the most recent reporting period against the projections that were provided at the beginning of the same period. See the "Financial Summary & Reporting" sheet in the Excel budget template for the calculated variance (for example, column AD, starting on row 29 for period 1). The budget template also includes an "Analytics" sheet for convenient calculation of variances.

- Did the project spend more or less than anticipated? At a high level, what are the reasons for spending more or less than projected (such as, programmatic changes, delays in recruitment, etc.)?

- Please provide a detailed explanation for any expense category in which the variance was greater than 10%. This should include an explanation of relevant project decisions or changes to actual costs compared to prior assumptions.

Future Period Projections

In the Excel budget template, please provide estimated expenditures ("projections") for all future periods of the grant (for example, in the "Financial Summary & Reporting" sheet, after period 1, populate the yellow cells in column T and following). It is important that your projections are realistic and consider the latest plan of activities with updated costs. For example, projections should not just carry forward previously unspent budget amounts into the next period without reassessing expected spending for each future period. In total, the projections should not exceed the total approved grant amount. The budget template's "Analytics" sheet may be helpful for comparing current to previous projections.

Note: Over the course of a grant's life, the foundation may adjust the timing and amounts disbursed to the grantee. This is done to avoid oversized cash balances on grantee bank accounts when spending is slower than originally budgeted. Realistic and up-to-date projections are a crucial input for right-sizing disbursements.

❖ How have your projections for the remaining periods changed compared to your previous projections (from your most recent progress report, or original proposal if this is the first report) and why? Please discuss any project adjustments and shifts in scope, assumptions, timing, decisions etc. which will affect spending. If you had previous discussions with the foundation on changes, please mention.

Subawards (if applicable)

❖ If you are an Indian organization/entity subject to India's Foreign Contribution Regulation Act (FCRA), then in compliance with FCRA, do you confirm that your project does not include any subgrantees? This prohibition does not preclude subcontracts in the ordinary course of business.

___Not applicable ___Confirmed ___Not confirmed (please explain)

❖ In the table below, please provide information for all subgrantees or subcontractors receiving grant funds. The total of the column titled *Amount disbursed to subawardee in most recent reporting period* should equal the actual subaward expenses reported for this period in the Excel budget template's "Financial Summary & Reporting" sheet. Add more rows as needed.

Type of Subaward (grant or contract)	**Organization Name**	**New and/or newly identified subawardee?**	**Amount disbursed to subawardee in most recent reporting period (USD)**	**Total amount disbursed to subawardee to date* (USD)**	**Total amount spent by the subawardee to date* (USD)**	**Total subaward amount (USD)**
		No/Yes	$	$	$	$
		No/Yes	$	$	$	$
		No/Yes	$	$	$	$
		Total	**$**	**$**	**$**	**$**

**As of the end of the most recent reporting period and since the grant start date.*

❖ If not already addressed in your answers above, please provide additional explanations on subaward spending below. In particular, for subawards that are large or where actual and projected spending are significantly different from prior projections, please explain. *Note: The foundation may request updated budget files for large subawards.*

Other Sources of Support and External Factors (if applicable)

❖ Please provide an update on other sources of support and external factors that affect the financial situation of this project. This may include information on co-funding (updates to the sources listed in the Funding Plan, recent commitments secured, funding gaps and contingency plans). Please also comment on interest earned/spent, and currency exchange impacts (as applicable).

Geography Served

❖ List all countries, sub-regions, and/or states that benefit from this work and the associated dollar amounts for the full term of the grant. The total of "Foundation Funding" amounts should match the approved and/or most recently amended grant total. If any of the amounts have changed since your last report (or original proposal if no prior report), please mark with an 'X' in the third column below. As a reminder, please avoid listing "World," unless the work truly serves beneficiaries globally. If geographies include the United States, indicate city and state. Add more rows as needed.

For additional information, see the Geography and Location of Work – Frequently Asked Questions.

Geography Served	**Foundation Funding Amount (USD)**	**Has the amount changed since the proposal or last report?** (if yes, mark with an 'X')
	$	
	$	
	$	

Location of Work Updates (if applicable)

- List all locations (countries, sub-regions, and/or states) where this work is being performed and the associated dollar amounts for the full term of the grant. The total of "Foundation Funding" amounts should match the approved and/or most recently amended grant total. If any of the amounts have changed since your last report (or original proposal if no prior report), please mark with an 'X' in the third column below. As a reminder, if locations include the United States, please indicate city and state. Add more rows as needed.

  For additional information, see the Geography and Location of Work – Frequently Asked Questions.

Location of Work	**Foundation Funding Amount (USD)**	**Has the amount changed since the proposal or last report?** (if yes, mark with an 'X')
	$	
	$	
	$	

- If you listed any TBD locations or locations that are not country-specific, and/or locations that are subject to U.S. government sanctions or believe that project work could reasonably occur in a location that is subject to U.S. government sanctions, please review the guidance in the Office of Foreign Assets Control (OFAC) Guidelines and confirm that: (a) you have discussed the location of work with your foundation contact, and (b) your organization will ensure compliance throughout the term of the project with the anti-terrorism clause in our grant agreement.

  ___Confirmed ___Not confirmed (please explain)

Feedback for the Foundation

- What are 1–3 ways that the foundation successfully enabled or supported your work during this project? What are 1–3 ways that the foundation can improve?

Grantee Confirmation

By submitting this report, I declare that I am authorized to certify, on behalf of the grantee identified under Section A – Investment Details, that I have examined the responses provided and related attachments, and that to the best of my knowledge, they are true, correct, and complete.

I also confirm that the grantee identified under Section A – Investment Details has complied with all of the terms and conditions of the grant agreement, including but not limited to the clauses regarding Use of Funds, Anti-Terrorism, Subgrants and Subcontracts, and Regulated Activities.

Your Name	**Report Submitted Date**
	[DD Month YYYY]

SECTION C – FINAL NARRATIVE

For completed grants only. At the end of your grant, complete this final narrative section and submit to your foundation contact by the date indicated in your grant agreement or latest amendment. If you have any questions or need support, please reach out to your foundation contact. Please find contact information under Section A – Investment Details.

This is your final update to the foundation, please include:

- The results you achieved for the entire project
- Your perspective on what you learned
- Final financial update
- Feedback you have on the foundation's support of your work

Grant Start Date	**Grant End Date**	**Final Report Due Date**	**Remaining / Unexpended Grant Funds** (if applicable)	**Remaining / Unexpended Interest** (if applicable)	**Remaining / Unexpended Currency Gains** (if applicable)
[DD Month YYYY]	[DD Month YYYY]	[DD Month YYYY]	$	$	$

Final Results

❖ Provide information about what you achieved during the grant and your progress towards achieving the project's desired outcomes and outputs.

Outputs/Funded Developments and Background Technology

❖ Please update the results table below to reflect completed and/or adjusted outputs, and new outputs/Funded Developments not previously reported. Add more rows as needed.

Number	**Output/Funded Development Description**	**Newly Added Output/Funded Development?**	**Status** (e.g., planned, in progress, complete, canceled)	**Target Completion Date***	**Actual Completion Date**	**Third-Party agreement required? If yes, by when?**	**Were any IP rights be filed/ generated?**
1		No/Yes		[DD Month YYYY]	[DD Month YYYY]	No/Yes	No/Yes
2		No/Yes				No/Yes	No/Yes
3		No/Yes				No/Yes	No/Yes
4		No/Yes				No/Yes	No/Yes

**Note: For items that are complete or canceled, this should be the date that was stated in the most recent progress report or in the proposal if this is the first report. For items that are not yet complete, either restate the most recent target date or propose a revised target date (e.g., ~~30 Nov 2023~~ 31 Jan 2024).*

❖ If necessary, please add further explanation below.

❖ Do you confirm that you have provided a full and accurate disclosure of all outputs/Funded Developments and Background Technology as applicable to the project?

___Yes ___No (please explain)

Global Access

- Do you confirm that you have ensured and will continue to ensure Global Access for the Funded Developments of the project and, as applicable, Background Technology?

  ___Yes ___No (please explain)

Financial Update

The purpose of this section is to help the foundation understand expenditures in relation to the work performed under this grant during the most recent reporting period. Please provide actual expenditures for past periods by updating the Excel budget template and use this narrative document to share additional explanations. Together, this narrative section and Excel budget should provide a complete quantitative and qualitative description of the financial state of the project.

For additional instructions, see the Grant Budgeting & Financial Reporting Detailed Instructions.

Latest Period Variance

"Latest period variance" compares expenditures that occurred in the most recent reporting period against the projections that were provided at the beginning of the same period.

- Did the project spend more or less than anticipated? At a high level, what are the reasons for spending more or less than projected?
- Please provide a detailed explanation for any expense category in which the variance was greater than 10%. This should include an explanation of relevant project decisions or changes to actual costs compared to prior assumptions.

Subawards (if applicable)

- If you are an Indian organization/entity subject to India's Foreign Contribution Regulation Act (FCRA), then in compliance with FCRA, do you confirm that your project did not include any subgrantees? This prohibition does not preclude subcontracts in the ordinary course of business.

  ___Not applicable ___Confirmed ___Not confirmed (please explain)

- In the table below, please provide information for all subgrantees or subcontractors receiving grant funds. The total of the column titled *Amount disbursed to subawardee in most recent reporting period* should equal the actual subaward expenses reported for this period in the Excel budget template's "Financial Summary & Reporting" sheet.

Type of Subaward (grant or contract)	**Organization Name**	**New and/or newly identified subawardee?**	**Amount disbursed to subawardee in most recent reporting period (USD)**	**Total amount disbursed to subawardee to date* (USD)**	**Total amount spent by the subawardee to date* (USD)**	**Total subaward amount (USD)**
		No/Yes	$	$	$	$
		No/Yes	$	$	$	$
		No/Yes	$	$	$	$
		Total	**$**	**$**	**$**	**$**

**Over the life of the grant, from start date to end date*

- If not already addressed in your answers above, please provide additional explanations on subaward spending below. In particular, for subawards that are large or where actual spending was significantly different from prior projections, please explain. *Note: The foundation may request separate updated budget files for sub-awards.*

Other Sources of Support and External Factors (if applicable)

- ❖ Please provide an update on other sources of support and external factors that affected the financial situation of this project. This may include information on co-funding, interest earned/spent, and currency exchange impacts (as applicable).

Geography Served

- ❖ List all countries, sub-regions, and/or states that benefit from this work and the associated dollar amounts for the full term of the grant. The total of "Foundation Funding" amounts should match the approved and/or most recently amended grant total. If any of the amounts have changed since your last report (or original proposal if no prior report), please mark with an 'X' in the third column below. As a reminder, please avoid listing "World," unless the work truly serves beneficiaries globally. If geographies include the United States, indicate city and state. Add more rows as needed.

For additional information, see the Geography and Location of Work – Frequently Asked Questions.

Geography Served	**Foundation Funding Amount (USD)**	**Has the amount changed since the proposal or last report?** (if yes, mark with an 'X')
	$	
	$	
	$	

Location of Work Updates (if applicable)

- ❖ List all locations (countries, sub-regions, and/or states) where this work is being performed and the associated dollar amounts for the full term of the grant. The total of "Foundation Funding" amounts should match the approved and/or most recently amended grant total. If any of the amounts have changed since your last report (or original proposal if no prior report), please mark with an 'X' in the third column below. As a reminder, if locations include the United States, please indicate city and state. Add more rows as needed.

For additional information, see the Geography and Location of Work – Frequently Asked Questions.

Location of Work	**Foundation Funding Amount (USD)**	**Has the amount changed since the proposal or last report?** (if yes, mark with an 'X')
	$	
	$	
	$	

- ❖ Do you confirm that you have complied throughout the term of the project with the anti-terrorism clause in our grant agreement?

___Confirmed ___Not confirmed (please explain)

Lessons Learned

- What are 1–3 lessons that you learned from this project? If you were to do this project again, how would you approach things differently?

Feedback for the Foundation

- What are 1–3 ways that the foundation successfully enabled or supported your work during this project? What are 1–3 ways that the foundation can improve?

Grantee Confirmation

By submitting this report, I declare that I am authorized to certify, on behalf of the grantee identified under Section A – Investment Details, that I have examined the responses provided and related attachments, and that to the best of my knowledge, they are true, correct, and complete.

I also confirm that the grantee identified under Section A – Investment Details has complied with all of the terms and conditions of the grant agreement, including but not limited to the clauses regarding Use of Funds, Anti-Terrorism, Subgrants and Subcontracts, and Regulated Activities.

Your Name	**Report Submitted Date**
	[DD Month YYYY]

QUICK START GUIDE

This page provides a quick overview of the BMGF budget template to orient you before getting started.
As you populate the sheets, you will find direct links to specific instructions with examples throughout the template.

*The **full instruction document** can be accessed here.*

Click here for a **video** explaining how to create a **simple budget**

***Frequently Asked Questions (FAQs)** can be accessed here.*

Click here for a **video** explaining how to create a **financial report**

Main Sheets
These first three sheets need to be filled out by the grantee for every grant.

Grantees do not enter information into this sheet, but can preview the auto-generated schedule.

General Information

*Enter the required basic information into this sheet **before** populating any of the other sheets.*

→

Budget Details

For each of the 6 expense categories, enter the items that collectively make up the grant budget into this sheet.

→

Financial Summary & Reporting

Review the summary tables from the Budget Detail sheet and add further information (where applicable). This sheet is also used to enter actual and projected expenditures as part of reporting.

→

Payment Schedule

This sheet creates an auto-generated payment schedule; BMGF may make changes before incorporating it into the grant agreement.

Important information for working with the template

Legend for cell formatting

Input **cells are colored** according to the following scheme. At the time of budgeting, populate only light yellow cells.

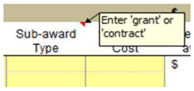
Enter information into light yellow cells
Enter actual expenditures into green cells
Blue cells are intended to be populated by BMGF

Cash vs. Projected Actuals (Accruals)

All **actual** amounts reported should be based on ***cash spent***, not PROJECTED (or ACCRUED) for the remainder of the period.

Hints for specific data entry fields

For some fields, a **red triangle in the upper right corner** indicates that a hint is available when hovering the mouse over the cell.



Error checking

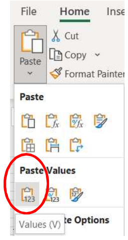
For some cells, an automatic error check will show its **value in red** if it appears to be populated incorrectly or if the calculation yields a result that is inconsistent with another value.

incorrect entry
01-Jan-22

Inconsistent result
$ 124,237

Copying data into the template

Use ***Paste Values*** whenever transferring data from an external source into the template. If not, the template can become corrupted



*In addition to the three sheets described above, there are **several additional sheets** included in the template, some of them optional and/or hidden. Grantees **do not need to use** these additional sheets, but for transparency, you will find an overview below.*

*If you would like to include additional information (or are asked for it by BMGF) in this file, it is **OK to add extra sheets** for this purpose. However, please **do not make any changes to the structure, formatting or formulas of the existing sheets.***

BMGF Work Sheets
These three sheets are primarily used by BMGF to analyze and manage the grant. You may use them yourself, but please do not enter or alter any data or formulas.

Payment Schedule

This sheet is used by the grantee to review an auto-generated preliminary payment schedule and by BMGF to make any changes before incorporating it into the grant agreement.

Analytics

Contains two analytic features: a set of Basic Charts and a Comparison Tool

Budget Pivot
(typically hidden)

Excel Pivot table that can be used to analyze the data in the Budget Detail sheet.

Historic or Background Information
These sheets are used by BMGF to preserve historic information throughout the grant or for administrative purposes. Please do not make any changes to these sheets.

[Historic Budget Details]
0, 1 or more sheets (typically hidden)

If a grant budget revision takes place, the previous version of the Budget Detail sheet can be preserved as a separate sheet for reference.

Historic Budget Summaries
(typically hidden)

If grant budget revisions take place, this sheet can be used to preserve previous versions of the Budget Summary for reference.

Config
(typically hidden)

Used for administrative purposes to populate dropdown lists etc. throughout the template.

HTS for small molecule drug development for henipaviruses
University of Chicago

BILL & MELINDA
GATES foundation

Prepared by: Megan Konley

Date submitted:

Template version 2022-01-04

Legend for cell formatting:
Enter information into light yellow cells
Enter actual expenditures into green cells
Blue cells are intended to be populated by BMGF

GENERAL INFORMATION

Go to instructions for this page…

Proposal Information

Organization Name	HTS for small molecule drug development for henipaviruses
Investment Name	University of Chicago
Investment ID	INV-048917
Is this a Subaward Budget?	No
Requested Grant Amount	**$2,954,167**
Total Project Cost	**$2,954,167**

Budgeting & Reporting Periods

Please ensure that you are selecting the correct reporting cadence before inputting the budget details. If you need to readjust the reporting periods, the budget details will need to be revised as well to reflect the date changes.
Note that if your grant is an Expenditure Responsibility (ER) grant, you must select "**Align with fiscal year**" as the reporting cadence.

Anticipated Start Date	01-Oct-22
Anticipated End Date	30-Sep-25
Project Duration (months)	36

Preferred Reporting Cadence Custom periods

Reporting Periods	Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8	Period 9	Period 10
Period Start Date	**01-Oct-22**	**01-Jan-24**	**01-Jan-25**							
Period End Date	**31-Dec-23**	**31-Dec-24**	**30-Sep-25**							
Number of Months	15.0	12.0	9.0							
Custom Period End Dates	31-Dec-23	31-Dec-24	30-Sep-25							

Indirect Cost Rate(s)

Please enter indirect cost rates in accordance with the indirect cost policy:
Read BMGF Indirect Cost policy…

Organization Type U.S. university

Indirect Cost Rate on Primary Grantee's Portion	10.00%	*Maximum rates for selected organization type are 10%.*
Primary Grantee's Indirect Cost Rate on Subaward Portion	10.00%	

Other Budget Factors

More info..	**Will the financials of this project be managed in a currency other than USD?**	No
More info..	**Will the budget be broken down by an additional dimension?** (e.g. outcomes/outputs, project components/phases)	No
More info..	**Will the total cost of this project require contributions of funding from sources other than BMGF?** (e.g. either by your organization or others)	No
More info..	**Is this a new version of a previously approved budget?** (e.g. supplement)	No

BUDGET DETAILS

Go to instructions for this page…

Enter budget details for each expenditure category below.

	Description	Purpose	Unit Cost	Period 1 Oct-22 - Dec-23 Quantity	Period 2 Jan-24 - Dec-24 Quantity	Period 3 Jan-25 - Sep-25 Quantity	Period 4 - Quantity	Period 5 - Quantity	Addtl. Info	Addtl. Info	Period 1 Oct-22 - Dec-23 Amount	Period 2 Jan-24 - Dec-24 Amount	Period 3 Jan-25 - Sep-25 Amount	Period 4 - Amount	Period 5 - Amount	TOTAL AMOUNT	TOTAL UNITS	% of Total Direct Cost
Personnel				**4.07**	**3.45**	**2.58**	**-**	**-**			**$ 350,354**	**$ 293,690**	**$ 219,881**	**$ -**	**$ -**	**$ 863,925**	**10.10**	**32.2%**
	Name	Job Title	Annual Salary per FTE	Period 1 FTE Allocation	Period 2 FTE Allocation	Period 3 FTE Allocation	Period 4 FTE Allocation	Period 5 FTE Allocation	% Inflation (Annualized)	% Benefits	Period 1 Personnel Cost	Period 2 Personnel Cost	Period 3 Personnel Cost	Period 4 Personnel Cost	Period 5 Personnel Cost	Total Personnel Cost	Total FTE Allocation	% of Total Direct Cost
1	Lijun Rong	Professor	$168,318	0.13	0.10	0.08			0%	37%	$ 28,860	$ 23,088	$ 17,316	$ -	$ -	$ 69,265	0.30	2.6%
2	Irina Gaisina	Research Associate Professor	$86,554	0.38	0.30	0.22			0%	37%	44,909	35,618	26,327	-	-	106,854	0.90	4.0%
3	Ryan Bott	Research Scientist	$60,000	0.63	0.50	0.38			0%	37%	51,439	41,151	30,863	-	-	123,453	1.50	4.6%
4	Malaika Argade	Research Scientist	$56,925	0.69	0.55	0.41			0%	37%	53,683	42,946	32,210	-	-	128,839	1.65	4.8%
5	Laura Cooper	Postdoctoral Associate	$60,000	1.25	1.00	0.75			0%	37%	102,878	82,302	61,727	-	-	246,906	3.00	9.2%
6	TBN	Research Technician	$50,000	1.00	1.00	0.75			0%	37%	68,585	68,585	51,439	-	-	188,609	2.75	7.0%
7											-	-	-	-	-	-	-	0.0%
8											-	-	-	-	-	-	-	0.0%
9											-	-	-	-	-	-	-	0.0%
10											-	-	-	-	-	-	-	0.0%
11											-	-	-	-	-	-	-	0.0%
12											-	-	-	-	-	-	-	0.0%
13											-	-	-	-	-	-	-	0.0%
14											-	-	-	-	-	-	-	0.0%
15											-	-	-	-	-	-	-	0.0%

← Unhide additional rows if needed

Travel											**$ 3,000**	**$ 3,000**	**$ 3,000**	**$ -**	**$ -**	**$ 9,000**	**3.00**	**0.3%**
	Travel Cost Item Description	Purpose of Trip	Cost per Item	Period 1 No. of Items	Period 2 No. of Items	Period 3 No. of Items	Period 4 No. of Items	Period 5 No. of Items	N/A	N/A	Period 1 Travel Cost	Period 2 Travel Cost	Period 3 Travel Cost	Period 4 Travel Cost	Period 5 Travel Cost	Total Travel Cost	Total Number of Units	% of Total Direct Cost
1	Antiviral research meetings	To attend and present our work	$3,000	1	1	1					$ 3,000	$ 3,000	$ 3,000	$ -	$ -	$ 9,000	3.00	0.3%
2											-	-	-	-	-	-	-	0.0%
3											-	-	-	-	-	-	-	0.0%
4											-	-	-	-	-	-	-	0.0%
5											-	-	-	-	-	-	-	0.0%
6											-	-	-	-	-	-	-	0.0%
7											-	-	-	-	-	-	-	0.0%
8											-	-	-	-	-	-	-	0.0%
9											-	-	-	-	-	-	-	0.0%
10											-	-	-	-	-	-	-	0.0%
11											-	-	-	-	-	-	-	0.0%
12											-	-	-	-	-	-	-	0.0%
13											-	-	-	-	-	-	-	0.0%
14											-	-	-	-	-	-	-	0.0%
15											-	-	-	-	-	-	-	0.0%

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Consultants											**$ 15,000**	**$ 12,000**	**$ 9,000**	**$ -**	**$ -**	**$ 36,000**	**120**	**1.3%**
	Consultant Name	Role / Purpose of Engagement	Billing Rate / Project Cost	Period 1 Billable Units	Period 2 Billable Units	Period 3 Billable Units	Period 4 Billable Units	Period 5 Billable Units	Billing Unit / Expense	N/A	Period 1 Consultants Cost	Period 2 Consultants Cost	Period 3 Consultants Cost	Period 4 Consultants Cost	Period 5 Consultants Cost	Total Consultants Cost	Total Number of Units	% of Total Direct Cost
1	Norton Peet	supervises ADME/Tox and PK stud	$300	50	40	30					$ 15,000	$ 12,000	$ 9,000	$ -	$ -	$ 36,000	120.00	1.3%
2											-	-	-	-	-	-	-	0.0%
3											-	-	-	-	-	-	-	0.0%
4											-	-	-	-	-	-	-	0.0%
5											-	-	-	-	-	-	-	0.0%
6											-	-	-	-	-	-	-	0.0%
7											-	-	-	-	-	-	-	0.0%
8											-	-	-	-	-	-	-	0.0%
9											-	-	-	-	-	-	-	0.0%
10											-	-	-	-	-	-	-	0.0%

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Capital Equipment											**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**-**	**0.0%**
	Equipment Description	Purpose of Equipment	Cost per Item	Period 1 Quantity	Period 2 Quantity	Period 3 Quantity	Period 4 Quantity	Period 5 Quantity	N/A	N/A	Period 1 Equipment Cost	Period 2 Equipment Cost	Period 3 Equipment Cost	Period 4 Equipment Cost	Period 5 Equipment Cost	Total Equipment Cost	Total Number of Units	% of Total Direct Cost
1											$ -	$ -	$ -	$ -	$ -	$ -	-	0.0%
2											-	-	-	-	-	-	-	0.0%
3											-	-	-	-	-	-	-	0.0%
4											-	-	-	-	-	-	-	0.0%
5											-	-	-	-	-	-	-	0.0%

← Unhide additional rows if needed

Other Direct Costs											**$ 253,371**	**$ 138,000**	**$ 122,250**	**$ -**	**$ -**	**$ 513,621**	**26**	**19.1%**
	Item description	Purpose of Item	Cost per Item	Period 1 Quantity	Period 2 Quantity	Period 3 Quantity	Period 4 Quantity	Period 5 Quantity	N/A	N/A	Period 1 Other Direct Cost	Period 2 Other Direct Cost	Period 3 Other Direct Cost	Period 4 Other Direct Cost	Period 5 Other Direct Cost	Total Other Direct Cost	Total Number of Units	% of Total Direct Cost
1	ChemDiv Antiviral library	For HTS to identify new hits	$124,621	1.00	-	-					$ 124,621	$ -	$ -	$ -	$ -	$ 124,621	1.00	4.6%
2	HTS supplies (plates, tips. Reagents, etc)	For Rong Lab HTS	$15,000	1.00	-	-					15,000	-	-	-	-	15,000	1.00	0.6%
3	Tissue culture work supplies	for Rong Lab bioassays	$15,000	1.00	1.00	1.00					15,000	15,000	15,000	-	-	45,000	3.00	1.7%
4	Reagents for DNA and protein work	for Rong Lab supplies	$12,000	1.00	1.00	1.00					12,000	12,000	12,000	-	-	36,000	3.00	1.3%
5	Chemicals and solvents	Compounds syntheses	$23,000	1.25	1.00	0.75					28,750	23,000	17,250	-	-	69,000	3.00	2.6%
6	Chromatography supplies	Purification	$3,000	1.00	1.00	1.00					3,000	3,000	3,000	-	-	9,000	3.00	0.3%
7	Glassware and plasticware	Synthesis and storage	$3,000	1.00	1.00	1.00					3,000	3,000	3,000	-	-	9,000	3.00	0.3%
8	Instrument fees	NMR/MS service fees	$17,000	1.00	1.00	1.00					17,000	17,000	17,000	-	-	51,000	3.00	1.9%
9	ADME/Tox and PK screening	Characterization of Lead compds	$35,000	1.00	1.71	1.43					35,000	60,000	50,000	-	-	145,000	4.14	5.4%
10	publication costs		$5,000		1.00	1.00					-	5,000	5,000	-	-	10,000	2.00	0.4%
11											-	-	-	-	-	-	-	0.0%
12											-	-	-	-	-	-	-	0.0%
13											-	-	-	-	-	-	-	0.0%
14											-	-	-	-	-	-	-	0.0%
15											-	-	-	-	-	-	-	0.0%
16											-	-	-	-	-	-	-	0.0%
17											-	-	-	-	-	-	-	0.0%
18											-	-	-	-	-	-	-	0.0%
19											-	-	-	-	-	-	-	0.0%
20											-	-	-	-	-	-	-	0.0%

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Subawards											**$ 454,377**	**$ 371,778**	**$ 436,906**	**$ -**	**$ -**	**$ 1,263,061**	**1,263,061**	**47.0%**
	Subaward Organization	Scope of Subaward	N/A	Period 1 Amount	Period 2 Amount	Period 3 Amount	Period 4 Amount	Period 5 Amount	Subaward Type	% Indirect Cost	Period 1 Subaward Cost	Period 2 Subaward Cost	Period 3 Subaward Cost	Period 4 Subaward Cost	Period 5 Subaward Cost	Total Subaward Cost	Total Number of Units	% of Total Direct Cost
1	The University of Texas Medical Branch at Galveston	in vitro and in vivo studies		$260,000	$210,000	$310,000			Contract	10%	$ 260,000	$ 210,000	$ 310,000	$ -	$ -	$ 780,000		29.0%
2	The University of Washington Seattle	Stuctural studies of drug/RdRp		$194,377	$161,778	$126,906			Contract	10%	194,377	161,778	126,906	-	-	483,061		18.0%
3											-	-	-	-	-	-		0.0%
4											-	-	-	-	-	-		0.0%
5											-	-	-	-	-	-		0.0%
6											-	-	-	-	-	-		0.0%

7											-	-	-	-	-	-	0.0%
8											-	-	-	-	-	-	0.0%
9											-	-	-	-	-	-	0.0%
10											-	-	-	-	-	-	0.0%

← Unhide additional rows if needed

FINANCIAL SUMMARY & REPORTING

Expand/Collapse sections to see/hide the budget and actuals & projections →

Budget

Prepared by: Megan Konley

*Go to **budgeting** instructions for this page…*

*Go to **reporting** instructions for this page…*

	Period 1	Period 2	Period 3	Period 4	Period 5	
	Oct-22 - Dec-23	**Jan-24 - Dec-24**	**Jan-25 - Sep-25**	**-**	**-**	
	Budget	**Budget**	**Budget**	**Budget**	**Budget**	

Cash Flow Summary (BMGF Funds Only)

Note: Grantees are not expected to populate the blue cells

Revenue						TOTAL
BMGF Payment(s)						$ -
Interest Earned	-	-	-	-	-	-
Currency Gains / (Losses)	-	-	-	-	-	-
Carry-over Amount from Prior Period	-	(1,183,712)	(2,084,026)	(2,954,167)	(2,954,167)	N/A
TOTAL CASH AVAILABLE BY PERIOD	**-**	**(1,183,712)**	**(2,084,026)**	**(2,954,167)**	**(2,954,167)**	**N/A**
Expenditure						
BMGF Funds Spent on Direct Cost	1,076,102	818,468	791,037	-	-	2,685,607
BMGF Funds Spent on Indirect Cost	107,610	81,847	79,104	-	-	268,561
Interest Spent	-	-	-	-	-	-
TOTAL EXPENDITURE BY PERIOD	**1,183,712**	**900,315**	**870,141**	**-**	**-**	**2,954,167**
BALANCE AT PERIOD END	**$ (1,183,712)**	**$ (2,084,026)**	**$ (2,954,167)**	**$ (2,954,167)**	**$ (2,954,167)**	**N/A**

Summary by Expense Category

Category						TOTAL	*% of Total Direct Cost*
Personnel	$ 350,354	$ 293,690	$ 219,881	$ -	$ -	$ 863,925	*32%*
Travel	3,000	3,000	3,000	-	-	9,000	*0%*
Consultants	15,000	12,000	9,000	-	-	36,000	*1%*
Capital Equipment	-	-	-	-	-	-	*0%*
Other Direct Costs	253,371	138,000	122,250	-	-	513,621	*19%*
Subawards	454,377	371,778	436,906	-	-	1,263,061	*47%*
TOTAL DIRECT COST	**1,076,102**	**818,468**	**791,037**	**-**	**-**	**2,685,607**	***100%***
Indirect Cost	107,610	81,847	79,104	-	-	268,561	*10%*
TOTAL COST	**$ 1,183,712**	**$ 900,315**	**$ 870,141**	**$ -**	**$ -**	**$ 2,954,167**	***110%***

Breakdown along Additional Dimension

						TOTAL	*% of Total Direct Cost*
N/A	$ -	$ -	$ -	$ -	$ -	$ -	
N/A	-	-	-	-	-	-	
N/A	-	-	-	-	-	-	
N/A	-	-	-	-	-	-	
N/A	-	-	-	-	-	-	
TOTAL DIRECT COST	-	-	-	-	-	-	

Total Project Cost

Category						TOTAL	*% of Total*
Personnel						$ -	*0%*
Travel						-	*0%*
Consultants						-	*0%*
Capital Equipment						-	*0%*
Other Direct Costs						-	*0%*
Sub-awards						-	*0%*
Indirect Costs						-	*0%*
TOTAL PROJECT COST	**$ 1,183,712**	**$ 900,315**	**$ 870,141**	**$ -**	**$ -**	**2,954,167**	***100%***

Funding Plan

Sources of Funding							TOTAL	*% of Total*
	BMGF - Direct Cost	$ -	$ -	$ -	$ -	$ -	$ -	
	BMGF - Indirect Cost	-	-	-	-	-	-	
1							-	
2							-	
3							-	
4							-	
5							-	
TOTAL FUNDING PLAN		-	-	-	-	-	-	
Total BMGF and Secured Funding							-	
Total Potential Funding		-	-	-	-	-	-	
OVER/(UNDER) FUNDING		**$ (1,183,712)**	**$ (900,315)**	**$ (870,141)**	**$ -**	**$ -**	**$ (2,954,167)**	
Cumulative overage / (underage)		(1,183,712)	(2,084,026)	(2,954,167)	(2,954,167)	(2,954,167)	(2,954,167)	

Currency

Exchange Rates					
Budget rate used to convert from … to USD	-	-	-	-	-
… spot rate at end of period					

Bank Balances (BMGF project funds only)					
Balance held in USD at end of period					
Balance held in … at end of period					
Balance held in other currencies at end of period					

From: Rong, Lijun on behalf of Rong, Lijun <lijun@uic.edu>
To: Broder, Christopher; Geisbert, Thomas W.; David Veesler
Subject: FW: PAD henipavirus RFP
Date: Monday, July 25, 2022 6:10:13 PM
Attachments: INV-048917_UIC_Investment Document_proposal-07-25-22.docx
Copy of FINAL_ INV-048917_UIC_budget_proposalJuly2022 (003).xlsx

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Hi all,

Fyi, we just submitted the full proposal to Gates Foundation. I assume they will work with us to make it fit their requirements.
Hopefully we will be funded (cautiously optimistic).

Best,
Lijun

Lijun Rong, PhD
Professor
Dept of Microbiology and Immunology
College of Medicine
University of Illinois at Chicago (UIC)
8133 COMRB, 909 S Wolcott Ave
Chicago, IL 60612
312-355-0203 (office phone)
lijun@uic.edu

From: Rong, Lijun
Sent: Monday, July 25, 2022 1:51 PM
To: Lisa Galaites <Lisa.Galaites@gatesfoundation.org>; Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Konley, Megan E. <mkonley@uic.edu>
Subject: RE: PAD henipavirus RFP

Dear Rob, Linu, and Lisa,

Attached are the full proposal from us. Please acknowledge when you receive it. We look forward to working with you to make this “perfect”. Please kindly let us know if we miss anything. Thank you so much for your guidance!

Best,
Lijun

Lijun Rong, PhD
Professor
Dept of Microbiology and Immunology

College of Medicine
University of Illinois at Chicago (UIC)
8133 COMRB, 909 S Wolcott Ave
Chicago, IL 60612
312-355-0203 (office phone)
lijun@uic.edu

From: Lisa Galaites <Lisa.Galaites@gatesfoundation.org>
Sent: Monday, July 25, 2022 1:46 PM
To: Rong, Lijun <lijun@uic.edu>; Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Konley, Megan E. <mkonley@uic.edu>
Subject: RE: PAD henipavirus RFP

Hi Lijun,
Submitting through email is preferred. If the files are too big to send via email, please send us a Dropbox link or I'm happy to set up a MS Teams site for file sharing.
Thanks!

Lisa Galaites (she/her)
Senior Program Coordinator
Global Health | Discovery & Translational Sciences

From: Rong, Lijun <lijun@uic.edu>
Sent: Monday, July 25, 2022 2:41 PM
To: Lisa Galaites <Lisa.Galaites@gatesfoundation.org>; Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Konley, Megan E. <mkonley@uic.edu>
Subject: RE: PAD henipavirus RFP

Dear Lisa,

Could you please let us know the link we need to submit the proposal? Thank you so much!

Best,
Lijun

Lijun Rong, PhD
Professor
Dept of Microbiology and Immunology
College of Medicine
University of Illinois at Chicago (UIC)
8133 COMRB, 909 S Wolcott Ave
Chicago, IL 60612
312-355-0203 (office phone)

lijun@uic.edu

From: Lisa Galaites <Lisa.Galaites@gatesfoundation.org>
Sent: Wednesday, July 13, 2022 1:11 PM
To: Rong, Lijun <lijun@uic.edu>; Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Konley, Megan E. <mkonley@uic.edu>
Subject: RE: PAD henipavirus RFP

Hi Lijun,
We don't require any signature at this point as we shape the proposal documents. Once both parties have agreed on the final proposal documents (we typically go back and forth on a few iterations of the Investment Document and budget), we will then create the Grant Agreement that references both proposal documents. It is the Grant Agreement that will need a signature from UIC.
Hope that helps!

Lisa Galaites (she/her)
Senior Program Coordinator
Global Health | Discovery & Translational Sciences

From: Rong, Lijun <lijun@uic.edu>
Sent: Monday, July 11, 2022 11:25 AM
To: Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Lisa Galaites <Lisa.Galaites@gatesfoundation.org>; Konley, Megan E. <mkonley@uic.edu>
Subject: RE: PAD henipavirus RFP

Thank you Rob for your prompt response!
Lisa, please advise us how to proceed.

Best,
Lijun

Lijun Rong, PhD
Professor
Dept of Microbiology and Immunology
College of Medicine
University of Illinois at Chicago (UIC)
8133 COMRB, 909 S Wolcott Ave
Chicago, IL 60612
312-355-0203 (office phone)
lijun@uic.edu

From: Rob Jordan <robert.jordan@gatesfoundation.org>
Sent: Monday, July 11, 2022 9:19 AM

To: Rong, Lijun <lijun@uic.edu>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Lisa Galaites <Lisa.Galaites@gatesfoundation.org>; Konley, Megan E. <mkonley@uic.edu>
Subject: RE: PAD henipavirus RFP

Hi Lijun,
I believe and email approval will be sufficient but I will let Lisa provide the final word here. Lisa is out of the office until Wednesday this week.
Best regards,
Rob

From: Rong, Lijun <lijun@uic.edu>
Sent: Monday, July 11, 2022 9:47 AM
To: Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Lisa Galaites <Lisa.Galaites@gatesfoundation.org>; Konley, Megan E. <mkonley@uic.edu>
Subject: RE: PAD henipavirus RFP

Hi Rob and Linu,

Megan will help us to get the proposal and budget together here at UIC. We need some clarification on the institutional approval.
What do you need from UIC, a letter or email from UIC? Thank you so much for the information.

Best regards,
Lijun

Lijun Rong, PhD
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lijun@uic.edu

From: Rong, Lijun
Sent: Wednesday, July 6, 2022 11:52 AM
To: Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Lisa Galaites <Lisa.Galaites@gatesfoundation.org>
Subject: RE: PAD henipavirus RFP

Hi Rob and all,

We look forward to talking with you tmmr.

Best,
Lijun

Lijun Rong, PhD
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lijun@uic.edu

From: Rob Jordan <robert.jordan@gatesfoundation.org>
Sent: Wednesday, July 6, 2022 10:41 AM
To: Rong, Lijun <lijun@uic.edu>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>; Lisa Galaites <Lisa.Galaites@gatesfoundation.org>
Subject: RE: PAD henipavirus RFP

Hi Lijun,
Thursday July 7 at 1p seems like a good time to meet. Please invite others who will participate in the proposal. Lisa Galaites (cc'd) will provide the grant template, budget template and instructions for the proposal. We would like to receive a full proposal for review by July 22. Two areas that we would like to discuss are your plans for medicinal chemistry and overlap with AViDD programs. I will send the meeting invitation for 1p PST July 7.
We look forward to speaking with you soon.
Best regards,
Rob

From: Rong, Lijun <lijun@uic.edu>
Sent: Friday, July 1, 2022 9:24 AM
To: Rob Jordan <robert.jordan@gatesfoundation.org>
Cc: Linu John <linu.john@gatesfoundation.org>; Gaisina, Irina N <igaysina@uic.edu>
Subject: RE: PAD henipavirus RFP

Dear Rob and Linu,

We have very pleased and honored to be accepted to submit a full proposal from the PAD program, and we are eager to learn about the next step, and seek your guidance on it.

I am available next week:

Thursday July 7, 3 pm-5 pm (Central time) or Pacific time: 1-3 pm
Friday July 8th 3-5 pm (CT), or PT: 1-3 pm

Does that work out for you?

Also is it OK if I include Dr. Irina Gaisina to this meeting since she will work with me on the full proposal?

We look forward to talking with you!

Best,
Lijun

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From: Rob Jordan <robert.jordan@gatesfoundation.org>
Sent: Friday, July 1, 2022 6:49 AM
To: Rong, Lijun <lijun@uic.edu>
Cc: Linu John <linu.john@gatesfoundation.org>
Subject: PAD henipavirus RFP

Dear Lijun,

Congratulations on being accepted to submit a full proposal for the PAD program. It would be good for us to have a quick call to answer questions and make sure we're on the same page with the process. We would like to have the full proposal submitted by July 22 if possible so that we can go through the internal review process and make any adjustments as needed. I will be the senior program officer (SPO) managing this proposal and my colleague Linu John will be helping as the secondary program officer.

Please let me know a few dates and times next week (after July 5) that you might be available for a call.

We look forward to working with you on this interesting project.
Best regards,
Rob

Robert Jordan, PhD
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SUMMARY STATEMENT

(Privileged Communication)

PROGRAM CONTACT:
Eun-Chung Park
240-627-3338
epark@niaid.nih.gov

Release Date: **06/24/2022**
Revised Date:

Application Number: **1 R01 AI168287-01A1**
Formerly: **1R01AI168287-01**

Principal Investigator

GURLEY, EMILY SUZANNE

Applicant Organization: JOHNS HOPKINS UNIVERSITY

Review Group: **CRFS**
Clinical Research and Field Studies of Infectious Diseases Study Section

Meeting Date: **06/16/2022**
Council: **OCT 2022**
Requested Start: **09/01/2022**

RFA/PA: **PA20-185**
PCC: **M32A B**

Project Title: **Solving Opportunities for Spillover (SOS): Frequency and Mechanisms of Cross-species Transmission of Henipaviruses in Bangladesh**
SRG Action: **Impact Score:20 Percentile:5**
Next Steps: **Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects: 30-Human subjects involved - Certified, no SRG concerns
Animal Subjects: 30-Vertebrate animals involved - no SRG concerns noted
Gender: 1A-Both genders, scientifically acceptable
Minority: 5A-Only foreign subjects, scientifically acceptable
Age: 1A-Children, Adults, Older Adults, scientifically acceptable

Project Year	Direct Costs Requested	Estimated Total Cost
1	499,647	671,913
2	499,444	671,640
3	498,172	669,930
4	499,809	672,131
5	498,639	670,558
TOTAL	2,495,711	3,356,172

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.
EARLY STAGE INVESTIGATOR
NEW INVESTIGATOR

1R01AI168287-01A1 GURLEY, EMILY

EARLY STAGE INVESTIGATOR
NEW INVESTIGATOR
ADMINISTRATIVE NOTE

RESUME AND SUMMARY OF DISCUSSION: This application proposes to investigate the drivers of spillover events of henipavirus from bats to domesticated animals to humans. The characterization of transmission routes as well as molecular and serological analyses to assess exposure to henipavirus are highly significant and address an important knowledge gap. The work is performed by an outstanding team of investigators with complementary expertise in virology, epidemiology, zoonotic diseases; as well as a solid track record of working together with collaborators in Bangladesh. Strengths of the application also include highly significant biomedical and public health problems being addressed, well-developed approach including comprehensive sampling and multiplex pan-henipavirus assays, the integration of modeling input on weather patterns, and the use of camera trapping observations to determine interactions between domesticated animals with bats. Most of the critiques from previous review are adequately addressed including additional details of biosafety protocols on handling the virus. A minor concern identified during the discussion is the lack of details on statistical analysis method. Overall, the application is considered outstanding and high enthusiasm is expressed by the review committee.

DESCRIPTION (provided by applicant): Until and unless we better understand and prevent spillovers of bat-borne viruses into intermediate hosts and humans, we will be severely limited in our ability to stop pandemics. Henipaviruses are bat-borne, RNA viruses that spillover through this route. Nearly half of all reported human henipavirus infections have been associated with contact with sick domesticated animals, though our understanding of drivers of henipavirus spillovers and specific transmission pathways remains severely limited. Building on preliminary data about bat, domesticated animal and human infections, this multidisciplinary study integrates epidemiology, ecology, and anthropology to identify spillover pathways for henipaviruses into domesticated animals in Bangladesh and the risk they pose to human health. Our first aim is to identify drivers of henipavirus spillovers into domesticated animals in Faridpur, Bangladesh. We will use multiplex pan-henipavirus assays to identify infections in Pteropus medius bats and domesticated animals living nearby this roost through cross-sectional and prospective studies. Combined with intensive studies of bat-domesticated animal interactions and weather data, we will build statistical models to identify the relative contribution of each of these factors. Our second aim is to describe which henipaviruses are being transmitted from bats to domesticated animals. Serologic studies of animals in Bangladesh show that they are frequently infected with non-Nipah henipaviruses. Through surveillance for and sampling of sick domesticated animals, we will describe the specific viruses that spillover from bats in Faridpur. Our third aim is to determine the risk of henipavirus transmission from domesticated animals to humans. We hypothesize that undetected henipavirus spillovers in humans are occurring through contact with sick domesticated animals and will conduct cross-sectional and prospective serosurveys of humans who have close contact with sick animals in Faridpur. We remain ignorant about henipavirus spillovers through intermediate hosts – including the specific viruses spilling over, the frequency and distribution of spillovers, and the pathways of transmission – at our own peril. The knowledge gained from this study will be immediately applicable to human and animal health programs in Bangladesh and other countries where henipaviruses circulate in bats. By learning about which henipaviruses infect humans, and how they are infected, we can advise public health surveillance programs on how to optimize detection and epidemiologic investigation of cases across Bangladesh. Our investigations about spillovers in Faridpur can also be scaled- up to other areas of Bangladesh and countries where henipaviruses circulate in bats so that we can truly begin to appreciate the scale of henipavirus spillovers in the global landscape.

PUBLIC HEALTH RELEVANCE: We remain ignorant about bat henipavirus spillovers through intermediate hosts – including the specific viruses spilling over, the frequency and distribution of spillovers, and the pathways of transmission – at our own peril. Until and unless we better understand and prevent spillovers of bat-borne viruses into intermediate hosts and humans, we will be severely limited in our ability to stop pandemics. The knowledge gained from this study will be immediately applicable to human and animal health programs because if we know which henipaviruses infect humans and domesticated animals, and how they are infected, we can advise public health surveillance programs on how to optimize detection and prevention of infections.

CRITIQUE 1:

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 3
Environment: 1

Overall Impact: This proposal addresses a highly significant and timely set of questions around spillover of viruses from animals to humans with a focus on the role of understudied intermediate hosts. The investigators propose to obtain a comprehensive picture of viral transmission at the animal-human interface, with a focus on henipavirus. There is a well-considered strategy for sampling both animals and humans that addresses a gap and bias in existing surveillance data identified by the investigators. The study team has tremendous experience both in the technical areas as well as working with this community, and this comes through clearly in the proposal. The revised proposal has addressed the major concerns from the initial review around biosafety with a select agent and have provided details about safety protocols for both the study team and for the collaborating laboratory in the US. There are some areas of the proposal where further detail about the statistical analysis plan could be further developed, but this was a minor concern. Overall, the successful completion of this project would yield novel insights into routes of viral spillover at the animal-human interface.

1. Significance:

Strengths

- Spillovers of henipavirus are a major concern, and the proposed work will provide a comprehensive understanding of how those spillovers occur.
- The role of intermediate hosts has been understudied due to typical sampling protocols.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- PI Dr. Gurley and collaborators have long track record working on Nipah virus in the region.
- Combined study team is highly interdisciplinary.
- Strong collaboration with researchers at icddr,b.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Interdisciplinary combination of epidemiology, anthropology, ecology, immunology is innovative.
- Focus on potential gaps in current reactive sampling schemes with a focus on intermediate hosts.
- Strong conceptual model that identifies weaknesses in current data.

Weaknesses

- None noted.

4. Approach:

Strengths

- Comprehensive sampling of multiple animal species and humans will allow for a comprehensive view of spillover pathways.
- Possibility to detect novel/unexpected viruses.

Weaknesses

- The statistical analysis/model for Aim 1could be better developed. These are complex time series data with many outcomes.
- As the investigators acknowledge, the number of events is unpredictable, which could limit the ability to make strong inferences.

5. Environment:

Strengths

- Facilities at Rocky Mountain labs appropriate for BSL-4 work.
- Facilities at USUHS and Johns Hopkins are appropriate for the proposed work.
- icddr,b has tremendous experience conducting field studies with humans and animals in Bangladesh.

Weaknesses

- None noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections.

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically.
- Race/Ethnicity: Distribution justified scientifically.

- Inclusion/Exclusion Based on Age: Distribution justified scientifically.

Vertebrate Animals:

YES, all criteria addressed.

Biohazards:

Acceptable protocols in place for handling the pathogens.

Resubmission:

The investigators were highly responsive to previous reviews, including comments about biosafety and questions about the assays.

Applications from Foreign Components:

icddr,b in Bangladesh is a key partner in this proposal and necessary for the completion of the Aims.

Select Agents:

BSL-4 work will be conducted at Rocky Mountain Laboratories in the US; Personnel in Bangladesh will handle shipments for shipping at BSL-2 and will inactivate sera prior to testing in the BSL-2 lab.

Resource Sharing Plans:

Acceptable.

Authentication of Key Biological and/or Chemical Resources:

Not included.

Budget and Period of Support:

Recommended as requested.

CRITIQUE 2:

Significance: 3
Investigator(s): 2
Innovation: 2
Approach: 3
Environment: 1

Overall Impact: This reversed application seeks to identify spillover pathways for henipaviruses into domesticated animals in Faridpur, Bangladesh and identify the risk they pose to human health. They report that nearly half of all reported human henipavirus infections have been associated with contact with sick domesticated animals, though our understanding of drivers of henipavirus spillovers and specific transmission pathways remains severely limited. Preventing spillovers of bat-borne viruses into intermediate hosts is critical to stopping pandemics. In Aim 1 they will identify drivers of henipavirus spillovers into domesticated animals. To do this they will use multiplex pan-henipavirus assays to identify infections in bats and domesticated animals living nearby roosts. They speculate that that there is also substantial temporal variation in domesticated animal spillovers driven by infection dynamics in bats, weather patterns, and domesticated animal contact with bats. They will build statistical models to identify the relative contribution of each of these factors to spillover into potential intermediate hosts. In Aim 2, they will describe which henipaviruses are being transmitted from bats to domesticated animals. They will sample sick animals in Faridpur and will sequence any detected henipaviruses. In Aim 3, they will determine the risk of henipavirus transmission from domesticated animals to humans. They will identify human henipavirus infections following contact with domesticated animals and identify the types of contact associated with transmission. Strengths of the application include the public health importance and potential relevance of findings to other zoonotic viruses, the superb study team with prior collaborative work in the study site, the clear enrollment and sampling strategy, and inclusion of children who have potential to care for sick animals. Weaknesses are minor and include reliance of aims on detecting virus and reliance of aims on prior aims although this is inherent in nature of this study and a mitigation plan is outlined. Overall enthusiasm for this application is high.

1. Significance:

Strengths

- Need to better understand spillovers of bat-borne viruses into intermediate host to improve ability to prevent pandemics.
- Spillovers into intermediate hosts represent important opportunities for bat-borne viruses as they adapt and become more infectious to humans.
- Goal of updating public health surveillance systems to reduce pandemic risk is critical.

Weaknesses

- Unclear generalizability of findings to other pathogens although given public health importance of knowledge gained this could be viewed as minor.

2. Investigator(s):

Strengths

- Expertise in epidemiology, virology, infectious disease, microbiology, zoonotic diseases, anthropology, modeling.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Use of multiplex pan-henipavirus assay.
- Planned field work to study pathway from bats to intermediate host to humans.
- Study setting is optimal for proposed analyses.

Weaknesses

- None noted.

4. Approach:

Strengths

- Optimal study site (Faridpur District) for proposed analyses.
- Use of the multiplex pan-henipavirus assay will be used to detect all five known henipaviruses (Nipah virus, Hendra virus, Cedar virus, Ghana virus and Mojiang virus).
- Planned modeling will factor in weather patterns, changes in domesticated animal-bat contacts; use of camera tracking for data collection.
- Responsive to prior reviewer comments and plan for keeping field worker/veterinarian going to assist with sick animals clearly outlined.
- Phylogenetic analysis is planned to examine relationships between viruses identified in domesticated animals and bats within each of the six-month sampling periods.
- Inclusion of children who have potential to care for sick animals.

Weaknesses

- They acknowledge they may identify too few spillovers to make strong inferences about the drivers of those spillovers and may not identify sick animals. However contingency plans outlined so this could be viewed as minor.
- Aim 3 reliant on success of prior two aims.
- Unclear number humans planned to be enrolled with discrepancy between research strategy and the 3000 included in targeted enrollment table.

5. Environment:

Strengths

- Superb for carrying out proposed analyses.
- Existing collaboration exists in Bangladesh.

Weaknesses

- None noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections.

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically.
- Race/Ethnicity: Distribution justified scientifically.
- Inclusion/Exclusion Based on Age: Distribution justified scientifically.

Vertebrate Animals:

YES, all criteria addressed.

Biohazards:

Acceptable.

Resubmission:

Responsive to prior comments.

Applications from Foreign Components:

Justified.

Select Agents:

Acceptable.

Resource Sharing Plans:

Acceptable.

Authentication of Key Biological and/or Chemical Resources:

Unacceptable. Not included.

Budget and Period of Support:

Recommend as Requested.

CRITIQUE 3:

Significance: 2
Investigator(s): 2
Innovation: 2
Approach: 2
Environment: 2

Overall Impact: This is a resubmission of an R01 investigating the potential risk of spillovers of henipaviruses into domesticated animals and subsequently into man. The proposal will define the drivers and variability factors of spillover into domesticated animals and whether certain henipaviruses are more often found outside of bats than others, and finally will assess the risk of subsequent transmission into humans. The significance of the work is strong, given the substantial risk from henipaviruses and repeated regional/seasonal outbreaks. There were major concerns about the safety of the sampling within the study, but these issues have been addressed in the resubmission. There are also other improvements as suggested, including broader screening for novel spillover pathogens. The proposed work by a very experienced team should lead to important insights into the ecosystem for henipaviruses at the intersection between wildlife, domesticated animals, and humans.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections.

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically.
- Race/Ethnicity: Distribution justified scientifically.
- Inclusion/Exclusion Based on Age: Distribution justified scientifically.

Vertebrate Animals:
YES, all criteria addressed.

Budget and Period of Support:
Recommended as requested.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS: ACCEPTABLE.

INCLUSION OF WOMEN PLAN: ACCEPTABLE.

INCLUSION OF MINORITIES PLAN: ACCEPTABLE.

INCLUSION ACROSS THE LIFESPAN: ACCEPTABLE.

VERTEBRATE ANIMALS: ACCEPTABLE.

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

ADMINISTRATIVE NOTE: Applications proposing the use of key biological and/or chemical resources are expected to include a one-page PDF attachment describing plans for Authentication of Key Biological and/or Chemical Resources (see NOT-OD-17-068). Reviewers were asked to consider information provided in this attachment as part of their evaluation of your application. This attachment was missing from your application and the information could not be assessed.

Footnotes for 1 R01 AI168287-01A1; PI Name: Gurley, Emily Suzanne

NIH has modified its policy regarding the receipt of resubmissions (amended applications).See Guide Notice NOT-OD-18-197 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

Clinical Research and Field Studies of Infectious Diseases Study Section
Infectious Diseases and Immunology B Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW
CRFS
06/16/2022 - 06/17/2022

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-22-044 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-22-044.html, including removal of the application from immediate review.

CHAIRPERSON(S)

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

From: Plowright, Raina on behalf of Plowright, Raina <raina.plowright@montana.edu>
To: Emily Gurley
Cc: Henrik Salje; clifton.mckee; Munster, Vincent (NIH/NIAID) [E]; Broder, Chris (USU-DoD); Dr. Mohammed Ziaur Rahman; Laing, Eric; islam_ausraf@icddrb.org; nadiarimi@icddrb.org; rebeca@icddrb.org; sluby@stanford.edu; Dr Sayera Banu
Subject: Re: R01 reviews
Date: Sunday, June 26, 2022 9:33:32 PM
Attachments: 1R01AI168287-01A1.pdf

Great reviews, Emily. It is interesting to see the panel is almost all human infectious disease people. So it's great that you were able to get a One Health proposal through and with such strong reviews.

Sent from my iPhone

> On Jun 26, 2022, at 7:13 AM, Emily Gurley <egurley1@jhu.edu> wrote:
>
> ****External Sender****
>
> Attached are the reviews, FYI.
>
> Emily
>
> ---
>
> **From:** Henrik Salje <hs743@cam.ac.uk>
> **Sent:** Wednesday, June 22, 2022 11:12 AM
> **To:** clifton.mckee <clifton.mckee@gmail.com>; Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>
> **Cc:** Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; Dr Sayera Banu <sbanu@icddrb.org>; Dr. Mohammed Ziaur Rahman <mzrahman@icddrb.org>; Emily Gurley <egurley1@jhu.edu>; Laing, Eric <eric.laing@usuhs.edu>; Raina Plowright <raina.plowright@montana.edu>; islam_ausraf@icddrb.org; nadiarimi@icddrb.org; rebeca@icddrb.org; sluby@stanford.edu
> **Subject:** Re: plans for R01 submission
>
> This makes me so happy. Well done Emily and team on leading this. 5th percentile!
> Looking forward to working with everyone.
> Henrik
>
> Henrik Salje
> Professor
> Pathogen Dynamics Group
> Department of Genetics
> University of Cambridge

www.pdg.gen.cam.ac.uk

From: Clif McKee <clifton.mckee@gmail.com>
Date: Wednesday, 22 June 2022 at 15:59
To: Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>, Dr Sayera Banu <sbanu@icddrb.org>, Dr. Mohammed Ziaur Rahman <mzrahman@icddrb.org>, Emily Gurley <egurley1@jhu.edu>, Laing, Eric <eric.laing@usuhs.edu>, Raina Plowright <raina.plowright@montana.edu>, Henrik Salje <hs743@cam.ac.uk>, islam_ausraf@icddrb.org <islam_ausraf@icddrb.org>, nadiarimi@icddrb.org <nadiarimi@icddrb.org>, rebeca@icddrb.org <rebeca@icddrb.org>, sluby@stanford.edu <sluby@stanford.edu>
Subject: Re: plans for R01 submission

Yesss!!! This is terrific news!

On Wed, Jun 22, 2022 at 5:42 PM Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov> wrote:

> Congrats!!!
>
> Vincent Munster, PhD
> Chief Virus Ecology Section
> Rocky Mountain Laboratories
> NIAID/NIH

> ---
>
> **From:** Emily Gurley <egurley1@jhu.edu>
> **Sent:** Wednesday, June 22, 2022 8:30 AM
> **To:** clifton.mckee <clifton.mckee@gmail.com>; islam_ausraf@icddrb.org; Laing, Eric <eric.laing@usuhs.edu>; Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; Dr. Mohammed Ziaur Rahman <mzrahman@icddrb.org>; sluby@stanford.edu; Raina Plowright <raina.plowright@montana.edu>; Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>; nadiarimi@icddrb.org; hs743@cam.ac.uk; rebeca@icddrb.org
> **Cc:** Dr Sayera Banu <sbanu@icddrb.org>
> **Subject:** [EXTERNAL] RE: plans for R01 submission
>
> Dear colleagues,

We've received scores back on our resubmission. Impact score is 20 and we're at the 5% percentile. With these numbers, we should expect to be funded. Congratulations.

I've heard from our Program Officer about timelines. Funding will start in fiscal year 2023, which begins on Oct 1, 2022. Her best guess is that we'll be funded to begin before the end of 2022, but exact timing will depend on when the US government approves their annual budget.

I look forward to working with you all on this exciting study.

Emily

From: Emily Gurley
Sent: Monday, January 10, 2022 4:13 PM
To: Clif McKee <clifton.mckee@gmail.com>; islam_ausraf@icddrb.org; hsalje@gmail.com; Laing, Eric <eric.laing@usuhs.edu>; christopher.broder@usuhs.edu; Dr. Mohammed Ziaur Rahman <mzrahman@icddrb.org>; sluby@stanford.edu; Raina Plowright <raina.plowright@montana.edu>; vincent.munster@nih.gov
Subject: plans for R01 submission

Dear colleagues,

Happy New Year to you all!

I'm writing to follow up and move us forward with plans to resubmit the R01. The date for submission is Feb 7th, but it's likely that our admin team will require documents well before that. Clif is working to put together our timeline now – updates shortly.

Based on my understanding, these are the updates that need to happen to respond to our last round of reviews:

1. Add in reference to preprint from Eric's group on the sero assay
2. Add in appendix with biosafety protocols from icddr,b, including staff safety, and perhaps letter from biosafety committee at icddr,b
3. Updating research plan to mention these updates, and address question of collecting some additional samples from sick animals (per reviewer comment)
4. Drafting one page response to reviewers to include in resubmission

I will draft the response to reviewers for your review and comment. Eric, grateful for updates and reference for your preprint. Rajib, look forward to receiving the biosafety SOPs (and thoughts on letter) as soon as you're able.

I assume that there are no changes required to budget. If this assumption is incorrect for you, please get in touch with me ASAP with the changes you'd like to include. However, there are new updates to biosketches that could be time consuming – Clif will send out guidance on this so please be on the lookout.

Look forward to getting this back in!

Emily

Professor of the Practice
Co-Director, Child Health and Mortality Prevention Surveillance (CHAMPS) project, Bangladesh
Department of Epidemiology
Department of International Health (Joint Appointment)
Johns Hopkins Bloomberg School of Public Health
615 N Wolfe St, E6545
Baltimore MD 21205
Link to my office hours booking site: https://emilysgurley.youcanbook.me

Expert Panel Discussion for Vaccine Library virus selection #2

1. Introduction/overview of next steps.

2. Presentation and discussion of Spillover program- Dr. Mazet

3. Questions for panel discussion.

What below factors in selecting viruses for a potential Disease X emergence do you believe are important in selecting a virus for a Disease X emergence?

1. RNA vs DNA virus with RNA>DNA
2. Virus characteristics: mutation rate of the virus, ability to reassort and/or recombine, fitness, tropism, number of recognized generic lineages ie genetic diversity?
3. Mode of human to human transmission: respiratory > oral-fecal > vector-borne (mosquito >tick>midge>sandflies) > sexual transmission > water-borne > fomite > others? A virus that uses several modes of transmission ie vector plus sexual would this increase its importance?
4. Zoonotic transmission: NHP>wild birds > bats>domestic animal ie (cattle>pigs>horses>birds>camels>fish) > market food (bush meat, civets, snakes, snails)> others?
5. Is the type of zoonotic transmission important and can it be ranked: wild-birds > domestic birds > cattle > pigs > sheep> horse > domestic dogs/cats > goats > camels > NHP > guinea pigs > others?
6. Should we consider viruses with a successful vaccine ie measles, chickenpox, polio or should we anticipate escape mutants that aren't protected by a current vaccine and develop vaccines against these pathogens that are more broadly protective?
7. R_0 if known of the virus or within a family?
8. Reported human outbreaks of viruses in a family and if they are: sporadic outbreaks, cyclical, annual, endemic, pandemic pathogens.
9. Number of spillover events from zoonotic to humans in total, per year; spillover to human with human-to-human transmission, spillover to vector to human?
10. If vector-borne, characteristics of the vector ie stable distribution, expanding due to environmental changes, number of viable vectors, phylogenetic distance ie diversity in the vectors?
11. Susceptible population: for example, restricted regional occurrence leaving a large population susceptible.
12. Potential disease impact/burden, mortality, and/or morbidity?

Do you think the human outbreak characteristics of one virus in a family could be a potential characteristic of other viral pathogens within a family ie do they share potentially common virulence factors? For example Lassa fever and Junin viruses would they elevate specific viruses in the viral family of arenaviruses that may share common characteristic but are not known human pathogens yet? Knowing the potential of SARS-Cov and MERS before Covid-19 would that have elevated the concerns for other coronaviruses

In the selection of viruses within a family, do you believe there is short-term heterotypic protection from one virus within or beyond a genera and this should be considered in selecting viruses for the vaccine library? For example, acute DENV-1 may provide short-term protection against other serotypes, JEV vaccination may provide short-term protection against DENV infection. If yes, is this specific for a virus family and not generic across many families?

4. Summary

Please highlight your 10 to 15 viruses selection by highlighting or filling in the box with red.

Genus	**Spillover rank of 887/risk score**	**Zoonotic**	**Human**									**Notes**
Metaavulavirus	*Avian metaavulavirus 2*	*Avian metaavulavirus 5*	*Avian metaavulavirus 6*	*Avian metaavulavirus 7*	*Avian metaavulavirus 8*	*Avian metaavulavirus 10*	*Avian metaavulavirus 11*	*Avian metaavulavirus 14*	*Avian metaavulavirus 15*	*Avian metaavulavirus 20*		*Avian paramyxovirus, migratory birds, natural reservoir, can cause high mortality.*
Orthoavulavirus	*Avian orthoavulavirus 1 (Newcastle disease)*	*Avian orthoavulavirus 9*	*Avian orthoavulavirus 12*	*Avian orthoavulavirus 13*	*Avian orthoavulavirus 16*	*Avian orthoavulavirus 17*	*Avian orthoavulavirus 18*	*Avian orthoavulavirus 19*	*Avian orthoavulavirus 21*			*Avian paramyxovirus, migratory birds, natural reservoir, can cause high mortality ie Newcastle disease..*
Praavulavirus	*Avian paraavulavirus 3*	*Avian paraavulavirus 4*										
Synodonvirus	*Synodus synodonvirus*											Isolated from a triplecross lizardfish
Aquaparamyxovirus	*Oncorhynchus aquaparamyxovirus*	*Salmo aquaparamyxovirus*										Isolated from Atlantic and Pacific Salmon
Ferlavirus	*Reptilian ferlavirus*											Isolated from a fer-de-lance snake and anaconda snakes.
Henipavirus	*Cedar henipavirus*	*Ghanaian bat henipavirus*	*Hendra henipavirus*	*Mojiang henipavirus*	*Nipah henipavirus 5/86*	*Paramyxovirus PREDICT PMV-61 411/56*						
Jeilongvirus	*Beilong jeilongvirus 54/70*	*Jun jeilongvirus*	*Lophuromys jeilongvirus 1*	*Lophuromys jeilongvirus 2*	*Miniopteran jeilongvirus*	*Myodes jeilongvirus*	*Tailam jeilongvirus 610/52*					Beilong virus widely distributed in rodents and shrews in China.
Morbillivirus	*Canine morbillivirus*	*Cetacean morbillivirus*	*Feline morbillivirus*	*Measles morbillivirus 100/66*	*Phocine morbillivirus*	*Rinderpest morbillivirus*	*Small ruminant morbillivirus*	*Paramyxovirus PREDICT PMV-83 72/68*	Paramyxovirus PREDICT PMV-83 72/68	Paramyxovirus PREDICT PMV-63 85/67	*Paramyxovirus PREDICT PMV-55 325/57*	Paper suggesting Measles virus adapted to dogs forming canine distemper.
Narmovirus	*Mossman narmovirus*	*Myodes narmovirus*	*Nariva narmovirus*	*Tupaia narmovirus*								Isolated from rodents
Respirovirus	*Bovine respirovirus 3*	*Caprine respirovirus 3*	*Human respirovirus 1*	*Human respirovirus 3 80/67*	*Murine respirovirus (Sendai virus) 70/68*	*Porcine respirovirus 1*	*Squirrel respirovirus*					Sendai virus pathogen of lab mice, etiology of a fatal epidemic of newborn pneumonitis in Japan 1952
Salemvirus	*Salem salemvirus*											
Orthorubulavirus	*Human orthorubulavirus 2*	*Human orthorubulavirus 4*	*Mammalian orthorubulavirus 5*	*Mammalian orthorubulavirus 6*	*Mapuera orthorubulavirus*	*Mumps orthorubulavirus*	*Porcine orthorubulavirus*	*Simian orthorubulavirus*				Mammalian orthorubulavirus 6 (Alston virus) isolated from bats and produces respiratory infection in a ferret model. Mapuera isolated from bats.
Pararubulavirus	*Achimota pararubulavirus 1*	*Achimota pararubulavirus 2*	*Hervey pararubulavirus*	*Menangle pararubulavirus*	*Sosuga pararubulavirus*	*Teviot pararubulavirus*	*Tioman pararubulavirus*	*Tuhoko pararubulavirus 1*	*Tuhoko pararubulavirus 2*	*Tuhoko pararubulavirus 3*		*Menangle in flying foxes, infected sows, human cases with an ILI.*
Cynoglossusvirus	*Cynoglossus cynoglossusvirus*											Gut from a flatfish.
Hoplichthysvirus	*Hoplichthys hoplichthysvirus*											Isolated from a fish.
Scoliodonvirus	*Scoliodon scoliodonvirus*											Isolated from a shark.
Unassigned		Ranking	Risk		Ranking	Risk		Ranking	Risk			
	Paramyxovirus PREDICT PMV-10	39	72.07902481	Paramyxovirus PREDICT PMV-47	248	59.11712896	Paramyxovirus PREDICT PMV-14	504	53.4084			
	Paramyxovirus PREDICT PMV-13	48	70.99553712	Paramyxovirus PREDICT PMV-104	252	58.83546349	Paramyxovirus PREDICT PMV-17	505	53.4084			
	Paramyxovirus PREDICT PMV-15	49	70.97716116	Paramyxovirus PREDICT PMV-172	254	58.83546349	Paramyxovirus PREDICT PMV-27	506	53.4084			
	Paramyxovirus PREDICT PMV-149	60	69.60909767	Paramyxovirus PREDICT PMV-103	251	58.83546349	Paramyxovirus PREDICT PMV-73	507	53.4084			
	Paramyxovirus PREDICT PMV-56	62	69.22202264	Paramyxovirus PREDICT PMV-109	253	58.83546349	Paramyxovirus PREDICT PMV-23	509	53.3879			
	Paramyxovirus PREDICT PMV-145	66	68.55846605	Paramyxovirus PREDICT PMV-130	257	58.71416092	Paramyxovirus PREDICT PMV-31	510	53.3631			
	Paramyxovirus PREDICT PMV-49	74	67.65025218	Paramyxovirus PREDICT PMV-93	258	58.68033907	Paramyxovirus PREDICT PMV-59	513	53.2416			
	Paramyxovirus PREDICT PMV-58	89	66.47281694	Paramyxovirus PREDICT PMV-96	260	58.64911534	Paramyxovirus PREDICT PMV-86	530	52.8982			
	Paramyxovirus PREDICT PMV-20	97	66.04101324	Paramyxovirus PREDICT PMV-54	265	58.43175242	Paramyxovirus PREDICT PMV-80	578	52.2184			
	Paramyxovirus PREDICT PMV-5	102	65.75010308	Paramyxovirus PREDICT PMV-68	267	58.37530581	Paramyxovirus IFBPV32-2012	605	51.8273			
	Paramyxovirus PREDICT PMV-3	101	65.75010308	Paramyxovirus PREDICT PMV-9	275	58.00599498	Paramyxovirus PREDICT PMV-18	606	51.7919			
	Paramyxovirus PREDICT PMV-6	103	65.75010308	Paramyxovirus PREDICT PMV-7	273	58.00599498	Paramyxovirus PREDICT PMV-129	609	51.7427			
	Rodent Paramyxovirus MpR12	109	65.38293715	Paramyxovirus PREDICT PMV-8	274	58.00599498	Paramyxovirus PREDICT PMV-123	613	51.598			
	Bat paramyxovirus BtHp-ParaV/GD2012	115	64.58634082	Paramyxovirus PREDICT PMV-41	276	57.87965224	Paramyxovirus PREDICT PMV-62	618	51.4239			
	Paramyxovirus PREDICT PMV-67	119	64.08792135	Paramyxovirus PREDICT PMV-88	279	57.86006414	Eidolon helvum paramyxovirus /UG23/U67N	645	51.2074			
	Paramyxovirus PREDICT PMV-66	118	64.08792135	Paramyxovirus PREDICT PMV-164	278	57.86006414	Paramyxovirus PREDICT PMV-133	646	51.1716			
	Bat paramyxovirus R_aeg_UPE766/525/122	121	63.98133582	Paramyxovirus PREDICT PMV-69	280	57.85533478	Paramyxovirus PREDICT PMV-160	647	51.1107			
	Paramyxovirus PREDICT PMV-134	130	63.70877156	Paramyxovirus PREDICT PMV-44	282	57.73493354	Paramyxovirus PREDICT PMV-30	648	51.0649			
	Paramyxovirus PREDICT PMV-174	136	63.60697594	Paramyxovirus PREDICT PMV-135	286	57.56373446	Paramyxovirus PREDICT PMV-101	650	51.0091			
	Paramyxovirus PREDICT PMV-117	138	63.42710609	Paramyxovirus PREDICT PMV-89	295	57.30044495	Paramyxovirus PREDICT PMV-25	652	50.9838			
	Paramyxovirus PREDICT PMV-173	142	63.38398556	Eidolon helvum paramyxovirus/TZ13	296	57.26059743	Paramyxovirus PREDICT PMV-156	663	50.7687			
	Paramyxovirus PREDICT PMV-185	143	63.3425238	Paramyxovirus PREDICT PMV-169	299	57.22316235	Paramyxovirus PREDICT PMV-53	666	50.5154			
	Paramyxovirus PREDICT PMV-90	145	63.30076335	Paramyxovirus PREDICT PMV-57	305	57.01221967	Paramyxovirus PREDICT PMV-131	669	50.4468			
	Paramyxovirus PREDICT PMV-175	147	63.16088738	Paramyxovirus PREDICT PMV-166	309	57.00411526	Paramyxovirus PREDICT PMV-97	675	50.4468			
	Paramyxovirus PREDICT PMV-179	148	63.07493714	Paramyxovirus PREDICT PMV-162	308	57.00411526	Paramyxovirus PREDICT PMV-163	674	50.4468			
	Paramyxovirus PREDICT PMV-181	154	62.4934672	Paramyxovirus PREDICT PMV-157	306	57.00411526	Paramyxovirus PREDICT PMV-161	673	50.4468			
	Paramyxovirus PREDICT PMV-46	156	62.47129485	Paramyxovirus PREDICT PMV-158	307	57.00411526	Paramyxovirus PREDICT PMV-152	672	50.4468			
	Paramyxovirus PREDICT PMV-40	155	62.47129485	Paramyxovirus PREDICT PMV-121	365	56.52161552	Paramyxovirus PREDICT PMV-147	671	50.4468			
	Paramyxovirus PREDICT PMV-178	157	62.43333078	Paramyxovirus PREDICT PMV-100	363	56.52161552	Paramyxovirus PREDICT PMV-138	670	50.4468			
	Paramyxovirus PREDICT PMV-143	162	62.1370011	Paramyxovirus PREDICT PMV-102	364	56.52161552	Paramyxovirus PREDICT PMV-127	668	50.4468			
	Paramyxovirus PREDICT PMV-150	165	62.1370011	Paramyxovirus PREDICT PMV-124	366	56.52161552	Paramyxovirus PREDICT PMV-137	691	50.171			
	Paramyxovirus PREDICT PMV-141	161	62.1370011	Pteropus poliocephalus paramyxovirus	369	56.52161552	Paramyxovirus PREDICT PMV-82	693	50.0438			
	Paramyxovirus PREDICT PMV-146	164	62.1370011	Paramyxovirus PREDICT PMV-126	367	56.52161552	Paramyxovirus PREDICT PMV-180	695	50.0213			
	Paramyxovirus PREDICT PMV-144	163	62.1370011	Paramyxovirus PREDICT PMV-94	368	56.52161552	Paramyxovirus PREDICT PMV-159	700	49.9077			
	Paramyxovirus PREDICT PMV-136	166	62.09647043	Paramyxovirus PREDICT PMV-112	370	56.47029096	Paramyxovirus PREDICT PMV-139	709	49.6404			
	Paramyxovirus PREDICT PMV-2	169	61.97950739	Paramyxovirus PREDICT PMV-39	376	56.32625774	Paramyxovirus PREDICT PMV-38	711	49.6378			
	Eidolon helvum paramyxovirus/U6B	172	61.74427326	Paramyxovirus PREDICT PMV-22	377	56.30788178	Paramyxovirus PREDICT PMV-50	712	49.6378			

Paramyxovirus PREDICT PMV-171	178	61.61400919	Paramyxovirus PREDICT PMV-142	400	55.93877764	Paramyxovirus PREDICT PMV-51	713	49.6378
Paramyxovirus PREDICT PMV-76	179	61.60386228	Paramyxovirus PREDICT PMV-140	399	55.93877764	Paramyxovirus PREDICT PMV-37	710	49.6378
Paramyxovirus PREDICT PMV-155	185	60.99651439	Paramyxovirus PREDICT PMV-154	410	55.72800524	Paramyxovirus PREDICT PMV-183	717	49.6183
Paramyxovirus PREDICT PMV-153	188	60.96575438	Paramyxovirus PREDICT PMV-72	413	55.66817555	Paramyxovirus PREDICT PMV-118	729	48.8244
Paramyxovirus PREDICT PMV-151	187	60.96575438	Paramyxovirus PREDICT PMV-177	428	55.18759243	Paramyxovirus PREDICT PMV-16	732	48.7682
Paramyxovirus PREDICT PMV-170	190	60.96047038	Paramyxovirus PREDICT PMV-60	429	55.15951619	Paramyxovirus PREDICT PMV-11	730	48.7682
Paramyxovirus PREDICT PMV-168	189	60.96047038	Paramyxovirus PREDICT PMV-106	430	55.06747828	Paramyxovirus PREDICT PMV-12	731	48.7682
Paramyxovirus PREDICT PMV-182	193	60.88116606	Paramyxovirus PREDICT PMV-111	435	54.90974338	Paramyxovirus PREDICT PMV-26	733	48.7682
Paramyxovirus PREDICT PMV-184	194	60.88116606	Paramyxovirus PREDICT PMV-95	436	54.90974338	Paramyxovirus PREDICT PMV-19	758	48.3653
Paramyxovirus PREDICT PMV-70	196	60.75480568	Paramyxovirus PREDICT PMV-148	450	54.89646659	Paramyxovirus PREDICT PMV-65	769	47.6318
Paramyxovirus PREDICT PMV-4	212	60.25288153	Paramyxovirus PREDICT PMV-122	451	54.85841881	Paramyxovirus PREDICT PMV-91	806	47.2885
Bat paramyxovirus B16-40	214	60.1167148	Paramyxovirus PREDICT PMV-81	454	54.7906063	Paramyxovirus PREDICT PMV-74	812	47.0732
Paramyxovirus PREDICT PMV-176	217	59.9602049	Paramyxovirus PREDICT PMV-105	452	54.7906063	Paramyxovirus PREDICT PMV-107	811	47.0732
Paramyxovirus PREDICT PMV-85	226	59.67857859	Paramyxovirus PREDICT PMV-110	453	54.7906063	Paramyxovirus PREDICT PMV-119	820	46.7887
Paramyxovirus PREDICT PMV-128	225	59.67857859	Paramyxovirus PREDICT PMV-28	458	54.69558064	Paramyxovirus PREDICT PMV-48	821	46.7699
Paramyxovirus PREDICT PMV-125	224	59.67857859	Paramyxovirus IFBPV25/2011	469	54.31677487	Paramyxovirus YN12103/CHN/2012	822	46.7516
Paramyxovirus PREDICT PMV-167	228	59.64139956	Paramyxovirus PREDICT PMV-1	476	54.23539929	Paramyxovirus PREDICT PMV-108	857	46.1956
Paramyxovirus PREDICT PMV-29	230	59.62481605	Paramyxovirus PREDICT PMV-43	480	54.10311505	Paramyxovirus PREDICT PMV-120	858	46.1956
Paramyxovirus PREDICT PMV-24	236	59.51815567	Paramyxovirus PREDICT PMV-42	479	54.10311505	Paramyxovirus PREDICT PMV-99	860	46.1956
Paramyxovirus PREDICT PMV-165	237	59.41345865	Paramyxovirus PREDICT PMV-79	481	54.07109249	Paramyxovirus PREDICT PMV-98	859	46.1956
Paramyxovirus PREDICT PMV-114	241	59.36018071	Paramyxovirus PREDICT PMV-36	484	53.96433785	Paramyxovirus PREDICT PMV-87	864	46.0741
Paramyxovirus PREDICT PMV-115	242	59.36018071	Paramyxovirus PREDICT PMV-64	499	53.44947053	Paramyxovirus PREDICT PMV-113	879	45.0182
Paramyxovirus PREDICT PMV-116	243	59.36018071	Paramyxovirus PREDICT PMV-92	500	53.43920561	Paramyxovirus PREDICT PMV-132	885	41.7002
Paramyxovirus PREDICT PMV-71	244	59.30507023	Paramyxovirus PREDICT PMV-75	508	53.40841088			

Please highlight your 10 to 15 viruses selection by highlighting or filling in the box with red.

Genus	**Spillover rank of 887/risk score**	**Zoonotic**	**Human**																																								**Notes**
Antennavirus	*Hairy antennavirus*	*Salmon antennavirus*	*Striated antennavirus*																																								Isolated from fish
Hartmanivirus	*Haartman hartmanivirus*	*Heimat hartmanivirus*	*Muikkunen hartmanivirus*	*Schoolhouse hartmanivirus*	*Setpatvet hartmanivirus*	*Zurich hartmanivirus*																																					Isolated from snakes
Mammarenavirus	*Allpahuayo mammarenavirus*	*Alxa mammarenavirus*	*Argentinian mammarenavirus (Junin virus) 96/66*	*Bear Canyon mammarenavirus*	*Brazilian mammarenavirus (Sabia virus)*	*Cali mammarenavirus*	*Chapare mammarenavirus*	*Chevrier mammarenavirus*	*Cupixi mammarenavirus*	*Flexal mammarenavirus*	*Gairo mammarenavirus*	*Guanarito mammarenavirus 110/65*	*Ippy mammarenavirus*	*Kitale mammarenavirus*	*Lassa mammarenavirus 1/91*	*Latino mammarenavirus*	*Loei River mammarenavirus*	*Lujo mammarenavirus*	*Luna mammarenavirus*	*Lunk mammarenavirus*	*Lymphocytic choriomeningitis mammarenavirus 10/85*	*Machupo mammarenavirus 99/66*	*Mariental mammarenavirus*	*Merino Walk mammarenavirus*	*Mobala mammarenavirus*	*Mopeia mammarenavirus 204/61 (subspecies Morogoro virus 171/62)*	*Okahandja mammarenavirus*	*Oliveros mammarenavirus*	*Paraguayan mammarenavirus*	*Pirital mammarenavirus*	*Planalto mammarenavirus*	*Ryukyu mammarenavirus*	*Serra do Navio mammarenavirus*	*Solwezi mammarenavirus*	*Souris mammarenavirus*	*Tacaribe mammarenavirus*	*Tamiami mammarenavirus*	*Wenzhou mammarenavirus*	*Whitewater Arroyo mammarenavirus 134/62*	*Xapuri mammarenavirus*	*Arenavirus PREDICT_ArenaV-1 68950*	Arenavirus PREDICT ArenaV-2 749/48	*Tacaribe and LCMV isolated from ticks recently.*
Reptarenavirus	*California reptarenavirus*	*Giessen reptarenavirus*	*Golden reptarenavirus*	*Ordinary reptarenavirus*	*Rotterdam reptarena*																																						Isolated from snakes

From: Luz Hermida on behalf of Luz Hermida <luz.hermida@cepi.net>
To: Eileen Fairclough; Timothy Endy; Daniel Bausch; WOOLHOUSE Mark; Broder, Christopher; Ksiazek, Thomas G.; Jason McLellan; ccs8@cdc.gov; Brian H Bird; feldmannh@mail.nih.gov; Jonna Mazet; Kathryn Hastie; Barrett, Alan; Wang Linfa
Cc: Mark Polhemus; Maïna L"Azou Jackson; Mike Whelan; Gabrielle Breugelmans; In-Kyu Yoon; Gerald Voss; Diana Magallanes; William Dowling; Jakob Cramer; Feldmann, Heinrich (NIH/NIAID) [E]
Subject: RE: Vaccine library virus selection available times for meetings #2
Date: Monday, April 4, 2022 1:55:21 PM
Attachments: 2022 0405 Apr - Experts Agenda meeting #2 questions for discussion.docx
2022 0405 Virus panel selection.xlsx

Dear all,

Please see attached the agenda for tomorrow’s Expert panel meeting #2.
Included also is the spreadsheet for the virus rankings for the paramyxo and arenaviruses.

Looking forward to a good discussion tomorrow.
Thank you. Best regards,

Luz on behalf of Tim

-----Original Appointment-----
From: Eileen Fairclough <eileen.fairclough@cepi.net>
Sent: Thursday, March 17, 2022 1:00 PM
To: Eileen Fairclough; Luz Hermida; Timothy Endy; Daniel Bausch; WOOLHOUSE Mark; Broder, Christopher; Ksiazek, Thomas G.; Jason McLellan; ccs8@cdc.gov; Brian H Bird; feldmannh@mail.nih.gov; Jonna Mazet; Kathryn Hastie; Barrett, Alan; Wang Linfa
Cc: Mark Polhemus; Maïna L'Azou Jackson; Mike Whelan; Gabrielle Breugelmans; In-Kyu Yoon; Gerald Voss; Diana Magallanes; William Dowling; Jakob Cramer (jakob.cramer@cepi.net); Feldmann, Heinrich (NIH/NIAID) [E]
Subject: Vaccine library virus selection available times for meetings #2
When: Tuesday, April 5, 2022 6:00 PM-8:00 PM (UTC+00:00) Dublin, Edinburgh, Lisbon, London.
Where: https://cepi-net.zoom.us/j/85749182660

Vaccine library virus selection available times for meetings #2

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+19292056099,,85749182660#,,,,*412457# US (New York)

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+1 301 715 8592 US (Washington DC)
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0 800 260 5801 United Kingdom Toll-free
0 800 358 2817 United Kingdom Toll-free
0 800 456 1369 United Kingdom Toll-free

Meeting ID: 857 4918 2660
Passcode: 412457
Find your local number: https://cepi-net.zoom.us/u/kekfEjM11E

Sensitivity: CEPI Internal

Arenaviridae

Sheli R. Radoshitzky, Michael J. Buchmeier, Rémi N. Charrel, J. Christopher S. Clegg, Jean-Paul J. Gonzalez, Stephan Günther, Jussi Hepojoki, Jens H. Kuhn, Igor S. Lukashevich, Víctor Romanowski, Maria S. Salvato, Manuela Sironi, Mark D. Stenglein and Juan Carlos de la Torre

The citation for this ICTV Report chapter is the summary published as Radoshitzky et al., (2019): ICTV Virus Taxonomy Profile: *Arenaviridae*, Journal of General Virology, 100, 1200–1201.

Corresponding author: Juan Carlos de la Torre (juanct@scripps.edu)

Edited by: Jens H. Kuhn, Stuart G. Siddell and Peter J. Walker
Posted: May 2019, updated September 2020
PDF: ICTV_*Arenaviridae*.pdf

Summary

Members of the family *Arenaviridae* produce enveloped virions containing genomes consisting of 2 to 3 single-stranded RNA segments totaling about 10.5 kb (Table 1.*Arenaviridae*). Arenaviruses are currently classified into four genera (*Antennavirus*, *Hartmanivirus, Mammarenavirus*, and *Reptarenavirus*). These viruses infect fish (antennaviruses), snakes (hartmaniviruses and reptarenaviruses) and mammals (mammarenaviruses). Some reptarenaviruses cause boid inclusion body disease in captive snakes, whereas some mammarenaviruses can infect humans and other primates, causing mild, severe, and sometimes fatal diseases.

Table 1.*Arenaviridae.* Characteristics of members of the family *Arenaviridae.*

Characteristic	Description*
Typical member	lymphocytic choriomeningitis virus Armstrong 53b [S segment: AY847350; L segment: AY847351], species *Lymphocytic choriomeningitis mammarenavirus*, genus *Mammarenavirus.*
Virion	Enveloped, pleomorphic virions 40–200 nm in diameter with trimeric surface spikes
Genome	Two or three single-stranded, usually ambisense coding arrangement, RNA molecules called small (S), medium (M), and large (L)
Replication	Ribonucleoprotein (RNP) complexes are generated that contain anti-genomic

	RNA serving as coding templates for synthesis of genomic RNA
Translation	Proteins are produced from capped and non-polyadenylated mRNAs. The 5′-cap structure is derived by polymerase slippage or cap-snatching from cellular mRNAs
Host range	Fish (antennaviruses), predominantly small mammals (mammarenaviruses), and reptiles (hartmaniviruses and reptarenaviruses), but potentially also bats and ticks
Taxonomy	Realm *Riboviria*, phylum *Negarnaviricota*, subphylum *Polyploviricotina*, class *Ellioviricetes*, order *Bunyavirales*. The family includes 4 genera and 50 species

* mostly based on experiments with mammalian arenaviruses

Viruses assigned to each of the 4 genera form a monophyletic clade based on phylogenetic analysis of large protein/RNA-directed RNA polymerase (L/RdRP) and nucleoprotein (NP) sequences. Viruses from all four genera share one or more of the following characteristics: (i) enveloped spherical or pleomorphic virions; (ii) segmented single-stranded, ambisense RNA genome without polyadenylated tracts at the 3′-termini; (iii) genomic 5′- and 3′-end sequence complementarity; (iv) nucleotide sequences that could form one or more hairpin configurations within non-coding intergenic regions (IGRs) of genomic segments; (v) capped but not polyadenylated virus mRNAs; and (vi) induction of a persistent and frequently asymptomatic infection in reservoir hosts, in which chronic viremia and/or viruria occur (Radoshitzky et al., 2015).

Piscine host

Genus *Antennavirus*. This recently established genus currently includes 2 species for 2 viruses discovered in actinopterygian fish. Antennaviruses are notable for having genomes consisting of 3, rather than 2, genomic segments and likely not encoding the zinc -binding matrix (Z) protein, which is encoded by mammarenaviruses and reptarenaviruses.

Reptilian host

Genus *Hartmanivirus*. This recently established genus currently includes 4 species for 6 viruses discovered in captive snakes with boid inclusion body disease (BIBD). Hartmaniviruses are notable for genomes lacking a gene encoding the Z protein, which is encoded by mammarenaviruses and reptarenaviruses.

Genus *Reptarenavirus*. This genus currently includes 5 species for 8 viruses discovered in captive snakes, some of which were suffering from BIBD. Reptarenaviruses are notable for their transmembrane surface GP2 glycoproteins, which are more closely related to those of ebolaviruses (order *Mononegavirales*, family *Filoviridae*) than to those of antennaviruses, hartmaniviruses, mammarenaviruses or other bunyaviruses. Reptarenaviruses are also unusual in that they are prone to cause co-infections, with multiple distinct S and L segments, not necessarily in a 1:1 ratio, being detectable in snakes.

Mammalian host

Genus *Mammarenavirus*. The genus currently includes 39 species for 46 viruses. These viruses have been detected in rodent hosts, apart from Tacaribe virus (TCRV) which has been found only in phyllostomid bats and ixodid lone star ticks. Mammarenavirus infections of their natural rodent hosts are generally asymptomatic. In humans, some mammarenaviruses, such as Western African Lassa virus (LASV) or several viruses of South American origin, can cause severe and often fatal diseases with hemorrhagic manifestations. Lymphocytic choriomeningitis virus (LCMV), the typical mammalian arenavirus, can also cause disease in humans and poses a serious threat to immunocompromised individuals.

Virion

Morphology

Virions are spherical or pleomorphic in shape, 40–200 nm in diameter, with dense lipid envelopes (Figure 1**.***Arenaviridae*). The virion surface layer is covered with club-shaped projections with distinctive stalk and head regions. These projections are made of trimeric spike structures of two virus-encoded membrane glycoprotein (GP) subunits (GP1 and GP2) and in case of some arenaviruses, a third component (stable signal peptide [SSP]). Isolated RNP complexes are organized into "beads-on-a-string"-like structures (Hetzel et al., 2013, Li et al., 2016, Neuman et al., 2005, Buchmeier 2002, Charrel and de Lamballerie 2003, Jay et al., 2005, Meyer et al., 2002, Hepojoki et al., 2018).

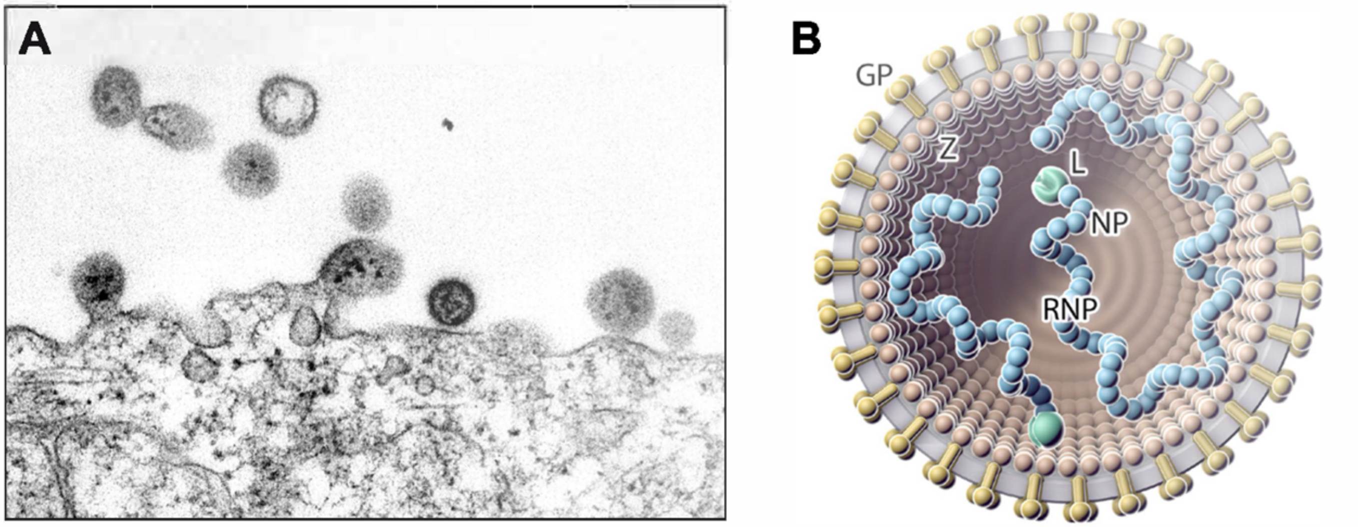


Figure 1.*Arenaviridae.* A) Electron micrograph of (mammalian) arenavirus particles, showing dark internal inclusion bodies (Latin: *arena*, sand), budding from an infected cell. **B)** Schematic illustration of an arenavirus particle. Shown is the spherical and enveloped (grey) particle that is spiked with glycoproteins (GP, gold) around a layer of zinc-binding matrix proteins (Z, brown; missing in hartmaniviruses). The small (S) and large (L) ribonucleoprotein (RNP) complexes inside the particle consist of nucleoprotein (NP, blue) and RNA-directed RNA polymerase (L, green).

Physicochemical and physical properties

Mainly known for members of the genus *Mammarenavirus* (see section on genus page).

Nucleic acid

Arenavirions typically contain 2 or 3 linear, ambisense or negative-sense single-stranded RNA segments that are encapsidated independently. These RNAs are uncapped (Leung et al., 1977) and contain a single non-templated G at each of the 5′-ends (Garcin and Kolakofsky 1990, Raju et al., 1990, Shi et al., 2018). No poly(A) tracts are present at the 3′-termini. The termini of the RNAs ends have inverted complementary sequences encoding transcription and replication initiation signals (Hepojoki et al., 2018, Salvato et al., 1989, Harnish et al., 1993, Young and Howard 1983).

Proteins

Arenaviruses express 3 (hartmaniviruses) or 4 (antennaviruses, mammarenaviruses, reptarenaviruses) structural proteins. The most abundant structural protein in virions is the nucleoprotein (NP), which encapsidates the virus genomic segments. The least abundant protein is the RNA-directed RNA polymerase (L), which mediates virus genome replication and transcription. The zinc-binding matrix (Z) protein, which is absent in antennaviruses and hartmaniviruses, is a matrix protein. Glycoproteins (GP1 or G1, GP2 or G2) are derived by post-translational cleavage of an intracellular GP precursor, the “glycoprotein-cell-associated” preprotein (GPC) by the cellular S1P/SKI protease. A third GPC cleavage product, the signal peptide, stays attached to the GP complex in hartmaniviruses and mammarenaviruses (stable signal peptide [SSP]), but not in reptarenaviruses (signal peptide [SP]). The GP structure of antennaviruses is unknown (Hepojoki et al., 2018, Shi et al., 2018, Buchmeier et al., 1987, Kunz et al., 2003, Lenz et al., 2001, Koellhoffer et al., 2014, Bederka et al., 2014, Eichler et al., 2003, York et al., 2004).

Lipids

Only known for members of the genus *Mammarenavirus* (see section on genus page).

Carbohydrates

Only known for members of the genus *Mammarenavirus* (see section on genus page).

Genome organization and replication

The arenavirus genome typically consists of two or three single-stranded, typically ambisense RNA molecules, termed S, (M), and L. Some of these RNAs encode two proteins in non-overlapping open reading frames (ORF) of opposite polarities (ambisense coding arrangement) that are separated by non-coding intergenic regions (IGRs) (Figure 2.*Arenaviridae*). The S RNA encodes NP in the virus genome-complementary sequence, and, in many cases, the GPC in the virus genome-sense sequence. The L RNA encodes L in the virus genome-complementary sequence, and, in some case, Z in the virus genome-sense sequence. Antennaviruses and hartmaniviruses lack the Z ORF, and antennaviruses encode at least one protein of unknown function. The IGRs form one or more energetically stable stem-loop (hairpin) structures and which function in structure-dependent transcription termination and in virion assembly and budding.

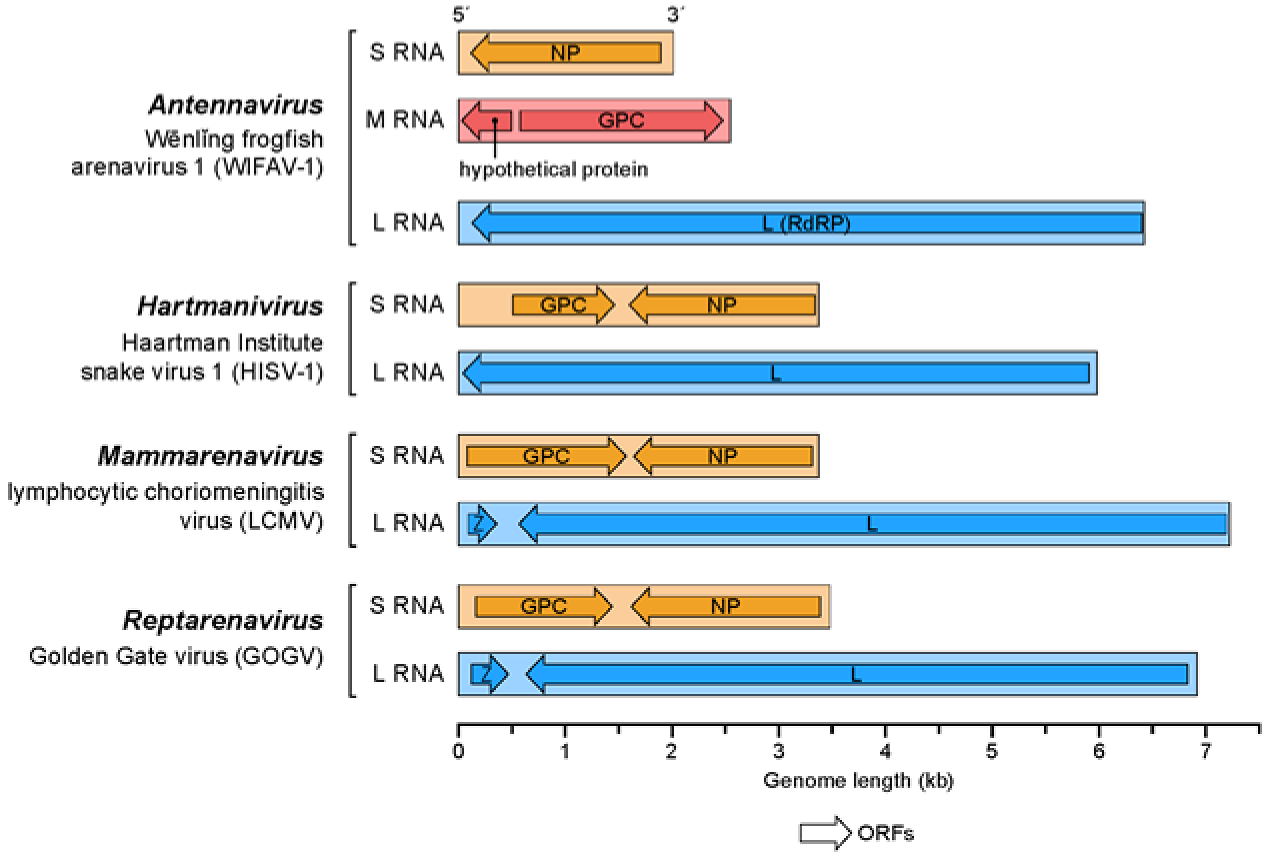


Figure 2.*Arenaviridae*. Schematic representation of the bi- or tri-segmented arenavirus genome organization. The 5′- and 3′-ends of all segments (S, [M], and L) are complementary at their termini, likely promoting the formation of circular ribonucleoprotein complexes within the virion. GPC, glycoprotein precursor; L, RNA-directed RNA polymerase; NP, nucleoprotein; Z, zinc-binding matrix

protein. Open reading frames are separated by non-coding intergenic regions (IGRs), with predicted hairpin structures (not shown).

Arenavirus infection starts with attachment to cell-surface receptors and entry via the endosomal route (Martinez et al., 2007, Vela et al., 2007, Borrow and Oldstone 1994, Radoshitzky et al., 2007, Cao et al., 1998, Raaben et al., 2017, Glushakova and Lukashevich 1989) (Figure 3.*Arenaviridae*). pH-dependent fusion with late endosomes releases the virion RNP complex into the cytoplasm. In the case of some mammalian arenaviruses (LASV), this fusion event involves a pH-dependent switch to an intracellular receptor, lysosomal associated membrane protein 1 (LAMP1) (Jae et al., 2014). The virus RNP directs both RNA genome replication and gene transcription (Meyer et al., 2002). During replication, L reads through the IGR transcription-termination signal and generates uncapped antigenomic and genomic RNAs (Leung et al., 1977). Because these RNAs contain a single non-templated G at the 5′-ends (Garcin and Kolakofsky 1990, Raju et al., 1990), replication initiation might involve a slippage mechanism of L on the nascent RNA (Garcin and Kolakofsky 1992). In case of ambisense coding arrangements, only mRNAs encoding NP or L can be synthesized from genomic RNAs. Transcription of mRNAs encoding GPC or Z occurs only after the first round of virus replication, during which S and L antigenomes are produced.

Virus proteins are synthesized from subgenomic capped mRNAs that lack terminal poly(A) (Meyer and Southern 1993, Singh et al., 1987, Southern et al., 1987). The 5′-ends of virus mRNAs contain several non-templated bases, suggesting that arenaviruses use either polymerase slippage or a cap-snatching mechanism similar to that used by other members of the subphylum *Polyploviricotina* (Garcin and Kolakofsky 1990, Raju et al., 1990, Meyer and Southern 1993). Cap-snatching would require an endonuclease presumed to be present in the N-terminal part of L, which cleaves cellular mRNAs to generate a cap leader that is subsequently used to prime arenavirus transcription. The 3′-termini of the mRNAs have been mapped to locations in the IGRs.

Virion budding occurs from the cellular plasma membrane, thereby providing the virion envelope (Dalton et al., 1968, Eichler et al., 2004, Perez et al., 2003, Strecker et al., 2003).

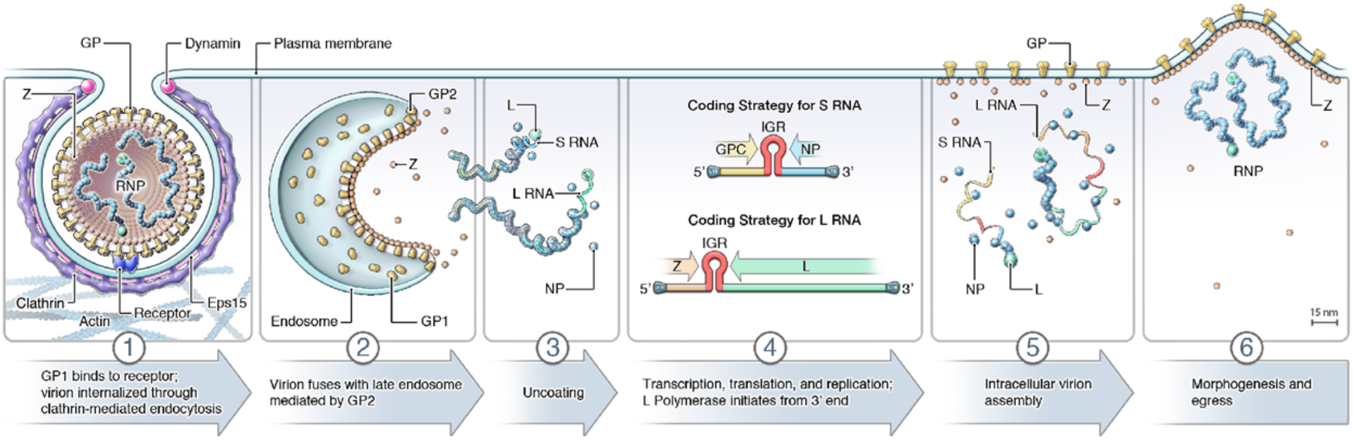


Figure 3.*Arenaviridae*. Lifecycle of arenaviruses. (1) Virion uptake; (2) virus-cell membrane fusion;

(3) uncoating; (4) transcription, translation, and replication; (5) virion assembly; and (6) virion budding. GP, glycoprotein; IGR, intergenic region; L, RNA-directed RNA polymerase; NP, nucleoprotein; RNP, ribonucleoprotein; Z, zinc-binding matrix protein. Note that antennaviruses and hartmaniviruses do not encode Z.

Biology

Arenaviruses are ecologically diverse: they have been isolated from fish (antennaviruses) (Shi et al., 2018), rodents, bats, and ticks (mammarenaviruses) (Downs et al., 1963, Sayler et al., 2014), and snakes (reptarenaviruses, hartmaniviruses) (Hetzel et al., 2013, Hepojoki et al., 2018, Hepojoki et al., 2015, Stenglein et al., 2012). The geographic distribution of arenaviruses overlaps with the distribution of their hosts. Most mammalian arenaviruses infect rodents of preferentially one or a few species and are, therefore, geographically constrained to their hosts, but LCMV, which infects the ubiquitous house mouse (*Mus musculus* Linnaeus, 1758) appears distributed globally (Childs 1993). The natural distribution of reptilian arenaviruses is unknown as they have only been detected in captive snakes thus far (Hetzel et al., 2013, Hepojoki et al., 2018, Hepojoki et al., 2015, Stenglein et al., 2012). A diverse range of vertebrate cell lines are permissive to mammalian arenavirus infection *in vitro*; certain reptilian cell lines support replication of reptilian arenaviruses (Hepojoki et al., 2018, Stenglein et al., 2012, Lukashevich et al., 1983).

Antigenicity

Systematic antigenicity studies have only been reported for mammarenavirions (see section on *Mammarenavirus* genus page).

Genus demarcation criteria

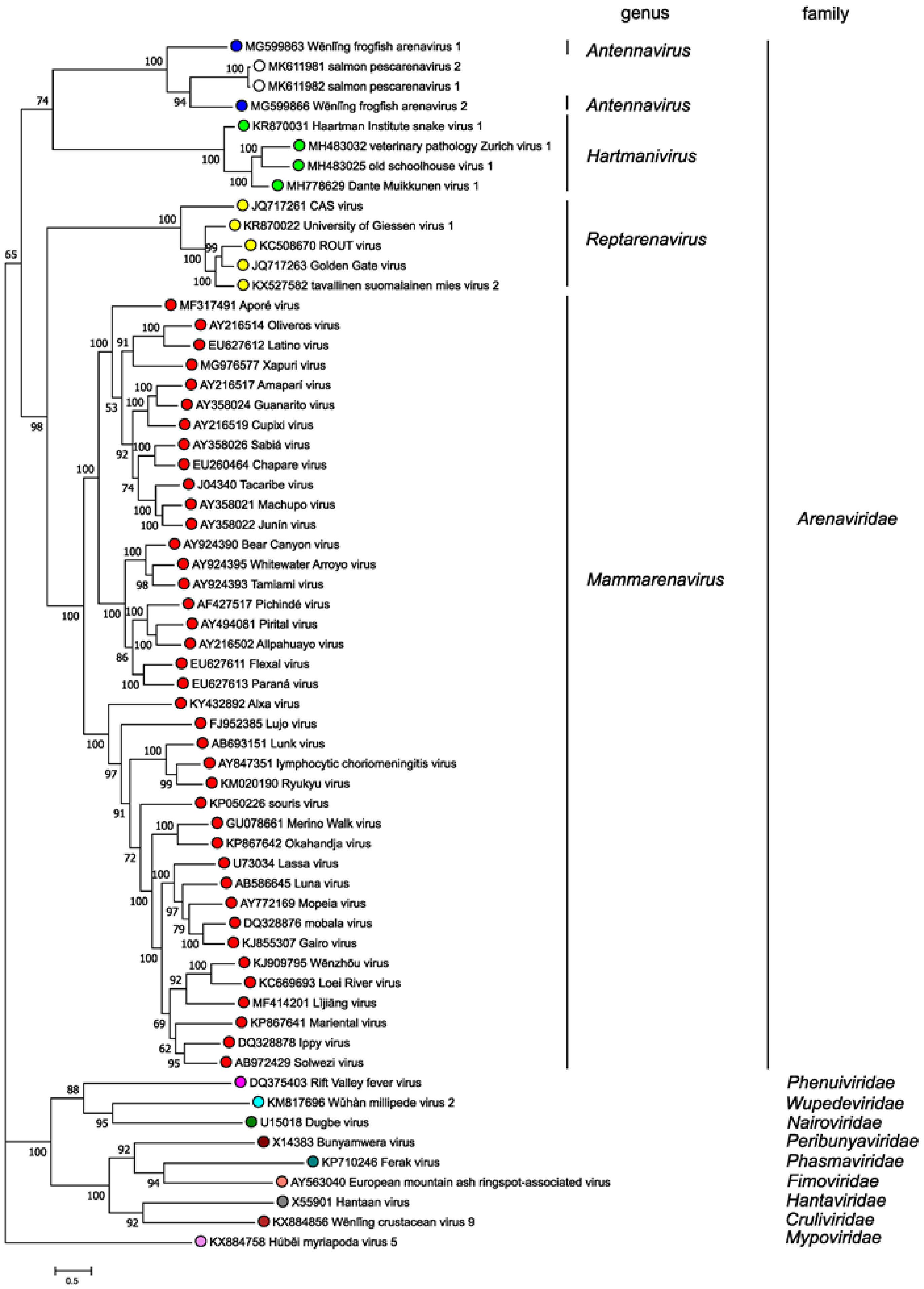
Classification of arenaviruses is currently based on pairwise sequence comparisons (PASC) of coding-complete genomes. Based on the most current sequence dataset, S segment and L segment nucleotide sequence identities for viruses within the same genus need to be higher than 40% and 35%, respectively (Radoshitzky et al., 2015). Four genera have been established to date. Viruses assigned to a genus form a monophyletic clade in well-supported maximum likelihood trees using complete L and NP nucleotide sequences and/or core L palm domain sequences. Use of L and NP for taxonomic purposes is justified by the presence of broadly conserved domains and the rarity of reassortment between genetic segments, at least in mammarenaviruses. Hence, the availability of at least coding-complete sequences of all genome segments may be sufficient for arenavirus classification in the absence of a cultured isolate. Classification is also possible when at least a coding-complete genomic S segment sequence is available together with a cultured isolate (Radoshitzky et al., 2015). However, at the present time, classification also includes the consideration of phenotypic characters such as significant differences in member virus genome architecture, virion antigenicity, and virus ecology (e.g., host range, pathobiology, and transmission patterns).

Derivation of names

Arenaviridae: from the Latin *arenosus* meaning “sandy” and *arena* meaning “sand,” in recognition of the “sandy” appearance of mammarenavirus particles observed in electron-microscopic thin sections (Rowe et al., 1970a).

Relationships within the family

Phylogenetic relationships across the family have been established from maximum likelihood trees generated using complete L amino acid sequences (Figure 4.*Arenaviridae*). Phylogenetic relationships between viruses assigned to more closely related genera and within genera can also be established using other structural protein genes, notably NP.


genus
family
100
MG599863 Wēnlǐng frogfish arenavirus 1
Antennavirus
100
MK611981 salmon pescarenavirus 2
MK611982 salmon pescarenavirus 1
74
94
MG599866 Wēnlǐng frogfish arenavirus 2
Antennavirus
KR870031 Haartman Institute snake virus 1
100
MH483032 veterinary pathology Zurich virus 1
100
MH483025 old schoolhouse virus 1
Hartmanivirus
100
MH778629 Dante Muikkunen virus 1
JQ717261 CAS virus
100
KR870022 University of Giessen virus 1
65
99
KC508670 ROUT virus
Reptarenavirus
100
JQ717263 Golden Gate virus
100
KX527582 tavallinen suomalainen mies virus 2
MF317491 Aporé virus
100
AY216514 Oliveros virus
100
91
EU627612 Latino virus
MG976577 Xapuri virus
100
AY216517 Amaparí virus
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AY358024 Guanarito virus
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AY216519 Cupixi virus
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AY358026 Sabiá virus
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EU260464 Chapare virus
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J04340 Tacaribe virus
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AY358021 Machupo virus
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AY358022 Junín virus
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AY924390 Bear Canyon virus
AY924395 Whitewater Arroyo virus
Arenaviridae
98
AY924393 Tamiami virus
Mammarenavirus
AF427517 Pichindé virus
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AY494081 Pirital virus
100
AY216502 Allpahuayo virus
86
EU627611 Flexal virus
100
EU627613 Paraná virus
KY432892 Alxa virus
FJ952385 Lujo virus
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AB693151 Lunk virus
100
AY847351 lymphocytic choriomeningitis virus
97
99
KM020190 Ryukyu virus
KP050226 souris virus
91
100
GU078661 Merino Walk virus
KP867642 Okahandja virus
72
U73034 Lassa virus
100
AB586645 Luna virus
100
AY772169 Mopeia virus
97
79
DQ328876 mobala virus
100
KJ855307 Gairo virus
100
100
KJ909795 Wēnzhōu virus
92
KC669693 Loei River virus
MF414201 Lìjiāng virus
69
KP867641 Mariental virus
62
DQ328878 Ippy virus
95
AB972429 Solwezi virus
88
DQ375403 Rift Valley fever virus
Phenuiviridae
KM817696 Wǔhàn millipede virus 2
Wupedeviridae
95
U15018 Dugbe virus
Nairoviridae
100
92
X14383 Bunyamwera virus
Peribunyaviridae
KP710246 Ferak virus
Phasmaviridae
94
AY563040 European mountain ash ringspot-associated virus
Fimoviridae
100
X55901 Hantaan virus
Hantaviridae
92
KX884856 Wēnlǐng crustacean virus 9
Cruliviridae
KX884758 Húběi myriapoda virus 5
Mypoviridae
0.5

Figure 4.*Arenaviridae*. Maximum likelihood phylogenetic tree inferred from PRANK alignment (Löytynoja and Goldman 2008) of the complete L amino acid sequences of 50 arenaviruses assigned to the genera *Antennavirus* (blue dots, blue rings for unclassified viruses the genus), *Hartmanivirus*(green dots), *Mammarenavirus* (red dots) and *Reptarenavirus* (yellow dots), along with representative viruses of other bunyavirus families (other colors of dots). The best-fit model of protein evolution (LG+G) was selected using ProtTest 3 (v. 3.4.2) (Darriba et al., 2011). The maximum likelihood tree with 1,000 bootstrap replicates was produced using RAxML (v. 8) (Stamatakis 2014). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap is shown next to branch nodes (when ≥ 70%). The tree was visualized using FigTree (http://tree.bio.ed.ac.uk) and is mid-point rooted. This phylogenetic tree and corresponding sequence alignment are available to download from the Resources page.

Relationships with other taxa

Arenaviruses are closely related to Húběi myriapoda virus 5 (*Bunyavirales*: *Mypoviridae*) (Shi et al., 2016).

Related, unclassified viruses

Additional unclassified arenaviruses that are probable members of existing genera are listed under individual genus descriptions.

Virus name	Accession number	Virus abbreviation
DF 20/00 virus (Granzow et al., 2014)	Not available	-
DF 26/02 virus (Granzow et al., 2014)	Not available	-
Hyriopsis cumingii Lea plague virus (Carella et al., 2016, Zhong et al., 2011)	Not available	HcPV

Virus names and abbreviations are not official ICTV designations.

Member taxa

- *Antennavirus*
- *Hartmanivirus*
- *Mammarenavirus*
- *Reptarenavirus*

Paramyxoviridae

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The citation for this ICTV Report chapter is the summary published as Rima et al., (2019):
ICTV Virus Taxonomy Profile: *Paramyxoviridae*, Journal of General Virology, 100:1593–1594

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Edited by: Jens H. Kuhn and Stuart G. Siddell
Posted: September 2019, updated August 2020
PDF: ICTV_*Paramyxoviridae*.pdf

Summary

The family *Paramyxoviridae* consists of large enveloped RNA viruses infecting mammals and birds, or in some cases reptiles and fish (Table 1.*Paramyxoviridae*). Many paramyxoviruses are host-specific and several such as measles virus, mumps virus, Nipah virus, Hendra virus and several parainfluenza viruses are pathogenic for humans. Virus transmission is horizontal, mainly through direct contact and airborne routes; no vectors are known.

Table 1.*Paramyxoviridae*. Characteristics of members of the family *Paramyxoviridae*

Characteristic	**Description**
Typical member	measles virus, Ichinose-B95a (AB016162), species *Measles morbillivirus*, genus *Morbillivirus*
Virion	Enveloped, pleomorphic (mostly spherical) virions with a diameter of 300–500 nm enclosing a ribonucleoprotein
Genome	Negative-sense, non-segmented RNA genomes of 14.6–20.1 kb
Replication	Cytoplasmic, by the virus ribonucleoprotein complex, involves replication of antigenome and transcription of 6–8 positive-sense mRNAs
Translation	Cytoplasmic, by cellular machinery from capped and poly-adenylated mRNAs
Host range	Mammals, birds, fish and reptiles

Taxonomy	Realm Riboviria, phylum *Negarnaviricota*, class *Monjiviricetes*, order *Mononegavirales*. Currently 4 subfamilies, 17 genera and 78 species

Virion

Morphology

Virions are 150 nm or more (up to 500nm) in diameter, pleomorphic, but usually spherical in shape in vitreous ice. Virions consist of a lipid envelope surrounding a nucleocapsid. The envelope is derived directly from the host cell plasma membrane by budding and contains two transmembrane glycoproteins (Figure 1.*Paramyxoviridae*). These are present as homo-oligomers and form spike-like projections, 8–12 nm in length, spaced 7–10 nm apart (depending on virus genus affiliation). Also, depending on the genus, one or two additional transmembrane proteins may be present. One non-glycosylated membrane or matrix protein is associated with the inner face of the envelope. The virus nucleocapsid consists of negative-sense virus genome RNA and the nucleocapsid protein (N). The nucleocapsid has helical symmetry and is approximately 18 nm in diameter with a 7 nm pitch; its length can be up to 1,000 nm in viruses of some genera. The ribonucleoprotein (RNP) complex in the virion consists of the nucleocapsid together with the polymerase-associated or phosphoprotein (P) and the L protein (L, including RNA-directed RNA polymerase, capping and cap methylation activities) (Lamb and Parks 2007). Multiploid virions are found, although the vast majority of virions contain a single functional genome.

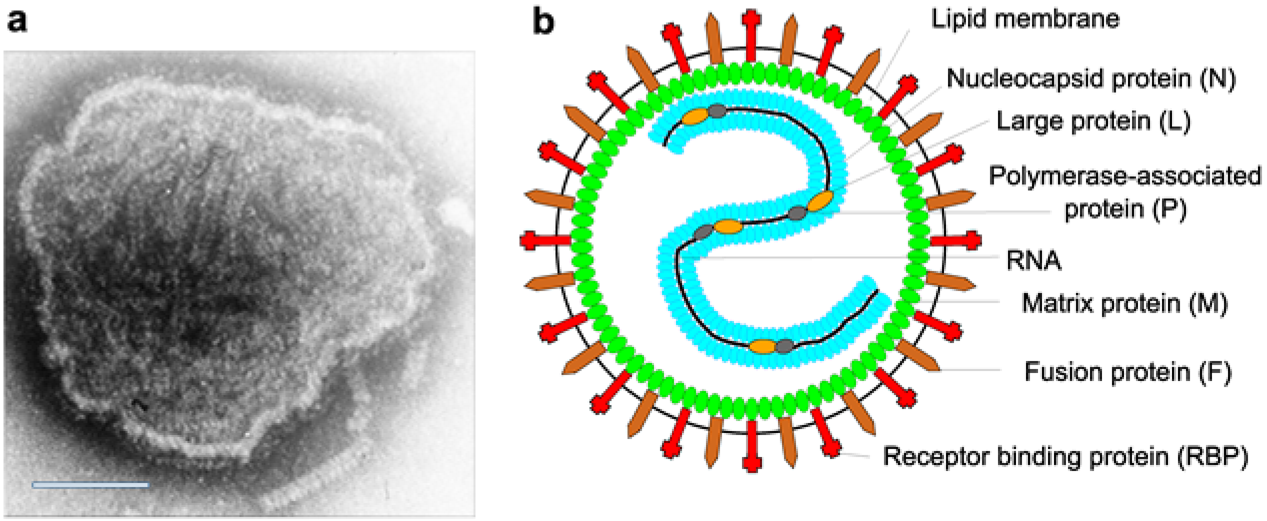

Figure 1.*Paramyxoviridae.* Paramyxovirus virion structure. (A) Negative-contrast electron micrograph of intact measles virus particle (genus *Morbillivirus*). Scale bar = 100 nm. (B) Schematic diagram of paramyxovirus particle in cross-section.

Physicochemical and physical properties

Virion Mr is about 500 ×10^6, and much greater for multiploid virions. Virion buoyant density in sucrose is 1.18–1.20 g cm^{-3}. Virion $S_{20,w}$ is at least 1000S. Virions are very sensitive to heat, lipid solvents, ionic and non-ionic detergents, formaldehyde and oxidizing agents.

Nucleic acid

Virions contain a single molecule of linear, negative-sense, single stranded RNA that is not infectious alone but is infectious if the RNP complex is introduced into the cytoplasm. The RNA genome varies from 14,296 nucleotides for Antarctic penguin virus B to 20,148 nt for Pohorje Myodes paramyxovirus 1. Genomes of all viruses in the family *Paramyxoviridae* are multiples of 6 nt, which is a requirement for efficient replication (Calain and Roux 1993). Some virions may contain positive-sense RNA and so partial self-annealing of extracted RNA may occur. Intracellularly, or in virions, genome-length RNA is found exclusively encapsidated in ribonucleocapsids (RNPs). The genome RNA does not contain a 5′-cap, nor a covalently linked protein. The genome 3′-end is not polyadenylated.

Proteins

Members of the family *Paramyxoviridae* encode 6–10 proteins (5–250 kDa) of which several can be derived either from gene editing events in the P locus and an overlapping ORF in the P gene itself (Figure 2.*Paramyxoviridae*). Virion proteins common to all genera include: three nucleocapsid-associated proteins, i.e., an RNA-binding nucleocapsid protein (N), a polymerase-associated phosphoprotein (P) and a large protein (L, including an RNA-directed RNA polymerase (RdRP), mRNA guanylyl- and methyltransferases, and methylation functions required for the capping of mRNAs), and three membrane-associated proteins, i.e., an unglycosylated inner membrane or matrix protein (M) and two glycosylated envelope proteins, comprising a fusion protein (F) and an attachment or receptor-binding protein (RBP, designated variably as HN, haemagglutinin-neuraminidase protein, H, haemagglutinin or G, glycoprotein). The F protein is synthesized within infected cells as a precursor (F_0) that is activated following cleavage by cellular protease(s) to produce the virion disulfide-linked F_1 and F_2 subunits (order: N-F_2-S-S-F_1-C). Some viruses also encode putative non-structural proteins (C), a cysteine-rich protein that binds Zn^{2+} (V) that can be structural or non-structural depending on the virus, a small integral membrane protein (SH) and transmembrane proteins (tM). Some virus genomes, such as that of the fer-de-lance virus, contain transcription units encoding proteins with unidentified functions. Virion enzyme activities include the RNA-directed RNA polymerase and mRNA guanylyl- and methyltransferases functionally encoded in the L protein. Variously represented among the genera are neuraminidases associated with the RBP.

Lipids

Lipids in the virus envelope are derived from host cell plasma membrane.

Carbohydrates

Virions are composed of approximately 6% carbohydrate by weight; composition is dependent on the host cell. Fusion and RBP proteins are glycosylated by *N*-linked carbohydrate side chains.

Genome organization and replication

The genome organization is illustrated in Figure 2.*Paramyxoviridae* for viruses representing 14 of the genera in the family.

Figure 2.*Paramyxoviridae.* Genome organization (3′-to-5′) of viruses in the family *Paramyxoviridae*. Each box represents a separately encoded coding sequence; slashes indicate where multiple distinct ORFs are present within mRNA transcripts. Co-transcriptional editing leads to expression of the V or the P protein: the first shown is derived from the unedited sequence. The lengths of the boxes are approximately to scale although the non-coding sequences (NCS) are not to scale. Certain viruses express additional C proteins by the using multiple secondary translational start sites within the P

gene. In human parainfluenza virus 1 and human parainfluenza virus 3 of the genus *Respirovirus*, the V ORF may be a non-expressed relic, the function of which may be partially compensated by an edited D protein. U is an additional transcription unit between the N and P genes in ferlavirus genomes.

After attachment to cell receptors, virion entry is achieved by fusion of the virion envelope with the cell surface membrane. This can occur at neutral pH. Virus replication occurs in the cell cytoplasm and is thought to be independent of host nuclear functions. The genome is transcribed processively from the 3′-end by the virion-associated RdRP into 6–8 separate positive-sense mRNAs. Transcription is guided by short (10–13 nt) conserved gene start (GS) and gene end (GE) signals flanking the intergenic sequence. The mRNAs are capped by the guanylyl- and methyltransferase activities of the L protein and possess 3′-poly(A) tracts synthesized by reiterative copying of U tracts in GE sequence. Intergenic regions are highly conserved in length (3 nt) and sequence (CUU with few exceptions see Table 2.*Paramyxoviridae* for details) in the orthoparamyxoviruses and metaparamyxoviruses. Neither, the length or sequence of the intergenic sequences is conserved in avulavirus or rubulavirus genomes. RNA replication occurs through an intermediate, the antigenome, which is an exact positive-sense copy of the genome.

RNP assembly occurs in the cytoplasm and is tightly linked to RNA synthesis. RNPs are enveloped by budding at the cell surface plasma membrane at sites containing virion envelope proteins. Orthoparamyxovirus genomes contain 6–8 transcriptional elements that encode 7–11 proteins. Each element encodes a single mRNA with the exception of the P/V element. This element is transcribed into an exact copy mRNA (P or V mRNA, depending on genus) and into alternative versions in which the RNA transcriptase ‘stutters’ on the template at an editing motif midway down the element. This stuttering results in the insertion of one or more pseudo-templated G nucleotides (“RNA editing”) and shifts the reading frame to access alternative ORFs. The exact copy and edited mRNAs synthesize two alternative proteins, P and V, which have identical amino-terminal domains but due to the insertions of G residues have different carboxy-terminal domains. Other truncated, or chimeric, proteins (called I, W, or D, depending on the virus) can be produced by shifting into the third reading frame. The C ORF present in henipavirus, morbillivirus, narmovirus, jeilongvirus, aquaparamyxovirus and respirovirus genomes overlaps the P ORF and can initiate synthesis at an AUG codon that is accessed by ribosomal choice or at alternative start codons in the same ORF.

Biology

Paramyxoviruses have been conclusively identified only in vertebrates and mostly in mammals and birds, although they have recently also been detected in reptiles and fish, including boneless fish. Most viruses have a narrow host range in nature but can infect a broader range of cultured cells. Infection of cultured cells is generally lytic, but temperate or persistent infections are common in this family *in vitro*and *in vivo*. Other features of infection include the formation of inclusion bodies and syncytia. Host cell surface molecules reported to serve as receptors for the attachment for members of the family vary (Thibault et al., 2017). Respiroviruses, some rubulaviruses and all avulaviruses use sialoglycoproteins and glycolipids as receptors. The cell surface proteins signaling lymphocytic activation molecule family member 1 (SLAMF1, aka CD150) and nectin cell adhesion molecule 4 (nectin 4) are major receptors for measles virus and other morbilliviruses. Henipaviruses use ephrin B2 (EFNB2) and B3 (EFNB3) proteins as cellular entry receptors (Table 2.*Paramyxoviridae*).

Table 2.*Paramyxoviridae.* Receptor and receptor binding protein properties of paramyxoviruses

Orthoparamyxovirinae

Genus	**Virus**	**RBP name / amino acid residues**[#]	**Sequence at start of RBP propeller blade 2**[*]	**Cell receptor**	**Intergenic trinucleotides**
Aquaparamyxovirus	Atlantic salmon paramyxovirus	HN 576	**NRKSCS**	? probably neuraminic acid	CUU + CAU (F-HN)
Aquaparamyxovirus	Pacific salmon paramyxovirus	HN 578	**NRKSCS**	? probably neuraminic acid	CUU
Ferlavirus	fer-de-lance virus	HN 564	**NRKSCS**	? probably neuraminic acid	CCU(3x)+ CUU(4x) alternating
Jeilongvirus	Beilong virus	“G” 734	NRRSCT	?	CUU
Jeilongvirus	Tailam virus	“G” 1052	NRRSCT	?	CUU
Jeilongvirus	J-virus	“G” 709	NRRSCS	?	CUU
Jeilongvirus	Pohorje Myodes paramyxovirus 1	“G” 1589	NRRSCT	?	CUU
Jeilongvirus	Mount Mabu Lophuromys virus 1	“G” 854	NRKSCT	?	CUU
Jeilongvirus	Mount Mabu Lophuromys virus 2	“G” 810	**NRKSCS**	? probably neuraminic acid	CUU
Jeilongvirus	Shaan virus	HN 588	**NRKSCS**	? probably neuraminic acid	CUU + CGU (F-SH)
Henipavirus	Hendra virus	G 604	TIHHCS	EFNB2/3	CUU

Henipavirus	Nipah virus	G 420	TVYHCS	EFNB2/3	CUU
Henipavirus	Cedar virus	G 622	QVINCV	EFNB2	CUU
Henipavirus	Mòjiāng virus	G 625	IINSCA	protein?	CUU
Henipavirus	Ghana virus	G 632	NYHSCT	EFNB2	CUU + CUG (F-G)
Morbillivirus	measles virus wt	H 617	DFSNCM	SLAMF1/NECTIN4	CUU + CGU (H-L)
Morbillivirus	measles Ed-Zag vac	H 617	DLSNCM	SLAMF1/NECTIN4/CD46	CUU + CGU (H-L)
Morbillivirus	canine distemper virus	H 607	KTKVCT	SLAMF1/NECTIN4	CUU + CUA (H-L)
Morbillivirus	canine distemper virus vaccine	H 607	KAKVCT	SLAMF1/NECTIN4/?	CUU + CUA (H-L)
Morbillivirus	phocine distemper virus	H 607	NTKICT	SLAMF1/NECTIN4t	CUU + CUA (H-L)
Morbillivirus	rinderpest virus	H 609	ELETCM	SLAMF1/NECTIN4	CUU + CGU (H-L)
Morbillivirus	peste des petits ruminants virus	H 609	DYRSCL	SLAMF1/NECTIN4	CUU
Morbillivirus	dolphin morbillivirus	H 604	GLNFCL	SLAMF1/ NECTIN4	CUU
Morbillivirus	feline morbillivirus	H 595	GMESCT	SLAMF1/ NECTIN4	CUU + CUA (M-F)
Narmovirus	Nariva virus	“H” 657	AYDGCA	protein?	CUU
Narmovirus	Mossman virus	“G” 632	VFDGCS	protein?	CUU + CGU (F-H)
Narmovirus	bank vole virus 1	“G” 625	LRDSCT	protein?	CUU + CUA (P-M and F-H and L-t); CAU (M-F)
Narmovirus	Tupaia paramyxovirus	“H” 665	NLRDCS	protein?	CUU
Respirovirus	human parainfluenza virus 1	HN 575	**NRKSCS**	Neuraminic acid	CUU + CGU (P-M)
Respirovirus	Sendai virus	HN 575	**NRKSCS**	Neuraminic acid	CUU + CCC (HN-L)

Respirovirus	giant squirrel virus	HN 574	**NRKSCS**	? probably neuraminic acid	CUU +CAU (HN-L)
Respirovirus	human parainfluenza virus 3	HN 572	**NRKSCS**	Neuraminic acid	CUU
Respirovirus	bovine parainfluenza virus 3	HN 572	**NRKSCS**	Neuraminic acid	CUU
Respirovirus	porcine parainfluenza virus 1	HN 576	**NRKSCS**	? probably neuraminic acid	CUU
Respirovirus	caprine parainfluenza virus 3	HN 574	**NRKSCS**	? probably neuraminic acid	CUU
Salemvirus	Salem virus	“G” 620	LSGKCT	protein?	CUU + CCU(P-M) + CGU (F-G)

Metaparaymyxovirinae

Genus	**Virus**	**RBP name / amino acid residues**[#]	**Sequence at start of RBP propeller blade 2**[*]	**Cell receptor**	**Intergenic trinucleotides**
Synodonvirus	Wēnlǐng triplecross lizardfish paramyxovirus	“HN”621	PAPSCP	protein?	CUU + CAUCUU (F-HN)

Rubulavirinae

Genus	**Virus**	**RBP name/ amino acid residues**[#]	**Sequence at start of RBP propeller blade 2**[*]	**Cell receptor**
Orthorubulavirus	mumps virus	HN 582	**NRKSCS**	Neuraminic acid
Orthorubulavirus	La Piedad Michoacán Mexico virus	HN 576	**NRKSCS**	? probably neuraminic.acid
Orthorubulavirus	Mapuera virus	HN 582	**NRKSCS**	? probably neuraminic acid
Orthorubulavirus	simian virus 41	HN 568	**NRKSCS**	? probably neuraminic acid
Orthorubulavirus	human parainfluenza virus 2	HN 571	**NRKSCS**	? probably neuraminic acid

Orthorubulavirus	human parainfluenza virus 4	HN 579	**NRKSCS**	? probably neuraminic acid
Orthorubulavirus	parainfluenza virus 5	HN 532	**NRKSCS**	Neuraminic acid
Orthorubulavirus	Alston virus	HN 565	**NRKSCS**	? probably neuraminic acid
Pararubulavirus	Menangle virus	“HN” 595	PVRTCS	protein?
Pararubulavirus	Tioman virus	“HN” 593	QARGCS	protein?
Pararubulavirus	Teviot virus	“HN” 595	QTRGCS	protein?
Pararubulavirus	Achimota virus 1	“HN” 595	VTYQCS	protein?
Pararubulavirus	Achimota virus 2	“HN” 583	FRRGCS	protein?
Pararubulavirus	Hervey virus	“HN” 543	PKRSCS	protein?
Pararubulavirus	Tuhoko virus 1	“HN” 580	WLRSCS	protein?
Pararubulavirus	Tuhoko virus 2	“HN” 588	VSRQCS	protein?
Pararubulavirus	Tuhoko virus 3	“HN” 582	RLYHCS	protein?
Pararubulavirus	Sosuga virus	“HN” 582	RLYHCS	protein?

Avulavirinae

Genus	**Virus**	**RBP name/** **amino acid residues**	**Sequence at start of RBP propeller blade 2[*]**	**Cell receptor**
Metaavulavirus	avian paramyxovirus 2	HN 580	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 5	HN 574	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 6	HN 613	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 7	HN 569	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 8	HN 577	**NRKSCS**	? probably neuraminic acid

Metaavulavirus	avian paramyxovirus 10	HN 575	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 11	HN 583	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 14	HN 580	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 15	HN 579	**NRKSCS**	? probably neuraminic acid
Metaavulavirus	avian paramyxovirus 20	HN 574	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	avian paramyxovirus 1 (NDV)	HN 571	**NRKSCS**	Neuraminic acid
Orthoavulavirus	avian paramyxovirus 9	HN 579	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	avian paramyxovirus 12	HN 614	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	avian paramyxovirus 13	HN 579	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	avian paramyxovirus 16	HN 618	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	Antarctic penguin virus A	HN 599	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	Antarctic penguin virus B	HN 591	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	Antarctic penguin virus C	HN 587	**NRKSCS**	? probably neuraminic acid
Orthoavulavirus	avian paramyxovirus 21	HN 567	**NRKSCS**	? probably neuraminic acid
Paraavulavirus	avian paramyxovirus 3	HN 577	**NRKSCS**	? probably neuraminic acid
Paraavulavirus	avian paramyxovirus 4	HN 569	**NRKSCS**	? probably neuraminic acid

[#] The nomenclature for RBP (G, H or HN) used in the accessions in the data bank submissions is shown in quotation marks.

[*] The canonical NRKSCS sequence at the start of propeller blade 2 (Langedijk et al., 1997) is shown in bold lettering; in the *Avulavirinae* and *Rubulavirinae* intergenic sequences vary widely in length and sequence and hence are not recorded in the Table.

Nucleocapsids associate with virus membrane proteins at the plasma membrane and are enveloped by budding out at the membrane.

Transmission of paramyxoviruses is horizontal, mainly through airborne and direct contact routes; no vectors are known. Paramyxovirus infection typically begins in the respiratory tract and may remain at that site (e.g., human parainfluenza virus 1 [HPIV-1]) or spread to secondary sites (e.g., lymphoid and endothelial tissues for measles virus (MV) (Griffin 2007), the parotid gland, CNS and endothelial tissues for mumps virus (MuV) (Carbone and Rubin 2007) or lung and CNS for Hendra virus (HeV) and Nipah virus (NiV) (Eaton et al., 2007). In general, paramyxovirus infections are limited, and eliminated, by host immunity. However, virus can sometimes be shed for periods of weeks or months in healthy and, especially, in immunocompromised individuals. Latent infection is unknown. However, long-term persistent infection is known for several morbilliviruses such as MV in subacute sclerosing panencephalitis, a rare complication that involves persistence of a defective measles virus in the CNS for periods of, on average, 8 years. Old dog distemper can involve persistence of defective or fully infectious canine distemper virus for weeks or months in healthy and, especially, immunocompromised animals. Feline morbillivirus has been shown to be shed for long periods from the kidneys of cats. The recurrence of neurological manifestations has also been noted in NiV patients more than 4 years after recovery from acute encephalitis (Eaton et al., 2007).

Antigenicity

The RBP and F proteins are of primary importance in inducing virus-neutralizing antibodies and immunity against reinfection. Antibodies to N and, variably, to other virus proteins also are induced by infection. Following processing into small peptides the virus proteins also stimulate cell-mediated immune responses.

Derivation of names

Avula: from avian and rubula

Cynoglossusvirus: from the genus *Cynoglossus* of the fish from which the virus sequence was obtained

Henipa: from Hendra and Nipah viruses, the first isolates assigned to this genus

Hoplichthysvirus: from the genus *Hoplichthys* of the fish from which the virus sequence was obtained

Meta: from Greek *meta*, meaning “after, beyond”.

Morbilli: from Latin *morbillus*, diminutive of *morbus*, “disease”.

Ortho: from Greek *orthos*, “straight”.

Paramyxo: from Greek *para*, “by the side of”, and *myxa*, “mucus”.

Pneumo: from Greek *pneuma*, “breath”.

Respiro: from Latin *respirare*, “respire, breathe”.

Rubula: from *Rubula inflans* – old name for the disease mumps from Latin *Rubula,* red; *inflans,* swelling or puffing up.

Scoliodonvirus: from binomial name *Scoliodon macrorhynchos* (Bleeker 1852) of Pacific spadenose shark from which the virus sequence was obtained

Subfamily, genus and species demarcation criteria

The current paramyxovirus taxonomic structure is based on a comparison of complete L protein amino acid sequences. The *Paramyxoviridae* Study Group decided to use this as a sole criterion on the basis of the likely monophyly of this large and complex virus protein (Wolf et al., 2018, Dolja and Koonin 2018)consequential upon the ICTV decision to classify viruses even if only known from their genome sequences (Simmonds et al., 2017) . The genetic-based classification reflected previous classifications based on biological characteristics, which are unlikely to be known for all the new paramyxovirus sequences that have become available (Rima et al., 2018). Four subfamilies have been established on the basis of their genetic distance from the node distinguishing the family *Paramyxoviridae* from the *Sunviridae*, which is the closest related outlier family. These distances (substitutions per site) are respectively 0.64 for the *Metaparamyxovirinae*, 0.80 for the *Avulavirinae*, 0.82 for the *Orthoparamyxovirinae* and 0.90 for the *Rubulavirinae*.

Relationships within the family

Phylogenetic analysis of complete L protein amino acid sequences (Figure 3.*Paramyxoviridae*) supports the classification of paramyxoviruses into four subfamilies and fourteen genera based on genetic distances; in addition, three viruses are members of species that are not assigned to a genus or subfamily

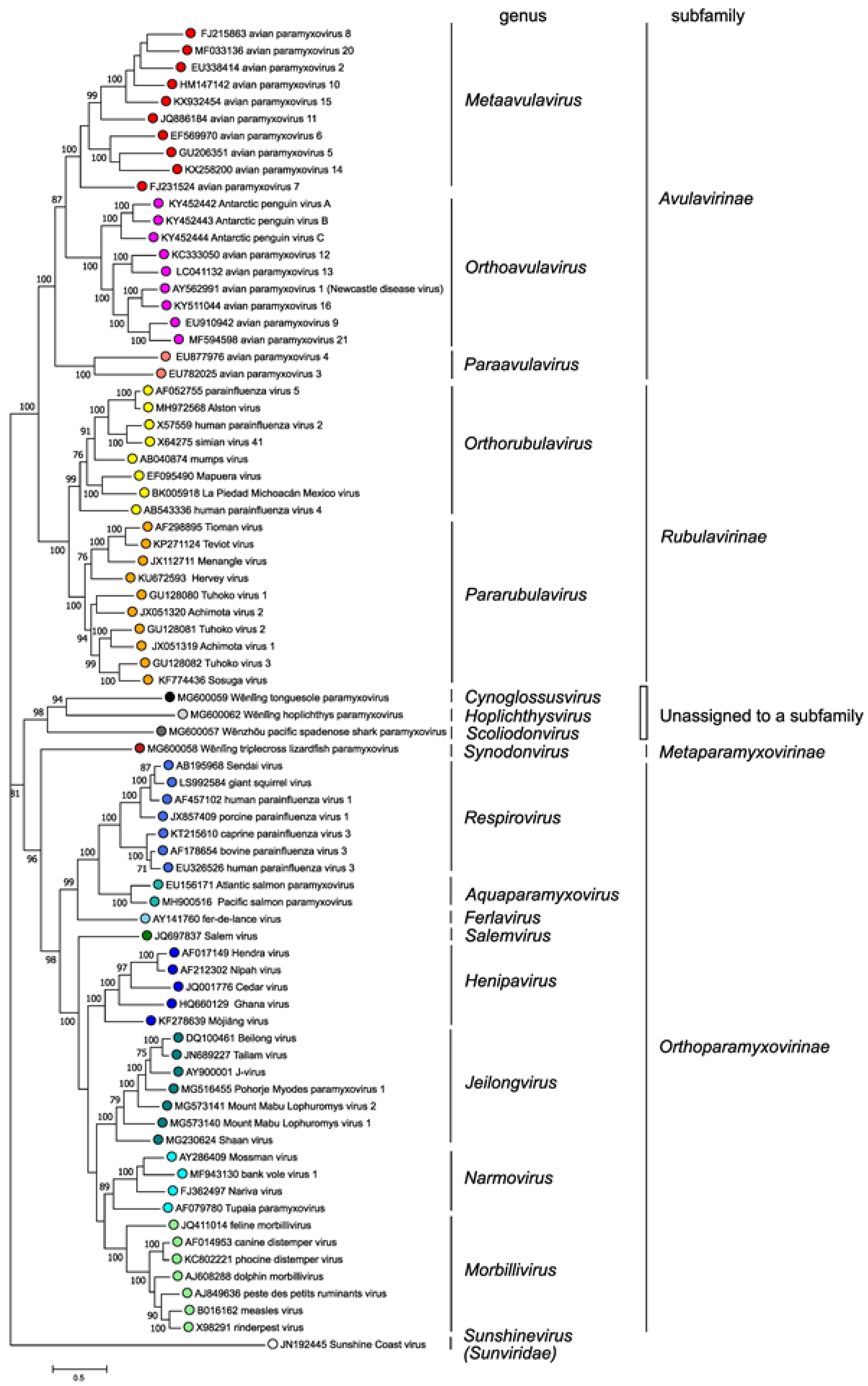
genus
subfamily
FJ215863 avian paramyxovirus 8
MF033136 avian paramyxovirus 20
EU338414 avian paramyxovirus 2
HM147142 avian paramyxovirus 10
KX932454 avian paramyxovirus 15
JQ886184 avian paramyxovirus 11
EF569970 avian paramyxovirus 6
GU206351 avian paramyxovirus 5
KX258200 avian paramyxovirus 14
FJ231524 avian paramyxovirus 7
Metaavulavirus
KY452442 Antarctic penguin virus A
KY452443 Antarctic penguin virus B
KY452444 Antarctic penguin virus C
KC333050 avian paramyxovirus 12
LC041132 avian paramyxovirus 13
AY562991 avian paramyxovirus 1 (Newcastle disease virus)
KY511044 avian paramyxovirus 16
EU910942 avian paramyxovirus 9
MF594598 avian paramyxovirus 21
Orthoavulavirus
EU877976 avian paramyxovirus 4
EU782025 avian paramyxovirus 3
Paraavulavirus
Avulavirinae
AF052755 parainfluenza virus 5
MH972568 Alston virus
X57559 human parainfluenza virus 2
X64275 simian virus 41
AB040874 mumps virus
EF095490 Mapuera virus
BK005918 La Piedad Michoacán Mexico virus
AB543336 human parainfluenza virus 4
Orthorubulavirus
AF298895 Tioman virus
KP271124 Teviot virus
JX112711 Menangle virus
KU672593 Hervey virus
GU128080 Tuhoko virus 1
JX051320 Achimota virus 2
GU128081 Tuhoko virus 2
JX051319 Achimota virus 1
GU128082 Tuhoko virus 3
KF774436 Sosuga virus
Pararubulavirus
Rubulavirinae
MG600059 Wēnlǐng tonguesole paramyxovirus
Cynoglossusvirus
MG600062 Wēnlǐng hoplichthys paramyxovirus
Hoplichthysvirus
MG600057 Wēnzhōu pacific spadenose shark paramyxovirus
Scoliodonvirus
Unassigned to a subfamily
MG600058 Wēnlǐng triplecross lizardfish paramyxovirus
Synodonvirus
Metaparamyxovirinae
AB195968 Sendai virus
LS992584 giant squirrel virus
AF457102 human parainfluenza virus 1
JX857409 porcine parainfluenza virus 1
KT215610 caprine parainfluenza virus 3
AF178654 bovine parainfluenza virus 3
EU326526 human parainfluenza virus 3
Respirovirus
EU156171 Atlantic salmon paramyxovirus
MH900516 Pacific salmon paramyxovirus
Aquaparamyxovirus
AY141760 fer-de-lance virus
Ferlavirus
JQ697837 Salem virus
Salemvirus
AF017149 Hendra virus
AF212302 Nipah virus
JQ001776 Cedar virus
HQ660129 Ghana virus
KF278639 Mòjiāng virus
Henipavirus
DQ100461 Beilong virus
JN689227 Tailam virus
AY900001 J-virus
MG516455 Pohorje Myodes paramyxovirus 1
MG573141 Mount Mabu Lophuromys virus 2
MG573140 Mount Mabu Lophuromys virus 1
MG230624 Shaan virus
Jeilongvirus
Orthoparamyxovirinae
AY286409 Mossman virus
MF943130 bank vole virus 1
FJ362497 Nariva virus
AF079780 Tupaia paramyxovirus
Narmovirus
JQ411014 feline morbillivirus
AF014953 canine distemper virus
KC802221 phocine distemper virus
AJ608288 dolphin morbillivirus
AJ849636 peste des petits ruminants virus
B016162 measles virus
X98291 rinderpest virus
Morbillivirus
JN192445 Sunshine Coast virus
Sunshinevirus
(Sunviridae)
0.5

Figure 3.*Paramyxoviridae.* Phylogenetic analysis of complete L protein amino acid sequences of members of the family *Paramyxoviridae*. Complete L protein amino acid sequences were aligned by Clustal W with gap generation penalties of 5 and extension penalties of 1 in both multi and pairwise alignments. The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model. The tree with the highest log likelihood (-258124.74) is shown. The percentage of 500 trees in which the associated taxa clustered together is shown next to the branches where this was > 70%. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 78 amino acid sequences. There were a total of 2745 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). This phylogenetic tree and corresponding sequence alignment are available to download from the Resources page.

Relationships with other taxa

The member viruses of the family *Paramyxoviridae* have a similar strategy of gene expression and replication and gene order to those of other mononegaviruses, specifically filoviruses and rhabdoviruses.

Member taxa

- *Avulavirinae*
  - *Metaavulavirus*
  - *Orthoavulavirus*
  - *Paraavulavirus*
- *Metaparamyxovirinae*
  - *Synodonvirus*
- *Orthoparamyxovirinae*
  - *Aquaparamyxovirus*
  - *Ferlavirus*
  - *Henipavirus*
  - *Jeilongvirus*
  - *Morbillivirus*
  - *Narmovirus*
  - *Respirovirus*
  - *Salemvirus*
- *Rubulavirinae*
  - *Orthorubulavirus*

  - *Pararubulavirus*
- Genera unassigned to a subfamily
  - *Cynoglossusvirus*
  - *Hoplichthysvirus*
  - *Scoliodonvirus*

Genus	**Spillover rank of 887/risk score**	**Zoonotic**	**Human**					
Antennavirus	*Hairy antennavirus*	*Salmon antennavirus*	*Striated antennavirus*					
Hartmanivirus	*Haartman hartmanivirus*	*Heimat hartmanivirus*	*Muikkunen hartmanivirus*	*Schoolhouse hartmanivirus*	*Setpatvet hartmanivirus*	*Zurich hartmanivirus*		
Mammarenavirus	*Allpahuayo mammarenavirus*	*Alxa mammarenavirus*	*Argentinian mammarenavirus (Junin virus) 96/66*	*Bear Canyon mammarenavirus*	*Brazilian mammarenavirus (Sabia virus)*	*Cali mammarenavirus*	*Chapare mammarenavirus*	*Chevrier mammarenavirus*
Reptarenavirus	*California reptarenavirus*	*Giessen reptarenavirus*	*Golden reptarenavirus*	*Ordinary reptarenavirus*	*Rotterdam reptarenavirus*			

Cupixi mammarenavirus	*Flexal mammarenavirus*	*Gairo mammarenavirus*	*Guanarito mammarenavirus 110/65*	*Ippy mammarenavirus*	*Kitale mammarenavirus*	*Lassa mammarenavirus 1/91*	*Latino mammarenavirus*	*Loei River mammarenavirus*	*Lujo mammarenavirus*

Luna mammarenavirus	*Lunk mammarenavirus*	*Lymphocytic choriomeningitis mammarenavirus 10/85*	*Machupo mammarenavirus 99/66*	*Mariental mammarenavirus*	*Merino Walk mammarenavirus*	*Mobala mammarenavirus*	*Mopeia mammarenavirus 204/61 (subspecies Morogoro virus 171/62)*	*Okahandja mammarenavirus*	*Oliveros mammarenavirus*

Paraguayan mammarenavirus	*Pirital mammarenavirus*	*Planalto mammarenavirus*	*Ryukyu mammarenavirus*	*Serra do Navio mammarenavirus*	*Solwezi mammarenavirus*	*Souris mammarenavirus*	*Tacaribe mammarenavirus*	*Tamiami mammarenavirus*	*Wenzhou mammarenavirus*

				Notes
				Isolated from fish
				Isolated from snakes
Whitewater Arroyo mammarenavirus 134/62	*Xapuri mammarenavirus*	*Arenavirus PREDICT_ ArenaV-1 68950*	Arenavirus PREDICT ArenaV-2 749/48	*Tacaribe and LCMV isolated from ticks recently.*
				Isolated from snakes

Non-Disclosure Agreement

Agreement Summary

[COUNTERPARTY] INFORMATION	
Name:	[●]
Mailing Address:	[●]
Project Lead:	[●]
Management Contact:	[●]

CEPI INFORMATION	
Name:	Coalition for Epidemic Preparedness Innovations (“**CEPI**”)
Mailing Address:	Marcus Thranesgate 2, PO Box 123 Torshov, N-0412 Oslo, Norway
Project Lead:	[●] Timothy P. Endy MD, MPH
Management Contact:	[●] timothy.endy@cepi.net

AGREEMENT INFORMATION	
Project Name:	[●] Virus Expert Panel for Disease X Vaccine Libraries
Effective Date:	January 17, 2022
Expiration Date:	[●] January 17, 2023
This Agreement includes and incorporates by reference:	The agreement (referred to as the “**Agreement**”) means this Agreement Summary together with the following: - Terms and Conditions (“T&Cs”) (Annex A)

THIS AGREEMENT is between the Counterparty and CEPI (each as defined above) and comes into force as of the Effective Date. Each party to this Agreement may be referred to individually as a "**Party**" and together as the "**Parties**".

This Agreement sets out the terms and conditions governing the exchange of information between the Parties and their representatives in the context of the selection of viruses for the Disease X vaccine library **(**the “**Purpose**”). As a pre-condition of this information exchange, the Parties enter into this Agreement by having their authorized representatives sign below.

Signed for and on behalf of [**COUNTERPARTY**] by:

Signature:……………………………………….

Name:…………………………………………..

Title:……………………………………………

Date:……………………………………………

Signed for and on behalf of **COALITION FOR EPIDEMIC PREPAREDNESS INNOVATIONS** by:

Signature:………………………………………

Name:…………………………………………..

Title:…………………………………………….

Date:…………………………………………….

Non-Disclosure Agreement - Terms and Conditions

1. Definitions

1.1 In this Agreement, terms shall have the following meaning:

1.2 "**Applicable Laws**" means all national and supranational laws and regulations and other mandatory professional regulations applicable to a Party or any activities carried out or to be carried in order to achieve the Purpose or a Party's activities or obligations described under or pursuant to this Agreement;

1.3 "**Business Day**" means a day which is not a Saturday or Sunday, or a bank or public holiday in England, Norway, and/or the country in which a Party's mailing address (as set out in the Agreement Summary) is located;

1.4 "**Confidential Information**" means any and all non-public information which by its nature or the manner of its disclosure is reasonably identifiable as Confidential Information, including but not limited to data, results, know-how, software (further including non-open source code), plans, details of research work, discoveries, inventions, intended publications, intended or pending patent applications, designs, technical information, business plans, budgets and strategies, business or financial information or other information in any medium, and any physical items, prototypes, compounds, samples, components, non-public regulatory filings, non-public submissions to regulatory authorities or other articles or materials disclosed on or after the Effective Date of this Agreement by one Party to the other Party whether orally or in writing or in any other form. Confidential information will not include:

a. information that is or was already known to the receiving Party at the time of disclosure, as shown by written records, without any obligation to keep it confidential;

b. information that is independently developed by employees of the receiving Party who have not had access to the Confidential Information of the disclosing Party as evidenced by contemporaneous written records;

c. information that at the time of being disclosed or obtained by the receiving Party or at any time thereafter, is published or otherwise generally available to the public other than due to default by the receiving Party of its obligations hereunder;

d. information properly obtained by the receiving Party from a source which, to the best knowledge of the receiving Party, is not known to be bound by a confidentiality agreement, fiduciary obligation or other legal or contractual restriction that may prohibit the disclosure of such Confidential Information;

e. information necessarily disclosed by the receiving Party pursuant to a statutory obligation and the Party required to make that disclosure has informed the other, within a reasonable time after being required to make such disclosure, of the requirement to disclose and the information required to be disclosed; and

f. information approved for release in writing by an authorised representative of the disclosing Party.

1.5 "**CEPI Group**" means the nodes of CEPI established in Norway, England, India, the United States of America and any other node of CEPI which may be established from time to time.

1.6 "**Group**" means in relation to a company (other than CEPI), that company, any subsidiary or any holding company from time to time of that company, and any subsidiary from time to time of a holding company of that company. Each company in a Group is a member of the Group;

1.7 all terms defined in the Agreement Summary shall have the same meaning throughout the Agreement.

2. Confidentiality; Restricted Use

2.1 All Confidential Information shall be used by the receiving Party ("**Receiving Party**") exclusively for the Purpose, unless otherwise expressly agreed to in writing by the disclosing Party ("**Disclosing Party**").

2.2 Each Party undertakes that during the term of this Agreement and for a period of five (5) years after its termination or expiry, it shall keep confidential and not disclose any Confidential Information of the other Party disclosed to or obtained by it in connection with this Agreement or the Purpose other than representatives, employees, agents, consultants, professional advisers, sub-contractors, regulatory authorities of itself or members of its Group and, in the case of CEPI, its funders or members of the CEPI Group, in each case who have a need to know Confidential Information of the other Party disclosed to or obtained by it in connection with the Purpose.

2.3 Each Party shall ensure that all representatives, employees, agents, consultants, professional advisers, sub-contractors (to the extent it can legally do so) and third parties to which Confidential Information of the other Party is disclosed are:

a. informed of the confidentiality provisions of this Agreement and

b. bound by obligations of confidentiality and non-use at least as protective as the provisions contained herein, it being understood that where such person is a natural person (i.e. individual), such person shall not be allowed to disclose the Confidential Information to any other person.

2.4 Each Party shall take reasonable security precautions to protect against unauthorized disclosure of Confidential Information.

2.5 **Required Disclosure.** The disclosure of information that is required to be disclosed by a competent Court or regulatory authority or otherwise by Applicable Law may be disclosed as required, provided that where it is free to do so, the Receiving Party shall give notice of such disclosure to the Disclosing Party as soon as reasonably practicable and all such information shall be marked as “Confidential”.

2.6 **Permitted Disclosures.** Notwithstanding the above, nothing in this Agreement shall restrict a Party’s (or any of its representatives, employees, agents, consultants, professional advisers, sub-contractors) right to

a. disclose the existence of a relationship between the Parties for the purpose of declaring a potential conflict of interest;

b. disclose Confidential Information to any committee or regulatory body in furtherance of Party’s statutory or regulatory duties.

3. Refusal

3.1 Each Party shall have the right to refuse to accept any information under this Agreement prior to any disclosure; information disclosed despite such a refusal is not covered by the confidentiality obligation under this Agreement. Nothing herein shall require either Party to disclose any particular information.

4. No Licence

4.1 Each Party reserves all rights in its Confidential Information. The disclosure of Confidential Information by one Party does not give the other Party or any other person any licence or other right in respect of any Confidential Information beyond the rights expressly set out in this Agreement.

5. No Warranty

5.1 Except as expressly stated in this Agreement, neither Party makes any express or implied warranty or representation concerning its Confidential Information, including but not limited to the accuracy or completeness of the Confidential Information.

5.2 The disclosure of Confidential Information by the parties shall not form any offer by, or representation or warranty on the part of, that party to enter into any further agreement with the other party in relation to the Purpose.

6. Termination

6.1 This Agreement shall come into force on the Effective Date and shall automatically expire five (5) years later. It may be prematurely terminated by mutual agreement of the Parties or by one Party upon thirty (30) days' prior written notice to the other Party of its intention to terminate. The rights and obligations of the Parties which have accrued prior to termination shall, however, survive the termination of this Agreement for a period of five (5) years.

7. Return

7.1 Within sixty (60) days after termination or expiry of this Agreement the disclosing Party may request in writing from the Receiving Party that the Receiving Party at the Disclosing Party's discretion either return or destroy all Confidential Information received from the Disclosing Party and stored electronically and/or on record-bearing media as well as any copies thereof. The Receiving Party shall confirm in writing such destruction or return the Confidential Information as well as any copies thereof to the Disclosing Party within fourteen (14) days after receipt of the Disclosing Party's request.

7.2 The Parties acknowledge that return and/or destruction of Confidential Information constitutes an administrative burden, and agree to keep requests for return and/or destruction of Confidential Information to the minimum required.

7.3 The provisions of Clause **Error! Reference source not found.** above shall not apply to copies of electronically exchanged Confidential Information made as a matter of routine information technology backup and to Confidential Information or copies thereof which must be stored by the receiving Party according to provisions of mandatory law or to the receiving Party's internal compliance guidelines, provided that such Confidential Information or copies thereof shall be subject to an indefinite confidentiality obligation according to the terms and conditions set forth herein until returned and/or destroyed, as the case may be.

8. Resolving Differences

8.1 **Escalation process**. Any question, difference or dispute which may arise concerning the construction, meaning or effect of this Agreement, or concerning the rights or liabilities of the Parties hereunder, or any other matter arising out of or in connection with this Agreement shall first be submitted to the Chief Executive Officer of CEPI and to the Chief Executive Officer of the other Party (the "**Senior Officers**") for resolution (each of whom may call on others to advise them as they see fit). The Senior Officers shall discuss the matter arising in good faith and in a timely manner and endeavour to reach a mutually agreeable solution. If the Parties are unable to resolve such dispute through such negotiations within sixty (60) days of such dispute being escalated to the Senior Officers, then in respect of any dispute, controversy or claim the Parties irrevocably submit to arbitration in accordance with Clause **Error! Reference source not found.**.

8.2 **Arbitration**. All disputes arising out of or in connection with this Agreement, including disputes on its conclusion, binding effect, amendment and termination, shall be resolved by arbitration, in accordance with the arbitration rules of the United Nations Commission of International Trade Law (UNCITRAL Arbitration rules) then-current in force. The appointing authority shall be the President of the Swiss Arbitration Association. The number of arbitrators shall be one, unless the parties agree otherwise. The arbitration proceedings shall take place in Geneva. The language of the arbitration shall be in English. The Parties agree to be bound by any award made by the arbitrator(s). An award shall be final and not be subject to any setting aside proceedings before any court absent fraud or misconduct. This clause shall be governed by and construed in accordance with the law of England and Wales without giving effect to any choice of law or conflict of law provisions or rules that would cause the application of the laws of any other jurisdiction.

8.3 **Good faith cooperation in resolving differences**: The Parties will cooperate in good faith to resolve differences and disputes pursuant to this Clause **Error! Reference source not found.**.

9. Limitation and exclusion of liability

9.1 **Liability cap**. Unless otherwise agreed by the Parties in writing, each Party's maximum liability in aggregate to the other Party arising out of this Agreement shall not exceed the higher of:

(a) US Dollars $10,000; and

(b) the amount (if higher) that such Party can recover from its insurer for the liability in question.

9.2 **Exclusions**. Neither Party shall be liable to the other Party for any loss of an indirect or consequential nature, nor for any loss of turnover, profits, business or goodwill, whether in contract, warranty, negligence, tort, strict liability or otherwise, arising out of any breach of or failure to perform any of the provisions of this Agreement.

9.3 **Exclusions from Liability Cap**. Notwithstanding the foregoing, nothing in this Agreement shall limit the liability of either Party in respect of: personal injury or death arising out of that Party's negligence or wilful misconduct; or fraud or fraudulent misrepresentation or wilful misconduct; or for any other liability which cannot be limited or excluded as a matter of Applicable Laws.

10. General Provisions

10.1 **Choice of law**. This Agreement and any dispute or claim (including non-contractual disputes or claims) arising out of or in connection with it or its subject matter or formation shall be governed by and construed in accordance with the law of England and Wales without giving effect to any choice of law or conflict of law provisions or rules that would cause the application of the laws of any other jurisdiction.

10.2 **No Rights for Third Parties**. A person who is not a Party to this Agreement has no right under the Contracts (Rights of Third Parties) Act 1999 or otherwise to enforce or to enjoy the benefit of any term of this Agreement.

10.3 **Notices**. Any notice to be given pursuant to this Agreement shall be in writing in the English language and shall be delivered by overnight courier, by registered, recorded delivery or certified mail (postage prepaid) to the address of the recipient Party provided in the Agreement Summary or such other address as a Party may from time to time designate by written notice. Any notice given pursuant to this clause shall be deemed to have been received on the day of receipt, provided receipt occurs on a Business Day of the recipient Party or otherwise on the next following Business Day of the recipient. The Parties agree that email and fax are not valid methods of giving notice under this Agreement.

10.4 **No Waiver**. Neither Party shall be deemed to have waived any of its rights or remedies under this Agreement unless the waiver is expressly made in writing and signed by a duly authorized representative of that Party.

10.5 **Assignment.** Neither this Agreement nor any rights and obligations under this Agreement may be assigned or delegated by either Party without the prior written consent of the other Party, such consent not to be unreasonably withheld.

10.6 **Entire Agreement**. This Agreement, including its Agreement Summary and Annex(es), constitutes the entire agreement and understanding between the Parties relating to its subject matter and together they supersede and replace all prior arrangements, whether written or oral, between the Parties relating to the subject matter of this Agreement.

10.7 **Export regulations.** The Parties shall abide by the applicable export license regulations of the respective country(ies) and, if required, the disclosing Party shall apply for an export license grant prior to any transmission of Confidential Information and to inform the receiving Party sufficiently of any existing limitation.

10.8 **Variation**. No variation, amendment, modification or supplement to this Agreement will be valid unless and until it is made in writing and signed by a duly authorised representative of each Party.

Expert Panel

*committed

Paramyxoviruses

PL Tim Endy timothy.endy@cepi.net
PM Luz Hermida luz.hermida@cepi.net

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Gerald Voss , gerald.voss@cepi.net

CEPI SAC
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Jason McLellan (UT Austin)* jmclellan@austin.utexas.edu
Christina Spiropoulou (CDC)* ccs8@cdc.gov
Daniel Bausch MD (FIND) * Daniel.Bausch@finddx.org
Brian Bird UC DAVIS* bhbird@ucdavis.edu
Heinz Feldmann (NIH RML)*- feldmannh@mail.nih.gov
Jonna Mazet UC Davis jkmazet@ucdavis.edu

Arenaviruses

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Gabrielle Bruegelmans* gabrielle.breugelmans@cepi.net
In-Kyu Yoon* in-kyu.yoon@cepi.net
Gerald Voss, gerald.voss@cepi.net

CEPI SAC
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Daniel Bausch MD* Daniel.Bausch@finddx.org
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Kathryn Hastie PhD* LaJolla Institute- kmhastie@lji.org
Tom Ksiazek (UTMB)* tgksiaze@UTMB.EDU
Brian Bird UC DAVIS* bhbird@ucdavis.edu
Jason McLellan (UT Austin)* jmclellan@austin.utexas.edu
Jonna Mazet UC Davis jkmazet@ucdavis.edu>

From: Timothy Endy on behalf of Timothy Endy <timothy.endy@cepi.net>
To: WOOLHOUSE Mark; Broder, Christopher; Ksiazek, Thomas G.; Jason McLellan; ccs8@cdc.gov; Daniel Bausch; Brian H Bird; feldmannh@mail.nih.gov; Jonna Mazet; Kathryn Hastie; Barrett, Alan; Wang Linfa
Cc: Nick Jackson; Melanie Saville; Kristine Rose; Luz Hermida; Mark Polhemus; Maïna L"Azou Jackson; Mike Whelan; Gabrielle Breugelmans; In-Kyu Yoon; Gerald Voss
Subject: FW: Vaccine library virus selection
Date: Sunday, January 16, 2022 4:39:25 PM
Attachments: Arenaviridae - Arenaviridae - Negative-sense RNA Viruses - ICTV.pdf
Paramyxoviridae - Paramyxoviridae - Negative-sense RNA Viruses - ICTV.pdf
Viruses.xlsx
Non-disclosure-Agreement-CEPI-01172022.docx
Expert Panel 12062021.docx
image001.png
image002.png
image003.png
image004.png
image005.png
image006.png

Dear Expert Panel, from all of us at CEPI we are grateful for your taking the time and agreeing to serve on our expert panel to select viruses for our vaccine library efforts to meet the 100-day mission of having a vaccine available for the next Disease X outbreak. The task at hand in which we need your expertise is to help us in selecting 10-15 viruses from each of our two pilot vaccine projects to develop vaccine libraries for the paramyxoviruses and the arenaviruses.

Organization of the Expert Panel: Attached is a listing of the participants in the expert panels for the paramyxoviruses and the arenaviruses. Some of the experts will be serving on both panels because of your broad expertise. Others will be serving on one due to time constraints or a particular interest in one family. However, if those serving on one panel would like to be involved in both please let me know as you will be welcome to participate. If you see an essential expert I should have invited but failed to please let me know.

Housekeeping: Per CEPI procedure we request that you complete with your information and sign the attached NDA. For members of our Scientific Advisory Committee your current NDA with us will suffice. For those of you who can accept an honorarium we would like to provide a $1,000 honorarium for your participation on each panel. This of course doesn’t cover your time commitment to this project but hope you will accept this as our appreciation for your efforts.

Background Information: Attached you will find some background information on the viruses from each family as listed in the ICTV by subfamilies and genera. I also highlighted those that are human pathogens and those that are zoonotic. In the excel file I merged in information from the UC Davis Spillover program on the spillover likelihood of some specific viruses. These are combined and also in a separate file. The spillover program lists viruses recently identified that are not yet listed in the ICTV or assigned a genera. Dr Bird and Dr Mazet who are on the expert panel are part of the DARPA sponsored Spillover program and will provide more information on this dataset in our general discussion meeting. Also attached are some general information and the latest phylogenetic trees from ICTV. As we progress we at CEPI are available to do the leg work on any additional information you will need ie research papers etc and please send me requests and I’ll make sure they are available prior to the meetings.

Organization of Meetings: Due to travel constraints all meetings will be held by zoom meetings. I will be sending out a doodle poll for availability for our first Zoom meeting. Our participants cross 5 different time zones so will try for early morning meetings US time which will be late afternoon for our UK members and early evening for those in SE Asia. For the first meeting I would like both expert panels for each virus family to participate as I'll give details in a presentation on our Disease X vaccine planning process as well as regulatory approach to meet the 100-day mission and the objectives for this panel to select 10-15 viruses with a likelihood for Disease X emergence for each virus family. I am planning a 1.5 hour first meeting leaving time for discussion and feedback. I envision that for each virus family we will need an additional two 1.5 hour meetings consisting of a general discussion on factors to be considered and a final meeting for virus selection. We can schedule additional meetings if needed. After the general discussion I'll send out a listing of the viruses and for each of you to select 10-15 viruses you think should go into the library. I'll then consolidate the information for our final virus selection meeting as I suspect we will have consensus on a portion of the viruses and can focus on the other viruses for discussion and a final consensus selection. First up will be the paramyxoviruses then the arenaviruses.

Please let me know if you would like additional information or recommendations on ways to improve this selection process.

Again thank you for your participation and very excited to have you involved in this.

Best,

Tim

Timothy Endy
Disease X Project Leader

CEPI New vaccines for a safer world

☎ +13153956450

✉ timothy.endy@cepi.net

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Sensitivity: CEPI Internal

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Australian Government
Department of Agriculture, Water and the Environment

Biosecurity Innovation Program

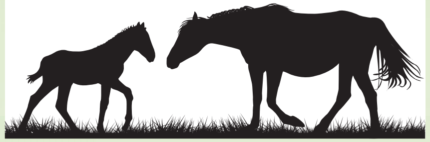
CSIRO

The Research Discovery of a variant genotype of Hendra virus (HeVg2) affording prospective spillover detection and revision of geographical spillover risk.

Dr Edward J Annand

University of Sydney | Sydney School of Veterinary Science and Sydney Institute of Infectious Diseases, Sydney, NSW.
CSIRO | Health and Biosecurity, Black Mountain, ACT.
EquiEpiVet | Equine Veterinary and One Health Epidemiology, Aireys Inlet, Vic.

Horses as Sentinels for Emerging Infectious Disease



with BAT ONE HEALTH

EQUINE VETERINARIANS AUSTRALIA

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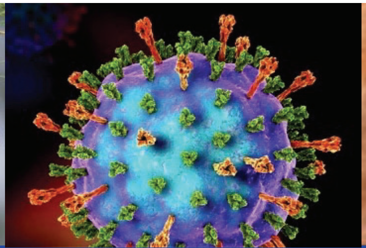
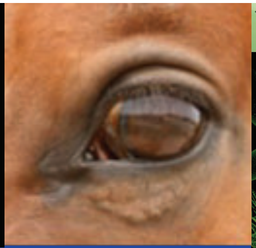
Department of Agriculture, Water and the Environment

Biosecurity Innovation Program





Queensland Government
Department of Agriculture, Fisheries and Forestry
Biosecurity Queensland

CSIRO

HeV spillover to horses and humans as a low sporadic incidence but HIGH CONSEQUENCE disease

Any single missed detection of an infected case (false negatives) place humans and animals at risk of fatal illness

And yet **missed detections are inevitable** due to LOW and SPORADIC INCIDENCE of disease

YEAR/S	**QLD**	**NSW**	**TOTAL**	**HORSE FATALITIES**	**Human**	
					Exposures / illnesses requiring intensive treatment	Fatalities
1994-2010	13	1	14	48	10 (7+3)	4 (3+1)
2011	10	8	18	24	0	0
2012	8	0	8	10	1	0
2013	4	4	8	8	(1*)	0
2014	3	1	4	4	6	0
2015	2	2	4	4	(1*)	0
2016	0	1	1	1	0	0
2017	1	3	4	4	3	0
2018	0	1	1	1	0	0
2019	0	1	1	1	0	0
2020	0	1	1	1	0	0
2021	0	1	1	1	0	0
TOTALS	41	24	65	107	22 (13)+2*	4

Horse cases listed in blue include recently identified HeVg2 disease events. Human cases listed in blue indicate received monoclonal antibody m102.4
Cases marked with an * represent human laboratory exposures (2013 US NiV 2015 CSIRO HeV) and thus are not included in the spillover tally.

HeV Outbreaks in Horses
1994 - 2021

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Department of Agriculture, Water and the Environment
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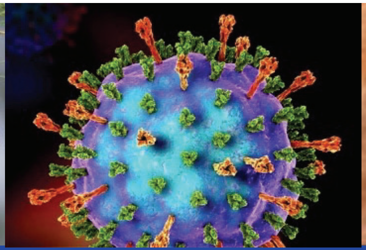
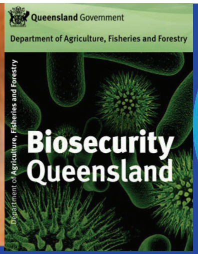




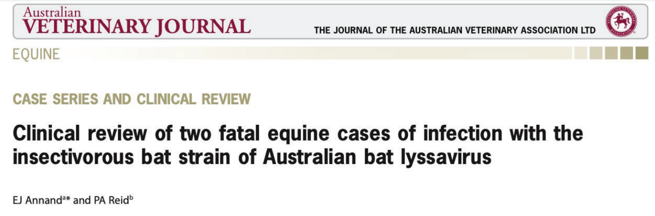
Queensland Government
Department of Agriculture, Fisheries and Forestry
Biosecurity Queensland

CSIRO

Veterinary Testing for unvaccinated horses

- Samples tested for Hendra virus exclusion
- Negative samples are not thoroughly investigated for other pathogens
- Significance realised with detection of two fatal cases of ABLV in 2013

Guidelines for veterinarians handling potential Hendra virus infection in horses



Australian VETERINARY JOURNAL THE JOURNAL OF THE AUSTRALIAN VETERINARY ASSOCIATION LTD

EQUINE

CASE SERIES AND CLINICAL REVIEW

Clinical review of two fatal equine cases of infection with the insectivorous bat strain of Australian bat lyssavirus

EJ Annand[a*] and PA Reid[b]

Veterinary Microbiology 173 (2014) 224–231

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

ELSEVIER

Australian bat lyssavirus infection in two horses

Mustaghfira Wafa Shinwari[a], Edward J. Annand[b], Luke Driver[a], David Warrilow[c], Bruce Harrower[c], Richard J.N. Allcock[d,e], Dennis Pukallus[c], Jennifer Harper[f], John Bingham[f], Nina Kung[g], Ibrahim S. Diallo[a,*]

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Australian Government
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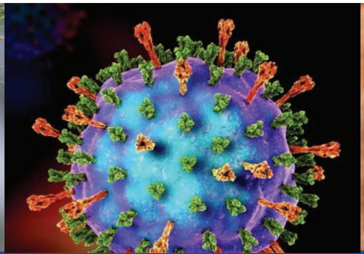

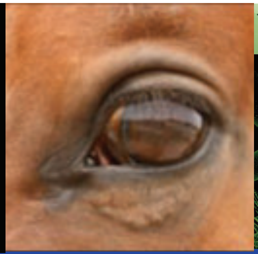

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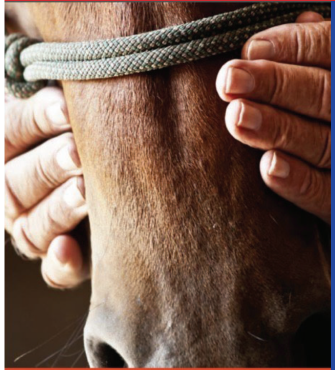


One Health

Combating the threat of infectious diseases to protect Equine and Human Health

Horses as Sentinels of Emerging Infectious Disease

Which pathogens are causing neurological and respiratory symptoms in Hendra negative horses?

Testing Hendra negative horses to Discover Emerging Pathogens causing Hendra like illnesses.

Driven by the hypothesis that:

Some cases of severe Hendra virus-like disease in Australian horses is due to spillover infection by related but divergent bat borne paramyxoviruses such as those in the Rubulavirus and Henipavirus families of which our awareness is emerging.

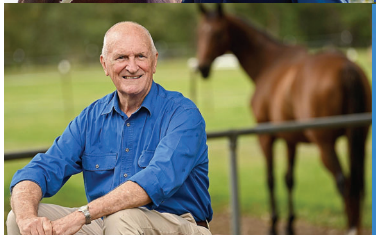
Researchers target new wave of emerging infectious diseases

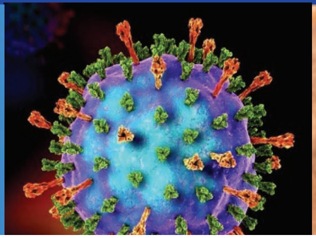
Penelope Arthur
16 Jul 2018, 5 a.m.

The work on emerging infectious zoonotic diseases associated with horses is funded by the Dalara Foundation and is a collaboration between the University of Sydney, School of Veterinary Science, Marie Bashir Institute for Infectious Diseases and Biosecurity and the CSIRO Australian Animal Health Laboratory (AAHL).

The project aims to:

- Identify pathogens in horse cases where the Hendra Virus was confirmed negative
- Confirm and characterise unknown pathogens
- Develop additional diagnostic testing tools for the diagnosis of equine respiratory and neurological diseases and see them incorporated into state diagnostic laboratories.





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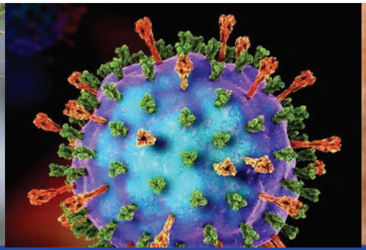

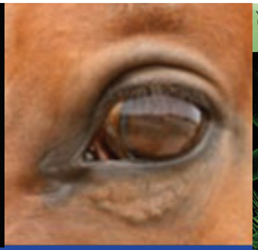

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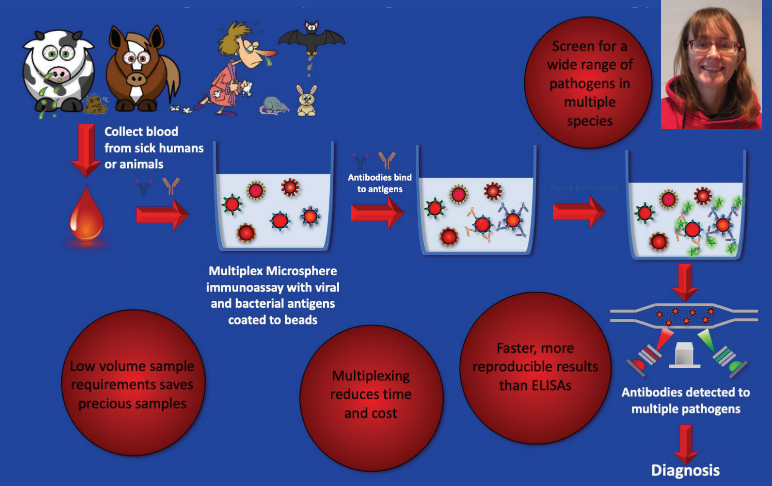
33 plex Microbead Fluorescent Immunoassay Targeting EIDs of Putative EID Relevance to Australian Geography and Horses



Antigens

- Brucella suis repA
- Brucella OMP
- Leptospira
- Chikungunya virus E1 protein
- Sindbis virus E1 protein
- Hepatitis E virus Capsid protein
- Hervey pteropid gammaretrovirus
- La Crosse virus N protein
- West Nile virus E protein

Paramyxoviruses
- Hendra
- Nipah
- Cedar
- Mojang
- Ghana
- Menangle (N and HN)
- Grove
- Yeppoon
- Tioman N

Filovirus
- EBOV (G)
- Bundibugyo
- Reston
- Marburg
- Ravn
- Lloviu
- Mengla
- Zaire N

Coronavirus
- SARS1
- SARS2 (S and N)
- MERS
- Rs4874

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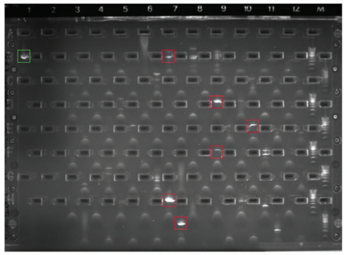
Sources: Commercial, collaborators-CSIRO, Burnett Institute, Vilnius University, Lithuania, Uniformed Services University

Nested Conventional RT-PCR and Total RNA NGS

Viral family pan-RT-PCR



Paramyxo, Corona, Filo, Alpha & Flaviviruses

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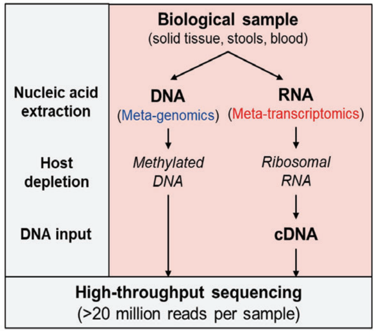
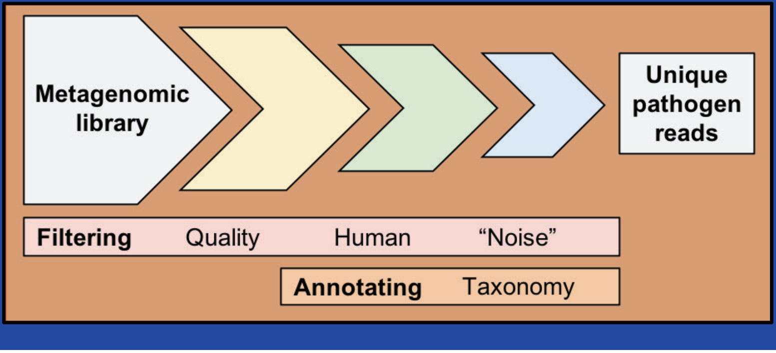
Deep RNA sequencing



Sensitive Bioinformatical Pipeline



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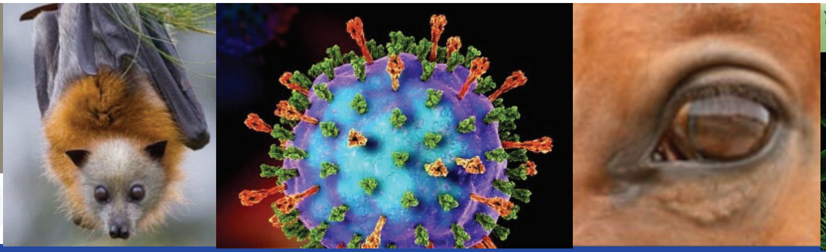

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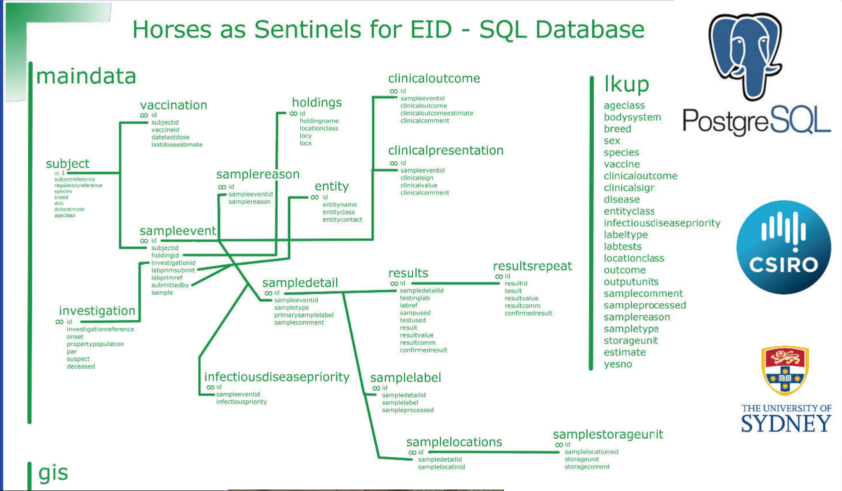
Horses as Sentinels – Targeted Disease Surveillance via research partnering with and extending routine Biosecurity

SQL database allowing syndromic classification, sample processing and multiple testing pipelines.



Horses as Sentinels for EID - SQL Database

maindata

subject
id 1
subjectreference
regulatoryreference
species
breed
dob
dobestimate
ageclass

vaccination
∞ id
subjectid
vaccineid
datelastdose
lastdoseestimate

holdings
∞ id
holdingname
locationclass
locy
locx

samplereason
∞ id
sampleeventid
samplereason

entity
∞ id
entityname
entityclass
entitycontact

clinicaloutcome
∞ id
sampleeventid
clinicaloutcome
clinicaloutcomeestimate
clinicalcomment

clinicalpresentation
∞ id
sampleeventid
clinicalsign
clinicalvalue
clinicalcomment

sampleevent
∞ id
subjectid
holdingid
investigationid
labprimsubmit
labprimref
submittedby
sample

investigation
∞ id
investigationreference
onset
propertypopulation
par
suspect
deceased

sampledetail
∞ id
sampleeventid
sampletype
primarysamplelabel
samplecomment

results
∞ id
sampledetailid
testinglab
labref
sampused
testused
result
resultvalue
resultcomm
confirmedresult

resultsrepeat
∞ id
resultid
tesult
resultvalue
resultcomm
confirmedresult

infectiousdiseasepriority
∞ id
sampleeventid
infectiouspriority

samplelabel
∞ id
sampledetailid
samplelabel
sampleprocessed

samplelocations
∞ id
sampledetailid
samplelocatinid

samplestorageunit
∞ id
samplelocationsid
storageunit
storagecoment

lkup
ageclass
bodysystem
breed
sex
species
vaccine
clinicaloutcome
clinicalsign
disease
entityclass
infectiousdiseasepriority
labeltype
labtests
locationclass
outcome
outputunits
samplecomment
sampleprocessed
samplereason
sampletype
storageunit
estimate
yesno

gis

PostgreSQL

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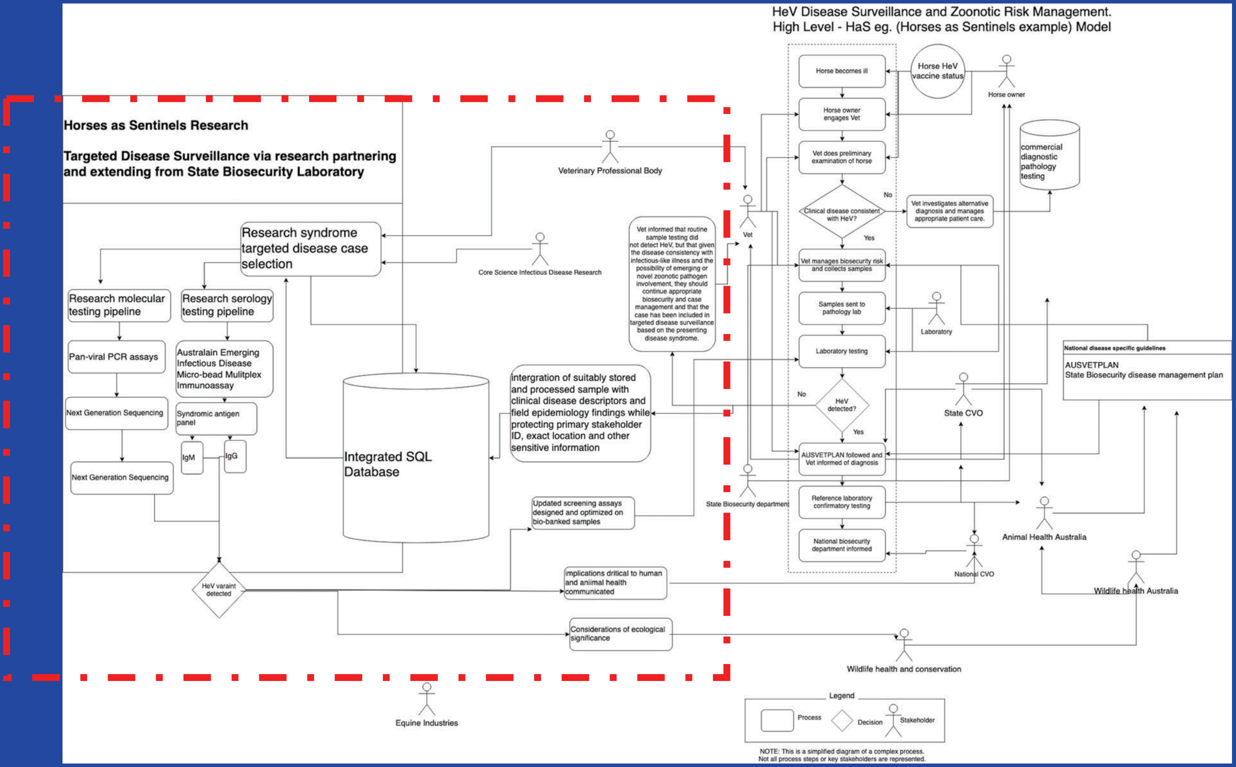
DATA



HeV Disease Surveillance and Zoonotic Risk Management.
High Level - HaS eg. (Horses as Sentinels example) Model

Horses as Sentinels Research

Targeted Disease Surveillance via research partnering and extending from State Biosecurity Laboratory

Research syndrome targeted disease case selection

Research molecular testing pipeline

Pan-viral PCR assays

Next Generation Sequencing

Next Generation Sequencing

Research serology testing pipeline

Australain Emerging Infectious Disease Micro-bead Mulitplex Immunoassay

Syndromic antigen panel

IgM

IgG

Integrated SQL Database

HeV varaint detected

intergration of suitably stored and processed sample with clinical disease descriptors and field epidemiology findings while protecting primary stakeholder ID, exact location and other sensitive information

Updated screening assays designed and optimized on bio-banked samples

implications dritical to human and aniimal health communicated

Considerations of ecological significance

Veterinary Professional Body

Core Science Infectious Disease Research

Vet informed that routine sample testing did not detect HeV, but that given the disease consistency with infectious-like illness and the possibility of emerging or novel zoonotic pathogen involvement, they should continue appropriate biosecurity and case management and that the case has been included in targeted disease surveillance based on the presenting disease syndrome.

Vet

State Biosecurity department

Equine Industries

Horse becomes ill

Horse HeV vaccine status

Horse owner

Horse owner engages Vet

Vet does preliminary examination of horse

Clinical disease consistent with HeV?

No

Yes

Vet investigates alternative diagnosis and manages appropriate patient care.

commercial diagnostic pathology testing

Vet manages biosecurity risk and collects samples

Samples sent to pathology lab

Laboratory

Laboratory testing

HeV detected?

No

Yes

AUSVETPLAN followed and Vet informed of diagnosis

Reference laboratory confirmatory testing

National biosecurity department informed

State CVO

National CVO

National disease specific guidelines

AUSVETPLAN
State Biosecurity disease management plan

Animal Health Australia

Wildlife health Australia

Wildlife health and conservation

Legend

▢ Process	◇ Decision	웃 Stakeholder

NOTE: This is a simplified diagram of a complex process.
Not all process steps or key stakeholders are represented.

Additional pathway extended from and supporting routine surveillance

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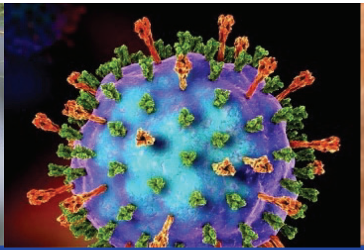
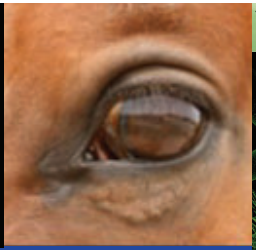

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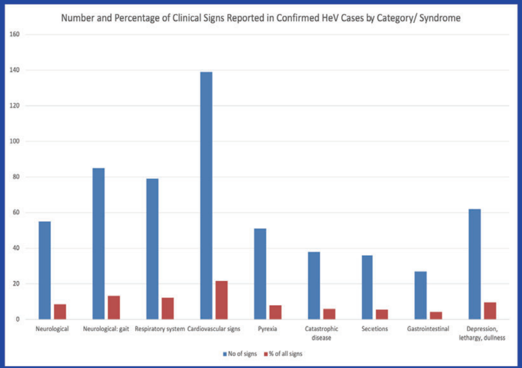
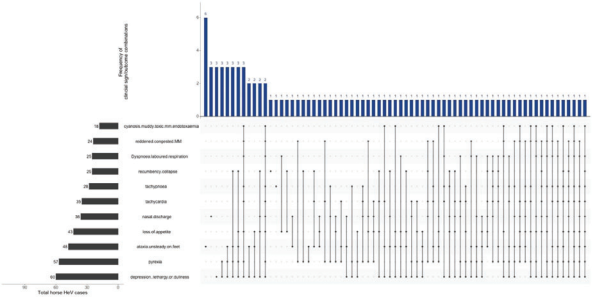
EquiEpiVet

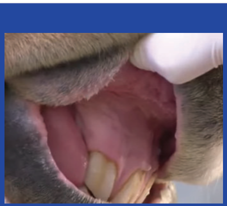
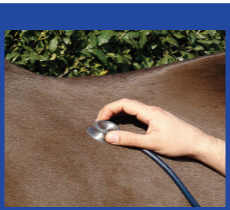
Horses as Sentinels – Targeted Disease Surveillance via research partnering with and extending routine Biosecurity

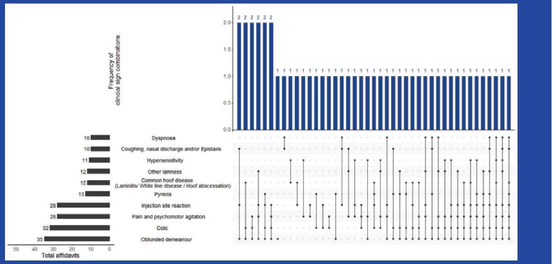
Understanding HeV disease in horses in its pathogenic basis and the syndromic presentation





Upset plot reported HeV cases



Upset plot AEFI

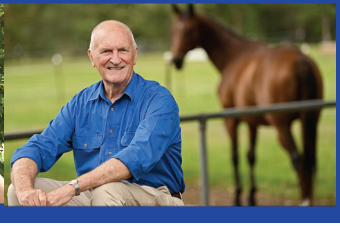
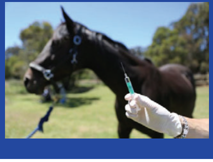




Table 1. Infectious disease prioritization categories (with examples) used in this study to identify Hendra-negative equine disease cases with highest likelihood of similar undiagnosed viral cause from larger cohort for further investigation

Infectious disease priority	Description	Example
Category 1 Highest infectious disease suspect	Case features ‘pyrexia’ or ‘abnormal mucous membranes AND one or more other clinical signs related to infectious disease OR the presence of either change AND ‘epidemiological observation indicative of infectious cause’ based on temporal and/or spatial relationship to similar disease cases	Pyrexia with tachycardia and acute onset respiratory consolidation and/or secretions. Pyrexia and neurological symptoms. Pyrexia and ‘injected/congested’ mucous membranes. ‘Congested/injected mucous membranes’ with acute severe respiratory dysfunction. Clustering of similar cases on same or neighboring properties
Category 2 High infectious disease suspect	Pyrexia OR other clinical signs associated with infectious disease of interest	Acute onset abnormal respiratory secretions. Fever of unknown origin. Colic with the presence of neurological symptoms
Category 3 Moderate infectious disease suspect	Clinical signs may be associated equally with infectious and non-infectious causes	Colic with the presence of dehydration and mucous membrane changes. Ataxia with the absence of pyrexia or known trauma
Category 4 Low infectious disease suspect	Non-infectious etiologies more common or most likely on differential diagnosis list, but infectious cause still possible	Ataxia following known traumatic event. Traumatic wounds following unusual behavioral event. Acute lethargy following chronic non-infectious disease condition
Category 5 No infectious disease suspect	No clinical signs of illness or no infectious cause considered likely	Traumatic wounds in the absence of underlying disease. Screening in unvaccinated horses to manage biosecurity risk prior to invasive procedures addressing non-infectious disease such as is a common requirement for dentistry or admission to equine hospitals in Australia
Category 6 Confirmed infectious disease	Other infectious disease confirmed via diagnostic testing	A case submitted for HeV testing, found negative and then testing positive for alternative known infectious disease such as ABLV, WNV, EHV or RRV*

*ABLV, Australian bat lyssavirus; EHV, Equine herpes virus; HeV, Hendra virus; RRV, Ross River Virus; WNV, West Nile virus

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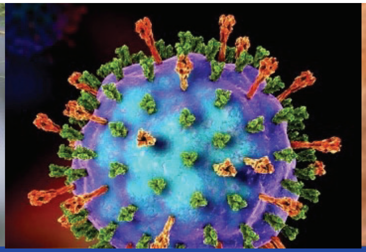

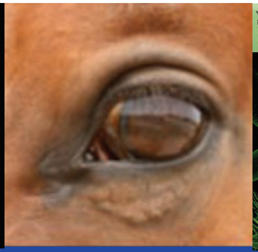

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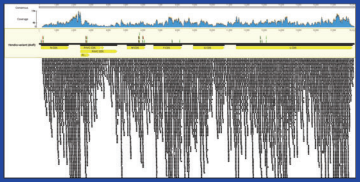
Important update for HeV surveillance in humans and animals.
Hendra-virus-variant - Horse South-East Qld 2015



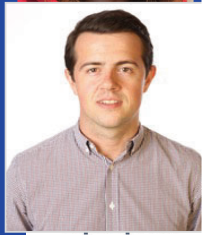
A Hendra virus variant (HeV-var) detected:

- 2015 South-east Queensland case of fatal equine disease;
- Previously found negative by routine state PCR testing for HeV at the time;
- Not detected by the molecular methods routinely relied upon due to sufficient nucleotide mismatches in genomic sequence (approx. 15%).
- Equine HeV disease caused by this variant was clinically indistinguishable from the most severe reported form of acute HeV disease.
- This 2015 horse HeV-variant shares near-identical genomic sequence to that detected in a grey-headed flying fox (*Pteropus poliocephalus*) from Adelaide in 2013.

2013 viral sequence was shared with us in good-will prior to publication will by relevant CSIRO scientists.

```
1. Hendra_ref_prototype_genome      CTTCGACAAAGACGGAACCAAAGTGGCATCTTTCATGCTCCATCTCGGGAATTTTGTCCGACGAGCTGG
                                                                  M CDS
FWD 2. M_fwd                        CTTCGACAAAGACGGAACCAA
FWD 3. M_prb                                             TGGCATCTTTCATGCTCCATCTCGG
REV 4. M_rev                                                                      AATTTTGTCCGACGAGCTGG
FWD 5. Hendra_var_QLD_horse_genome  TCTCGACAAGGACGGAGCTAAAGTGGCATCCTTCATGCTTCACCTTGGTAATTTTGTTCGACGAGCCGG
FWD 6. Hendra_var_SA_bat_genome     TCTCGACAAGGACGGAGCTAAAGTGGCATCCTTCATGCTTCACCTTGGTAATTTTGTTCGACGAGCCGG
FWD 7. Mv_fwd_1                     TCTCGACAAGGACGGAGCTAA
FWD 8. Mv_prb_1                                          TGGCATCCTTCATGCTTCACCTTGG
REV 9. Mv_rev_1                                                                   AATTTTGTTCGACGAGCCGG
```

Hendra virus variant primers shared with Human and Animal Health laboratory and biosecurity network 24th February 2021 courtesy of *'Horses as Sentinels Research' group in recommendation for urgent prepublication use on compassionate grounds. Primer design by JS Eden WIMR and I Smith CSIRO.*



Initial action:

- Timely communication with state biosecurity research partners and CVO allowing for immediate actions relevant to local stakeholders.
- Redesigned PCR assays suitable for routine biosecurity screening and shared these with state and national human and animal health laboratory network.
- Undertook genomic and phenotypic analysis of the HeV-variant to support the understanding that immune-protection should be afforded as for the prototypic HeV by both the Equivac HeV® vaccine in horses and the mono-clonal antibody m102.4 post-exposure therapy in humans.

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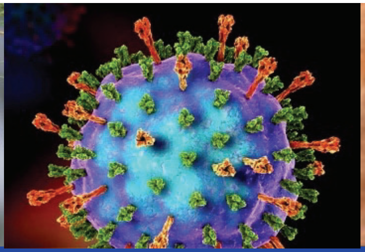

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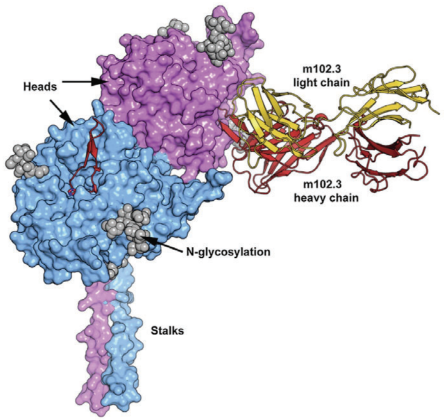
It should be emphasized, that the HeV RBP shares only 79% amino acid identity to NiV RBP, yet the HeV-sG subunit vaccine provides 100% protection against lethal challenge with both HeV and NiV in animal models (11).

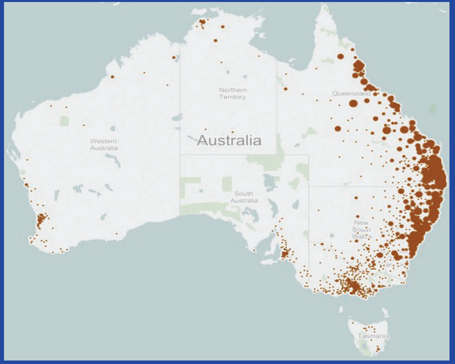
Both the higher similarity between the HeV-var and HeV RBP (92.5% amino acid identity) and structural consistency of critical epitopes mentioned above, suggest that current vaccination utilizing the HEV RBP will elicit similarly protective antibodies against this HeV-var. Current serological assays based on the HeV RBP are not expected to distinguish between exposure to the variants.

C.C. Broder et al. / Vaccine 34 (2016) 3525–3534



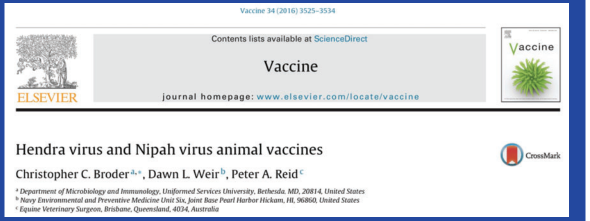
Neutralisation Titres on Vaccinated Horses

Serum	HeV	HeV-var/g2
1	64	64
2	256	256
3	128	128
4	128	128
5	64	64
6	2048	1024
7	16	16
8	1024	1024
9	2048	2048
10	64	64
11	512	1024
12	2048	2048
13	512	512
14	512	512



>150,000 horses vaccinated from 2012 to 2019

>650,000 doses administered.



Vaccine 34 (2016) 3525–3534

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Hendra virus and Nipah virus animal vaccines

Christopher C. Broder[a,*], Dawn L. Weir[b], Peter A. Reid[c]

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[b] Navy Environmental and Preventive Medicine Unit Six, Joint Base Pearl Harbor Hickam, HI, 96860, United States
[c] Equine Veterinary Surgeon, Brisbane, Queensland, 4034, Australia

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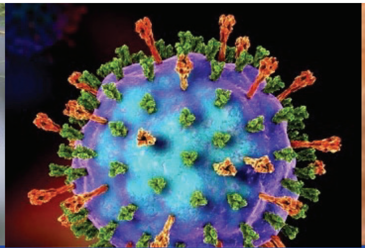

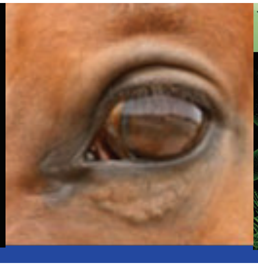

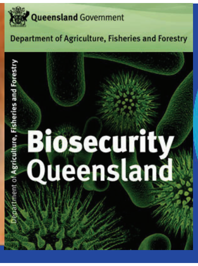
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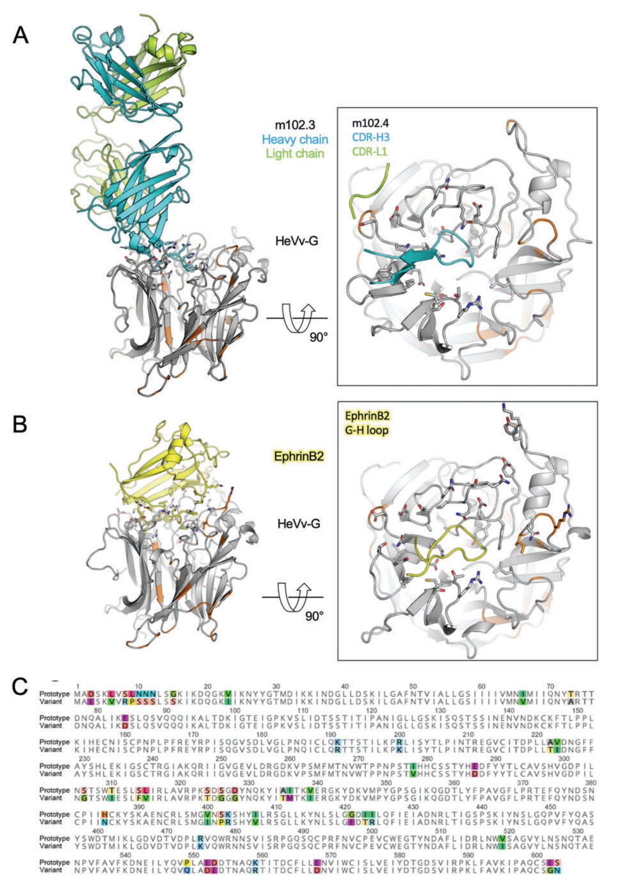
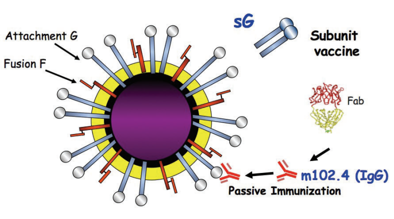
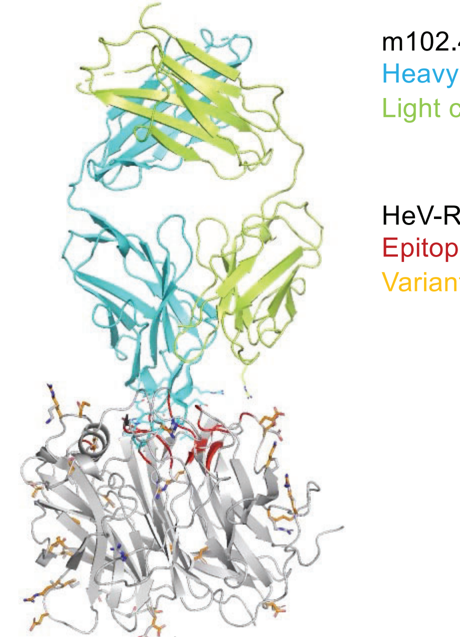


Comparison of the translated amino-acid sequence of this HeV-var and prototypic HeV RBP *in silico* revealed the ephrin-B2 entry receptor binding site and that of mAb m102.4 to be unchanged. Similarly, m102.4 neutralization was confirmed *in vitro* with HeV-var as for HeV. As such, it is expected that current PEP utilizing mAb m102.4 will remain effective against this HeV-var.





Dr Kai Xu Ohio State University College of Veterinary Medicine Department of Veterinary Biosciences, Columbus, Ohio, USA

Anticipated efficacy of sG vaccine for horses and post-exposure immunotherapy with m102.4 via *In silico* analysis of HeV variant receptor-binding protein structure based on established structure for HeV via x-ray crystallography.

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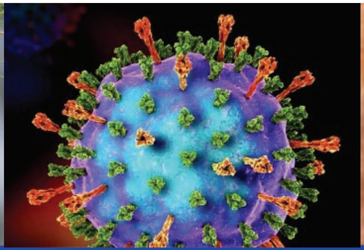

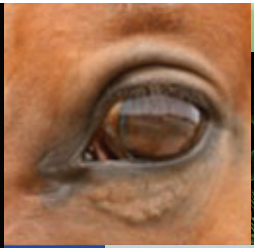

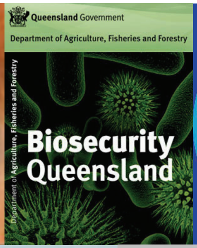

CSIRO

Timely communication of equivalent pathogenicity and immunogenicity with updated qPCR testing capacity shared for routine priority disease investigation.

EQUINE VETERINARIANS AUSTRALIA

Special Features

Hendra virus - Two Viruses, Same Fatal Disease

Edward J. Annand

University of Sydney | Sydney School of Veterinary Science |
Marie Bashir Institute for Infectious Diseases and Biosecurity, Sydney, NSW, AUS.
CSIRO | Health and Biosecurity, Black Mountain Laboratories, Canberra, ACT, AUS.
EquiEpiVet | Equine Veterinary and One Health Epidemiology, Airey's Inlet, Surf Coast, Vic., AUS.

bioRχiv
THE PREPRINT SERVER FOR BIOLOGY

Title: **Novel Hendra virus variant detected by sentinel surveillance of Australian horses.**

Authors: Edward J. Annand[1#], Bethany A. Horsburgh[1], Kai Xu, Peter A. Reid, Ben Poole, Maximillian C. de Kantzow, Nicole Brown, Alison Tweedie, Michelle Michie, John D. Grewar, Anne E. Jackson, Nagendrakumar B. Singanallur, Karren M. Plain, Mary Tachedjian, Brenda van der Heide, David T. Williams, Cristy Secombe, Eric D. Laing, Spencer Sterling, Lianying Yan, Louise Jackson, Cheryl Jones, Raina K. Plowright, Alison J. Peel, Ibrahim Diallo, Andrew C. Breed, Christopher C. Broder, Philip N. Britton*, Navneet K. Dhand*, Ina Smith*, John-Sebastian Eden*

EMERGING INFECTIOUS DISEASES®

[1]These authors contributed equally. *Shared senior authorship. [#]Corresponding author.

Manuscript Number: EID-21-1245 Submitted Mon, May 31, 2021, *In Review*.

Article Summary Line: A novel variant of Hendra virus was discovered and isolated from an Australian horse with fatal disease.

Running Title: HeV variant associated with fatal disease in a horse

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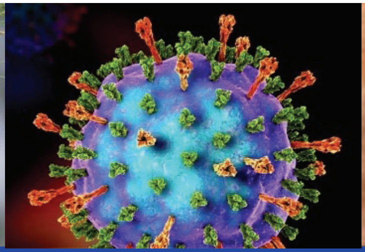
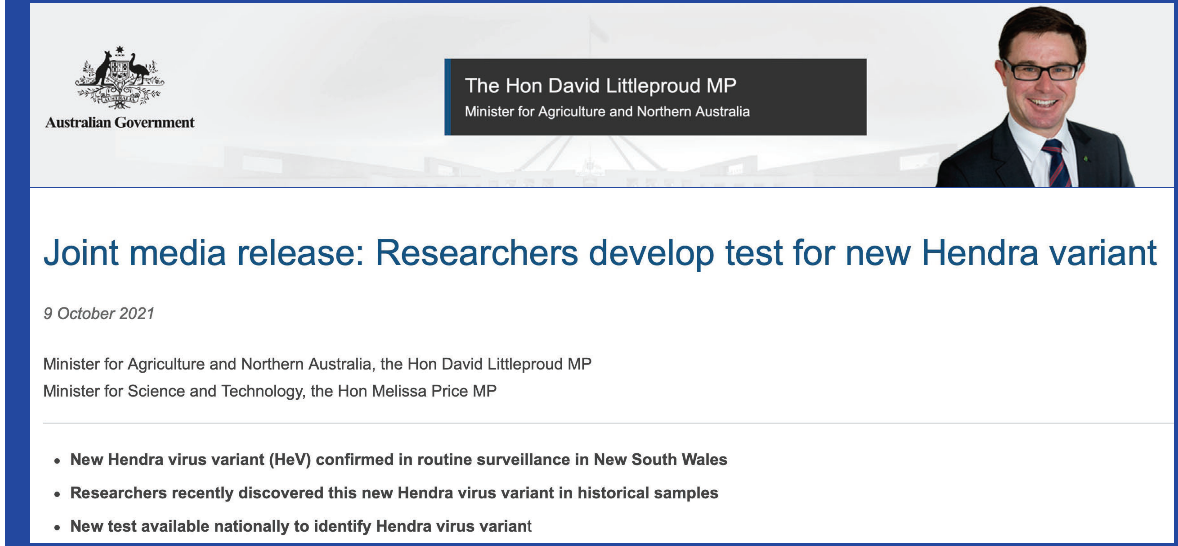


Queensland Government
Department of Agriculture, Fisheries and Forestry
Biosecurity Queensland

CSIRO

Timely Prepublication Sharing of Duplex qPCR for Variant (HeVg2) and Original HeV Genotypes Results in Prospective Detection *(Southern-most HeV Spillover)*



Australian Government

The Hon David Littleproud MP
Minister for Agriculture and Northern Australia

Joint media release: Researchers develop test for new Hendra variant

9 October 2021

Minister for Agriculture and Northern Australia, the Hon David Littleproud MP
Minister for Science and Technology, the Hon Melissa Price MP

- **New Hendra virus variant (HeV) confirmed in routine surveillance in New South Wales**
- **Researchers recently discovered this new Hendra virus variant in historical samples**
- **New test available nationally to identify Hendra virus variant**

NSW GOVERNMENT
Department of Primary Industries

Hendra variant case confirmed near Newcastle

8 Oct 2021

A variant Hendra virus strain has been confirmed in a 7-year-old unvaccinated Clydesdale from West Wallsend, near Newcastle.

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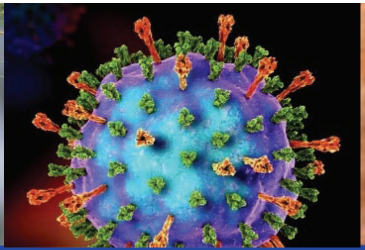

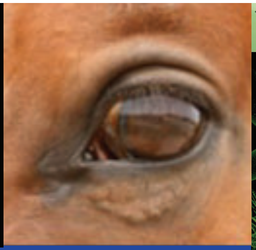

Queensland Government
Department of Agriculture, Fisheries and Forestry
Biosecurity Queensland

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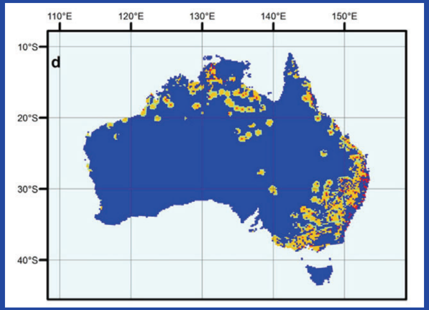
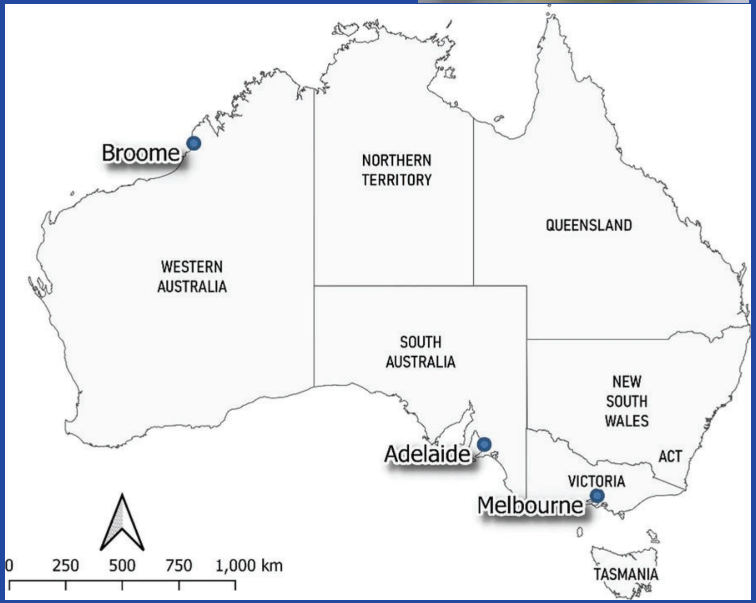
Numerous molecular detections of the same variant genotype of Hendra virus (HeVg2), in grey-headed flying foxes in South Australia and Victoria and a little red flying fox in Western Australia:

Wang, J., Anderson, D.E., Halpin, K. et al. A new Hendra virus genotype found in Australian flying foxes. Virol J 18, 197 (2021). https://doi.org/10.1186/s12985-021-01652-7.

The manuscript describes detections in bat samples (2013-2021) of the same consistent phenotypic second genotypic lineage detected retrospectively (2021) by this research to have caused fatal equine disease in QLD (2015).



East IJ, Wicks RM, Martin PAJ, Sergeant ESG, Randall LA, Garner MG. Use of a multi-criteria analysis framework to inform the design of risk based general surveillance systems for animal disease in Australia. Preventive Veterinary Medicine. 2013 Nov 1;112(3):230–47.



Map of Australia showing the locations of the HeV-g2 positive flying foxes collected between 2013 and 2021: one LRFF was from Broome, three GHFF were from Adelaide and 7 GHFF were from Melbourne.

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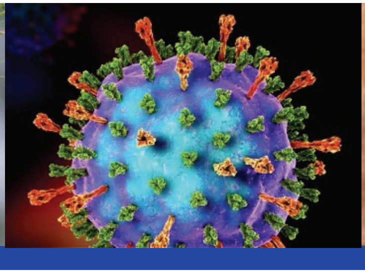

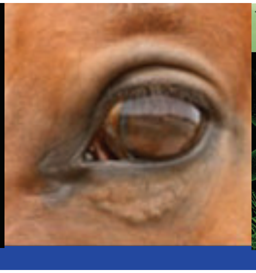

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Revised Geographic HeV Spillover Risk

Horses as Sentinels

with BAT ONE HEALTH

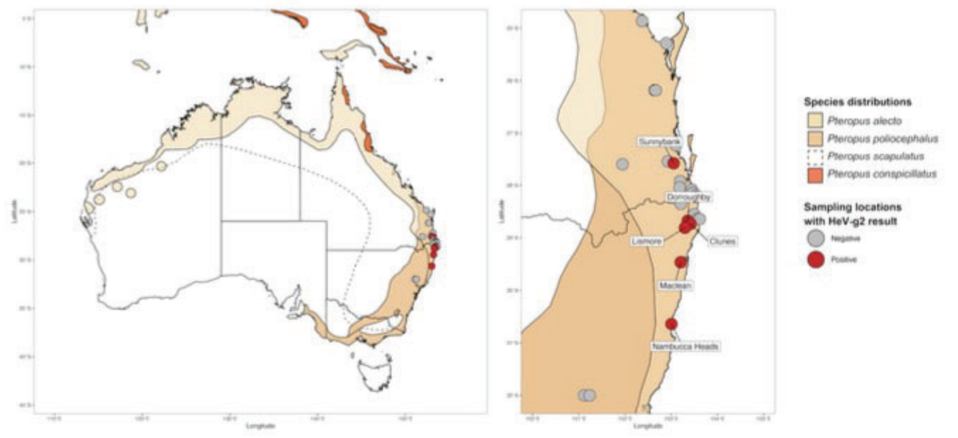
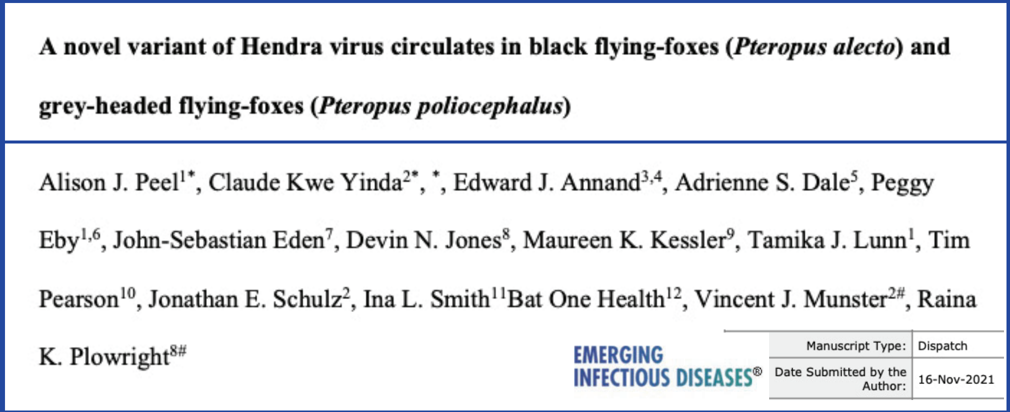


Figure 1: Map of Australia (left) and study area (right) showing the distribution of Australian flying foxes, the sampling locations and positive HeV-g2 detections.

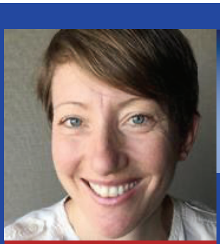


A novel variant of Hendra virus circulates in black flying-foxes (*Pteropus alecto*) and grey-headed flying-foxes (*Pteropus poliocephalus*)

Alison J. Peel[1*], Claude Kwe Yinda[2*], [*], Edward J. Annand[3,4], Adrienne S. Dale[5], Peggy Eby[1,6], John-Sebastian Eden[7], Devin N. Jones[8], Maureen K. Kessler[9], Tamika J. Lunn[1], Tim Pearson[10], Jonathan E. Schulz[2], Ina L. Smith[11]Bat One Health[12], Vincent J. Munster[2#], Raina K. Plowright[8#]

EMERGING INFECTIOUS DISEASES®

Manuscript Type:	Dispatch
Date Submitted by the Author:	16-Nov-2021



DR
Alison Peel

Griffith UNIVERSITY
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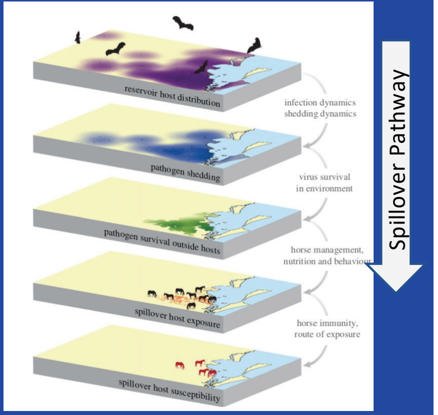
MONTANA STATE UNIVERSITY
Prof. Raina Plowright



Plowright RK *et al.* 2015

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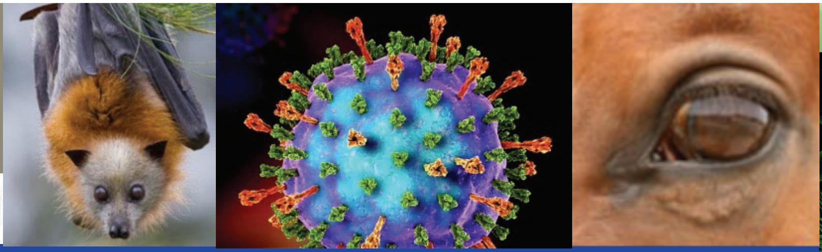

Queensland Government
Department of Agriculture, Fisheries and Forestry
Biosecurity Queensland

CSIRO

HeV and ABLV are both severe zoonotic diseases
that circulate amongst Australian bats
and are lethal to both horses and humans.

While they are likely ancient viruses,
our awareness of them is relatively recent and emerging.

Dr Ed Annand

BVSc(Hons) MANZCVS (Equine Surgery) & (Epidemiology) CertAVP (Equine Stud Medicine) PgCertVPS MRCVS

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CSIRO | Health and Biosecurity, Black Mountain, ACT.
EquiEpiVet | Equine Veterinary and One Health Epidemiology, Aireys Inlet, Vic.

ed.annand@sydney.edu.au

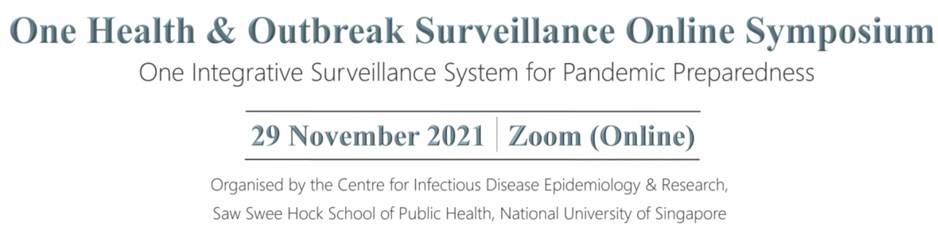
Horses as Sentinels of Emerging Infectious Diseases Research
Co-leadership: Dr Ina Smith, Dr Peter Reid, Dr John-Sebastian Eden A/Prof Navneet Dhand
Dr Philip Britton, Prof. Cheryl Jones, A/Prof Jenny-Ann Toribio
Co-Hosted by: The University of Sydney (USYD) (Sydney School of Veterinary Science and Faculty of Medicine and Sydney Institute for Biosecurity and Infectious Diseases) and CSIRO (Health and Biosecurity Business unit). **Supported by:** Westmead Institute for Medical Research**:** Queensland Government Biosecurity Sciences Laboratory and Queensland Department of Agriculture and Fisheries**;** Australian Centre of Disease Preparedness Diagnostic Surveillance and Response Laboratory**;** University of Sydney, Sydney Medical School; EquiEpiVet; JData and the Broder lab - Uniformed Services, University of Health Sciences, USA.
Additional funding contribution from: Dalara Foundation, philanthropic donation - Horses and Human Health; Marie Bashir Institute of Biosecurity and Infectious Diseases - Internal USYD seed funding; USYD on behalf of Australian Government – Department of Education Skills and Employment - Research Training Program scholarship.
Co-investigators and contributors: Molecular and Bioinformatical co-investigators: Dr Bethany Horsburgh and Miss Kate Cousins. Technical scientific contributors: Dr Maximillian de Kantzow, Miss Michelle Michie and Ms Allison Tweedie, Ms Karan Kim and Dr Karren Plain. Database, and data analysis contributors: Dr John Grewar, Dr Nicole Brown and Dr Nagendra Singanallur. Clinical disease and syndrome contributors: Dr Anne Jackson, A/Prof. Cristy Secombe, Prof. Cheryl Jones, Dr Tim Annand, Dr Ben Poole, Dr Carly Garling, Dr Greg Baldwin and Dr Doug English. Workshops and system processes: Dr Anna Gonzalez, Miss Nicole Popovic, Mr Tim Jackson and Dr Sandra Steele. Serology recombinant protein contributors: Prof. Christopher Broder, Dr Eric Laing, Dr Spenser Sterling, Dr Lianying Yan, Dr Rasa Burneikiene-Petraityte and Dr Aurelija Zvirbliene.
Federal Government DAWE research partner: Dr Andrew Breed; **project manager:** Mr David Bath and **research sponsor:** Dr Robyn Martin. **Queensland Government QDAF research partner:** Dr Ibrahim Diallo **and project governance:** Dr Louise Jackson
Additional university research governance: Prof. Ruth Zadoks. **Broader epidemiology engagement/advice:** Dr Ali Peel, Prof. Raina Plowright, Dr Andrew Way, Dr Kate Sawford, Dr Jonathan Happold and Dr Ian Langstaff. **Additional Federal Government DAWE support/consultation:** Dr William Wong, Dr Laura McFarlane-Berry and Dr Ariella Hayek. **Broader Scientific collaboration and contributions:** Dr David Williams, Dr Brenda van der Heide, Dr Sandy Crameri, Ms Jen Barr, Dr Mary Tachedjian and Dr Stacey Lynch and Dr Caroline Spelta.

From: Edward Annand on behalf of Edward Annand <ed.annand@sydney.edu.au>
To: John-Sebastian Eden; Broder, Christopher; Ina.Smith@csiro.au; Alison Peel; Plowright, Raina; Breed, Andrew; Ibrahim Diallo; Bethany Horsburgh; Peter Reid; John Grewar SAEHP; annejackson251@gmail.com; n.brown.bvsc@gmail.com; Xu, Kai
Cc: Navneet Dhand; Jenny-Ann Toribio; Ruth Zadoks; Philip Britton (SCHN); Cheryl Jones; inasmith23@gmail.com
Subject: Updating of One Health & Outbreak Surveillance Symposium 2021 lunchtime abstract presentation.
Date: Saturday, November 27, 2021 9:04:52 AM
Attachments: One-Health_Singapore_2021_EJAnnand_HeV-g2.pdf
image001.png

Hi JS, Ina, Chris, Peter, Kai, Ali, Raina, Andrew, Anne, Nicki, John, Ibrahim and Beth (Jenny-Ann, Ruth, Phil and Cheryl),

Updating here that I have had a late call up facilitated by Navneet (Cc'd - who is giving a keynote at the same event) to present the HeVg2 findings at:

One Health & Outbreak Surveillance Symposium 2021, Singapore (online) on Monday - https://blog.nus.edu.sg/ciderohs/ - in the lunchtime abstracts session.



One Health & Outbreak Surveillance Online Symposium

One Integrative Surveillance System for Pandemic Preparedness

29 November 2021 | Zoom (Online)

Organised by the Centre for Infectious Disease Epidemiology & Research,
Saw Swee Hock School of Public Health, National University of Singapore

The title of the abstract is: **Horses as Sentinels for Emerging Infectious Disease Research Discovery (2021) of a variant genotype of Hendra virus (HeVg2) affording prospective spillover detection (2021) and revision of geographical spillover risk.**

Sharing pdf of slides for your records.

I've had to send the slides in advance already but if anyone has any particular requests for edits let me know and I'll see if it can be accommodated. Ali and Raina – this is the same slide I used (while perhaps a little clearer) in the AVA VET Fest presentation on Thursday night which went very well by the way.

Cheers

Ed

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One Health Epidemiology and Virology
University of Sydney | Sydney School of Veterinary Science
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NIAID WORKSHOP ON PANDEMIC PREPAREDNESS

The Prototype Pathogen Approach to Accelerate Medical Countermeasures: Vaccines and Monoclonal Antibodies

NIH National Institute of Allergy and Infectious Diseases

PARAMYXOVIRIDAE

Dr. Paul Duprex, University of Pittsburgh, Co-Chair
Dr. Rebecca Dutch, University of Kentucky College of Medicine, Co-Chair

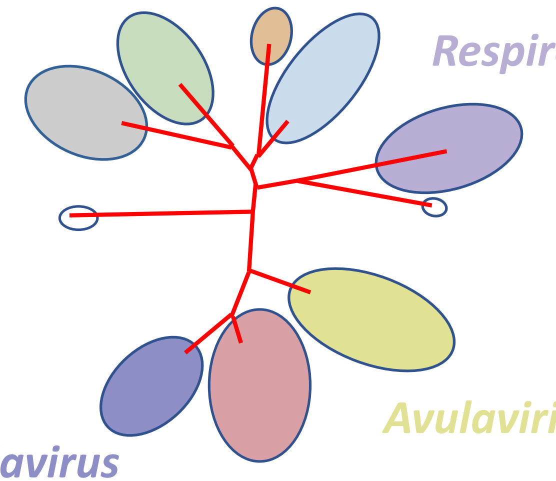
November 9, 2021

FOCUS GROUP PANELISTS

- **Dr. Christopher Broder**, Uniformed Services University
- **Dr. Emmie De Wit**, National Institute of Allergy and Infectious Diseases
- **Dr. Felix Drexler**, Charite - Universitätsmedizin Berlin
- **Dr. Benhur Lee**, Icahn School of Medicine at Mount Sinai
- **Dr. Anne Moscona**, Columbia University
- **Dr. Richard Plemper**, Georgia State University
- **Dr. Veronika von Messling**, German Federal Ministry of Education and Research
- **Dr. Linfa Wang**, Duke-NUS Medical School

PARAMYXOVIRIDAE TAXONOMY

Subfamily	***Genus***	***Virus***
Avulavirinae	***Orthoavulavirus*** ***Metaavulavirus*** ***Paraavulavirus***	avian paramyxovirus 2 avian paramyxovirus 1 avian paramyxovirus 3
Rubulavirinae	***Orthoarubulavirus*** ***Pararubulavirus***	mumps virus respiratory viruses human parainfluenza viruses 2 and 4
Orthoparamyxovirinae	***Respirovirus*** ***Aquaparamyxovirus*** ***Henipavirus*** ***Narmovirus*** ***Morbillivirus*** ***Salemvirus*** ***Ferlavirus*** ***Jeilongvirus***	human parainfluenza virus 1/3 Pacific salmon paramyxovirus Nipah virus Tupaia paramyxovirus Measles virus Salem virus *fer-de-lance* virus Beilong virus
Metaparamyxovirinae		Synodus synodonvirus



Metaparamyxovirinae

Orthoparamyxovirinae

Morbillivirus

Henipavirus

Respirovirus

Avulavirinae

Pararubulavirus

Orthorubulavirus

Rubulavirinae

PARAMYXOVIRIDAE TAXONOMY

Subfamily	***Genus***	***Virus***
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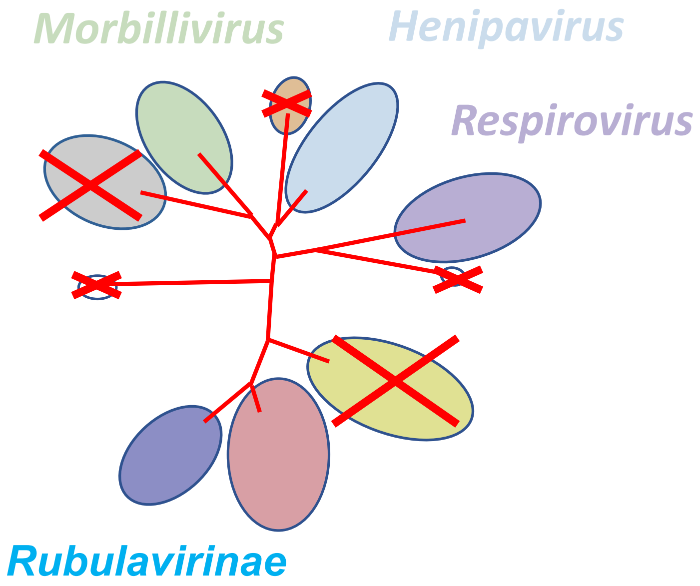
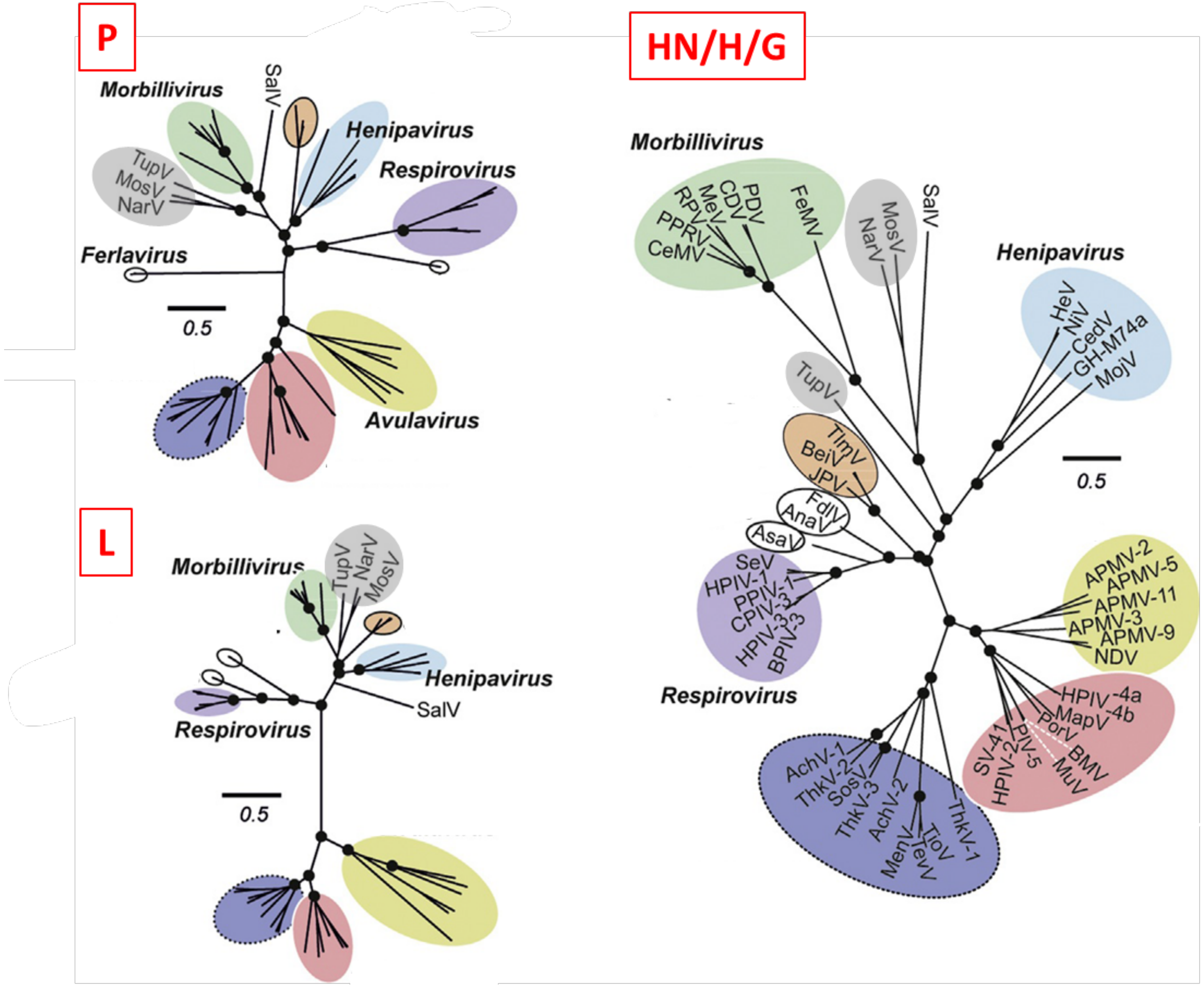
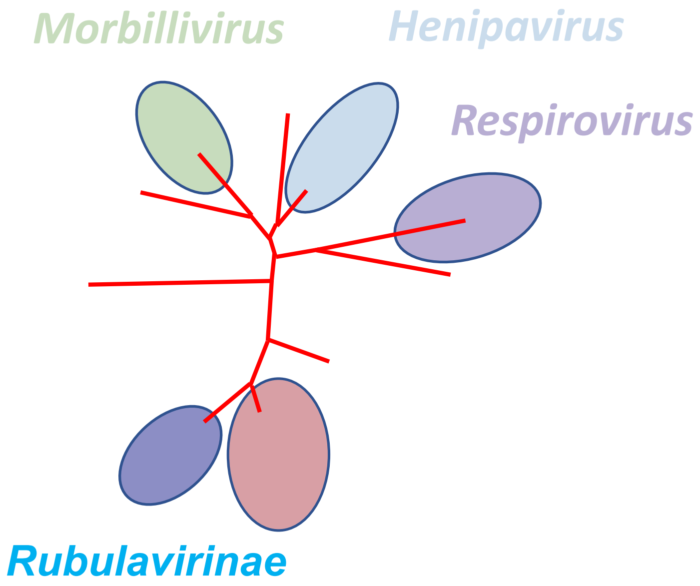
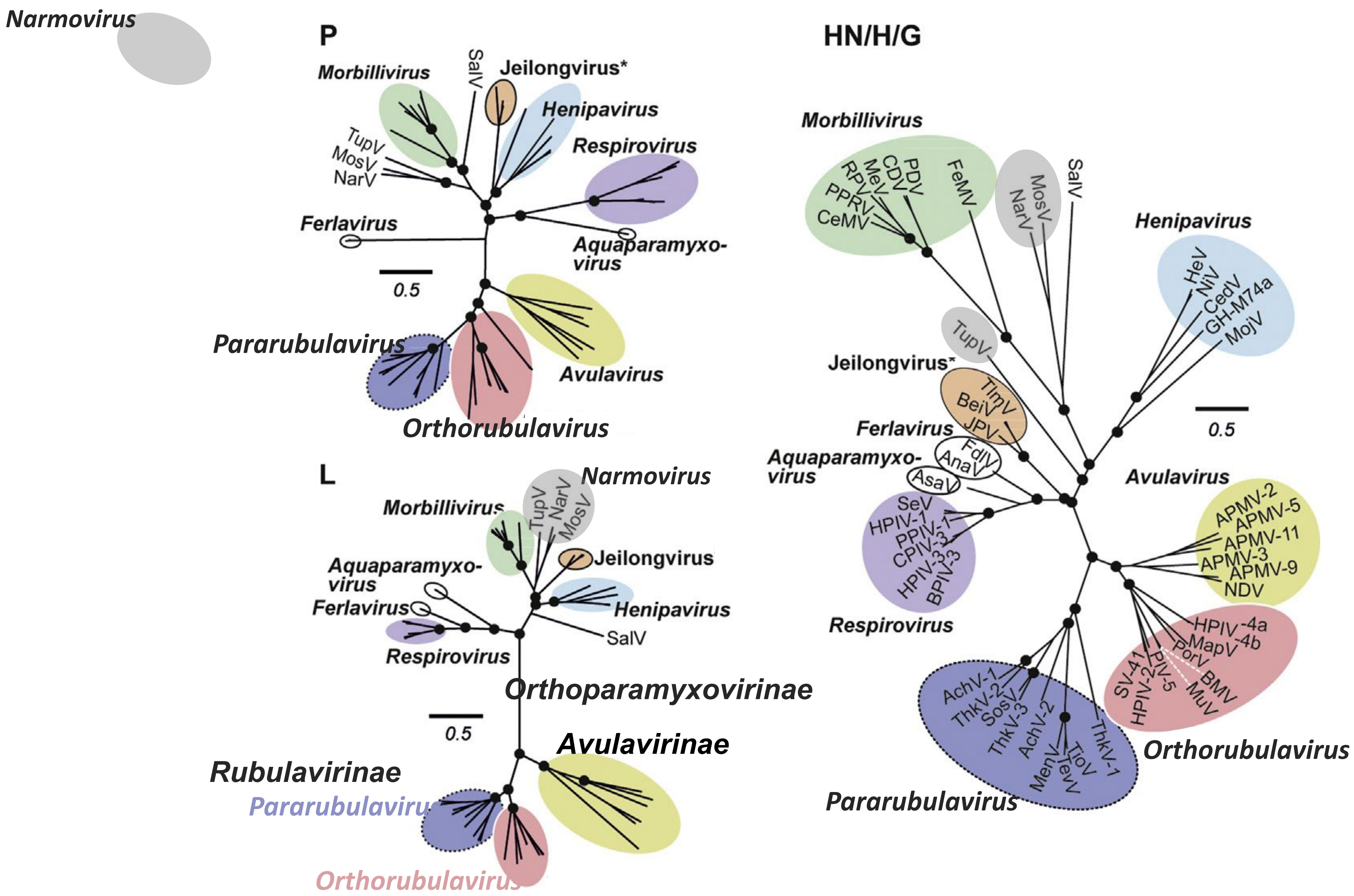


Paramyxoviridae






Narmovirus
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Morbillivirus
SalV
Jeilongvirus*
Henipavirus
Respirovirus
TupV
MosV
NarV
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Aquaparamyxo-
virus
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Avulavirus
Orthorubulavirus
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Narmovirus
Morbillivirus
TupV
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Jeilongvirus
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Rubulavirinae
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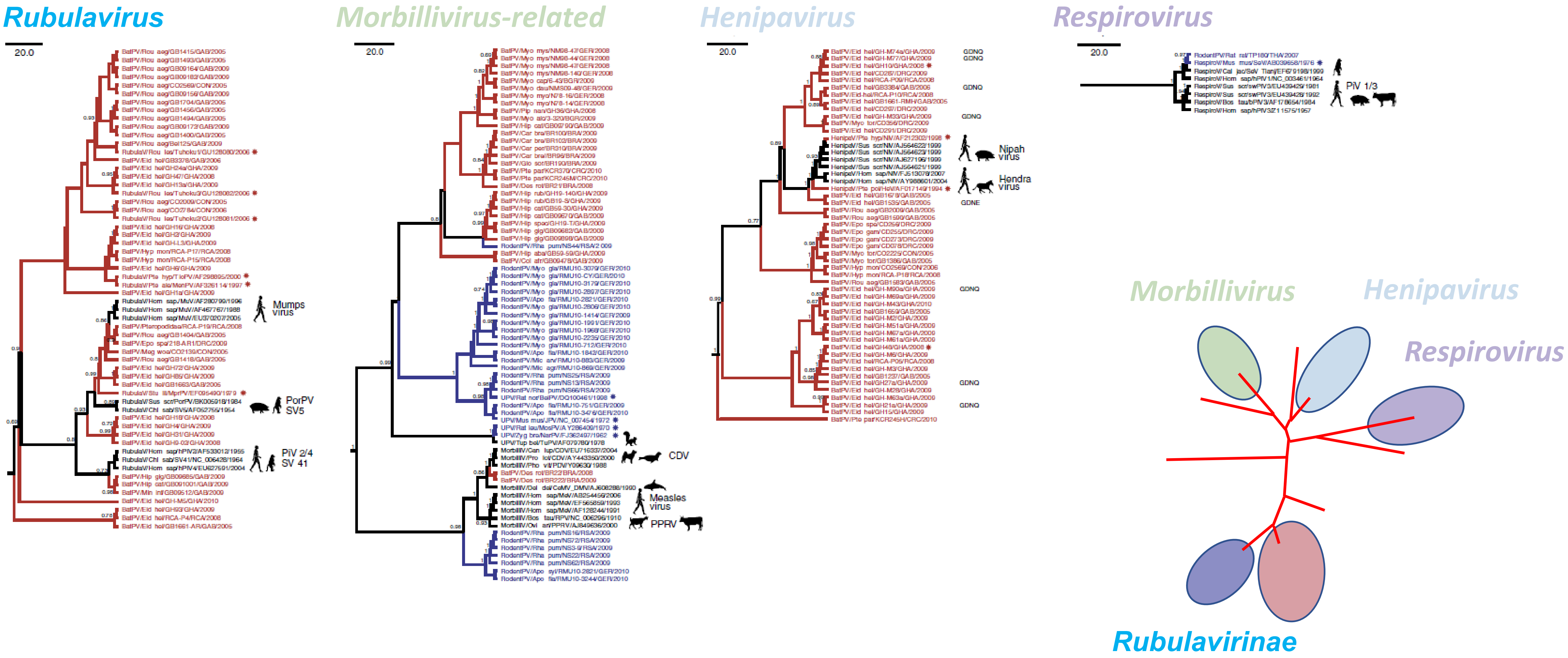
Orthorubulavirus
HN/H/G
Morbillivirus
PDV
CDV
MeV
RPV
PPRV
CeMV
FeMV
MosV
NarV
SalV
Henipavirus
HeV
NiV
CedV
GH-M74a
MojV
TupV
Jeilongvirus*
TlmV
BeiV
JPV
Ferlavirus
FdlV
AnaV
Aquaparamyxo-
virus
AsaV
0.5
Avulavirus
SeV
HPIV-1
PPIV-1
CPIV-3
HPIV-3
BPIV-3
Respirovirus
APMV-2
APMV-5
APMV-11
APMV-3
APMV-9
NDV
HPIV-4a
HPIV-4b
MapV
PorV
BMV
MuV
PIV-5
HPIV-2
SV-41
AchV-1
ThkV-2
SosV
ThkV-3
AchV-2
MenV
TevV
TioV
ThkV-1
Orthorubulavirus
Pararubulavirus

THE IMPORTANCE OF VIRAL ECOLOGY



Paramyxoviridae

- Brief history of the subfamilies we are focusing on – including info on recently identified members of the family.
- Also – do we need to summarize the basic biology of these???

Paul to finish tomorrow

BASIC BIOLOGY: KEY GAPS

- There are differences between clinical isolates laboratory-adapted strains. *Can we develop and provide model reagents for clinical strains to overcome this issue?*
- Wide breadth of immune memory. *How does the immune response vary between the different viruses? What would be the primary antigen target? Are there conserved key epitopes?*
- Persistence is common. *What is the mechanism? What are the implications for pathogen emergence?*
- Neurotropism is common. *What is the molecular basis of neurovirulence?*
- All existing vaccines are live-attenuated. *What is the molecular basis of attenuation? What can we learnt from what we have?*

BASIC BIOLOGY: KEY GAPS

- There is a difference in the level of protection after infection with a systemic versus a respiratory tract-limited virus. *Why are some paramyxoviruses systemic while some are limited to the respiratory tract?*
- Receptor binding is a hallmark. *Can we get a better understanding of glycoprotein evolution and determinants of cross-reactivity?*
- Non-structural protein are important. *How does viral protein variation affect innate immune activation?*
- Some genera are very diverse. *How stable are these viruses? What types of decontamination are effective and does this differ between paramyxoviruses?*

MODELS OF DISEASE: KEY GAPS

Cell culture/non-animal models:

- We need better human derived systems that are approachable and affordable.

Animal models of human pathogens alongside real animal diseases:

- Some paramyxoviruses don’t have good animal models (e.g. mumps).
- Some paramyxoviruses can only use NHP as good models (e.g. measles).
- CDV pathogenesis in ferrets is critical, systemic model – but to date this is not a human pathogen.
- While there are solid animal models for henipaviruses, the viruses are primarily BSL-4 (except for Cedar), making these challenging to work with for most researchers.
- Mouse models are not good for many (most) paramyxoviruses – we need to develop reagents for animal species beyond mice.
- Can we improve the predictive power of animals models for humans?

MEDICAL COUNTERMEASURES

Vaccines available for paramyxoviruses:

- Measles, mumps vaccines have been used for decades, though there are still questions about the molecular basis of attenuation and they a ALL live-attenuated.
- CDV vaccines have been developed, and there are many studies of the immune response in animals to these vaccines.
- A Hendra G protein vaccine is used in horses, and correlates of protection are currently being investigated. This vaccine is cross-protective for Nipah. It demonstrated subunit vaccines for paramyxoviruses are possible.

Monoclonals for paramyxoviruses:

- A henipavirus monoclonal antibody is through Phase 1 trials.

OVERALL SUMMARY: SCIENTIFIC GAPS

Basic Biology

- Mechanisms of viral transmission dynamics, cell entry, long-term persistence
- Systemic vs tissue-limited viral replication, disease manifestations (molecular and cellular basis)
- Differences between clinical isolates and lab viral strains; Glycoprotein evolution and determinants of cross-reactivity
- Immune response differences and similarities across viral family; Primary antigenic target; Immune epitope landscape (conserved key epitopes?); Role of innate immunity

Models

- Cell culture/non-animal models: human derived systems that are approachable and affordable
- Animal model development for some viruses in family
- Immunologic reagents for non-murine animal models (mouse - poor model for most family members)

Countermeasures

- Mechanisms of protection still unknown for successful licensed vaccines in family (e.g., measles)
- Correlates of protection for many paramyxoviruses
- Shorten mAb identification, development and production timelines
- Approaches to move promising candidates beyond Phase I trials (determine efficacy etc)


TOUGH
DECISIONS
AHEAD

PROTOTYPE PATHOGENS: ADDITIONAL CONSIDERATIONS

Henipaviruses

Issues with choosing as prototype pathogen:

- The BSL-4 nature creates considerable challenge for the wider research community.
- Significant funding is already directed towards vaccine development through CEPI.
- The consistent low R_0 value for Hendra and Nipah decreases the concerns about evolution to a highly transmissible agent.

However:

- The on-going outbreaks and high mortality rate make *Henipaviruses* a critical genus for continued research.
- Cedar virus could be considered as it is BSL-2+

PROTOTYPE PATHOGENS: POTENTIAL CHOICES

Morbilliviruses

- High transmissibility
- Propensity to jump species
- A number of emerging morbilliviruses have been identified
- *Key data needed*: what level of protection to novel morbilliviruses is provided by measles vaccination?
- Potential choices: canine distemper virus; bat morbilliviruses

PROTOTYPE PATHOGENS: POTENTIAL CHOICES

Respiroviruses

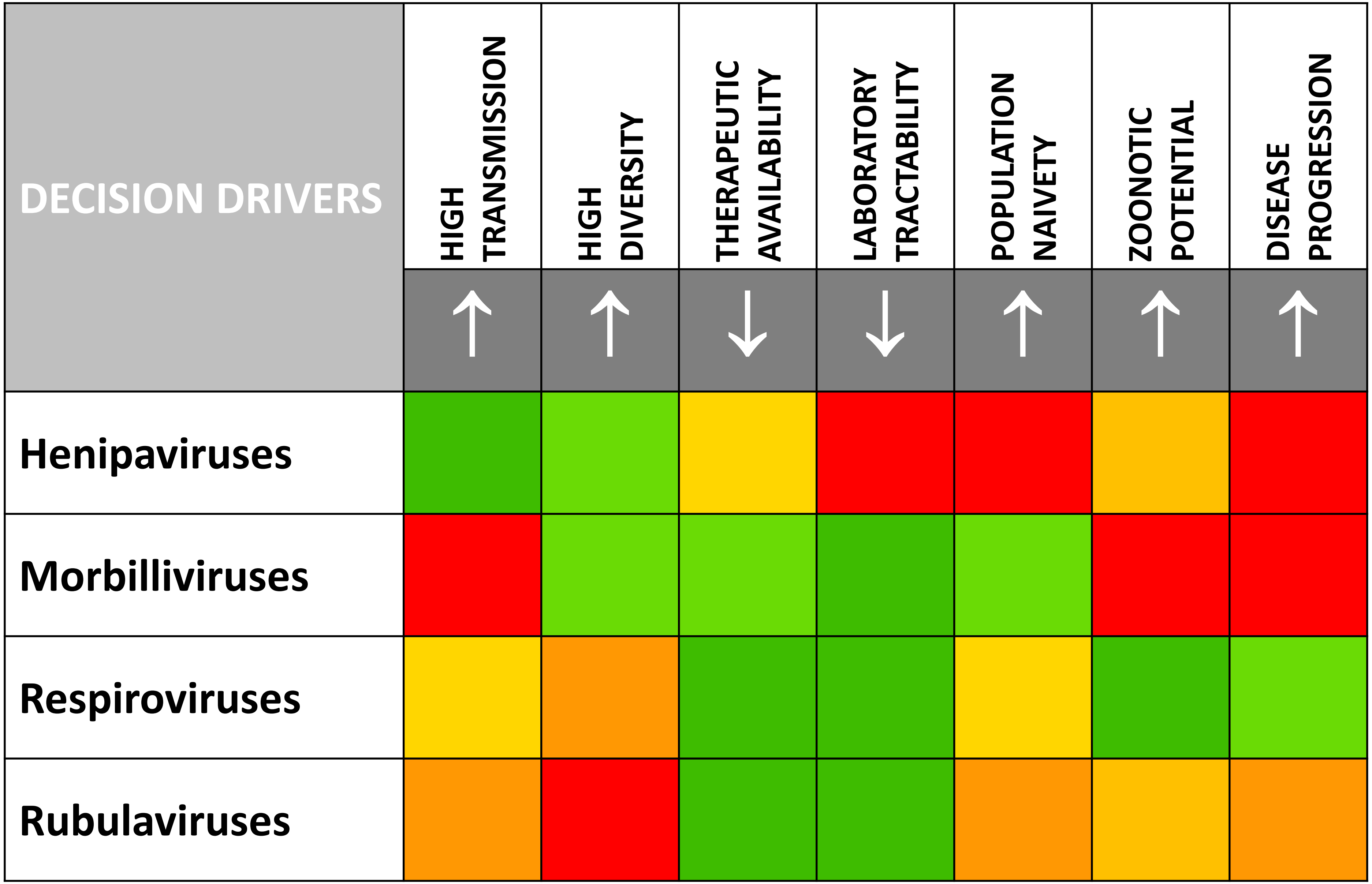
- Need to be prepared for both possible new systemic (e.g., pararubula) and respiratory-limited (respiroviruses) paramyxoviruses
- Novel respiroviruses have been recently identified, including from pangolins and squirrels
- Potential choices: HPIV1 or 3 (established pathogens), bat respiraoviruses

PROTOTYPE PATHOGENS: POTENTIAL CHOICES

Rubulaviruses

- High degree of viral diversity
- Novel members found in animal reservoirs, zoonotic transmission observed.
- Demonstrated ability to infect numerous cell types
- Current mumps vaccine doesn’t induce durable immunity, reducing potential protection to new members of this genus
- Potential choices: Menagle virus, Sosuga virus, Tioman virus, Achimotaviruses (all recently identified viruses); human parainfluenza virus 2, parainfluenza virus 5 or mumps (established) bat rubulaviruses

SUMMARY



DECISION DRIVERS	HIGH TRANSMISSION	HIGH DIVERSITY	THERAPEUTIC AVAILABILITY	LABORATORY TRACTABILITY	POPULATION NAIVETY	ZOONOTIC POTENTIAL	DISEASE PROGRESSION
	↑	↑	↓	↓	↑	↑	↑
Henipaviruses	🟩	🟩	🟨	🟥	🟥	🟨	🟥
Morbilliviruses	🟥	🟩	🟩	🟩	🟩	🟥	🟥
Respiroviruses	🟨	🟧	🟩	🟩	🟨	🟩	🟩
Rubulaviruses	🟧	🟥	🟩	🟩	🟧	🟨	🟧

NIAID WORKSHOP ON PANDEMIC PREPAREDNESS

The Prototype Pathogen Approach to Accelerate Medical Countermeasures: Vaccines and Monoclonal Antibodies

NIH National Institute of Allergy and Infectious Diseases

PARAMYXOVIRIDAE

Dr. Paul Duprex, University of Pittsburgh, Co-Chair
Dr. Rebecca Dutch, University of Kentucky College of Medicine, Co-Chair

November 9, 2021

From: Duprex, Paul on behalf of Duprex, Paul <pduprex@pitt.edu>
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Subject: NIH Pandemic Preparedness discussions: draft slides
Date: Friday, November 5, 2021 10:54:22 AM
Attachments: Paramyxoviridae presentation.pptx

Dear All,

We promised to get you a draft of the slides for next week. Becky and I are meeting this afternoon to finalize. We have been tag-teaming preparation, life and calendars.

Please don't worry about format just content.

ACTION

If anyone has useful slides to contribute to paramyxovirus background please can you send them to me – it would save us some time over the weekend

Please look at the summary slide we tried to capture what drover our recommendation, remembering not all columns are weighted equally. The colors are simply our best guess if anyone has strong opinions and how we have rated anything or if we have totally miscalled please let us know.

I have tried to really simplify the taxonomy … Benhur and Felix can you give this a good look at those slides.

If anyone finds it easier to chat I am around this afternoon from 14:00. Remember there's only 5 hours between EDT and CET at the minute.

Thanks so much in advance.

Kind regards

Paul and Becky

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The pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV) cause severe respiratory illness and encephalitis in both animals and humans with human case fatality rates ranging from 40-100%. The NiV-Bangladesh strain (NiV-B) is associated with multiple rounds of human-to-human transmission and is a pathogen of pandemic concern for which there are no vaccines or antivirals approved for human use. We have recently shown that the nonpathogenic Cedar henipavirus (CedV) is a useful surrogate platform for high throughput screening (HTS) for antiviral drug discovery, and for virus neutralization assays. This proposal focuses on developing antiviral small molecule and nanobody countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach. We created reporter gene-encoding recombinant CedVs and discovered a series of fused heterocycles, e.g., 4-aminoquinazoline-based, antivirals, and showed that a representative quinazoline CBS1437 was an effective inhibitor of rCedV replication and also both NiV-B and HeV, with EC_{50} values in the low µM range and minimal cytotoxicity. Neutralizing antibodies to the henipavirus F and G glycoproteins have also shown therapeutic promise, but antiviral nanobodies as potential immunotherapeutics represent a novel area of NiV and HeV countermeasure development. This proposal will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Aim 1 will focus on optimizing lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. Viral polymerase-targeting lead molecules will be selected using NiV-B and HeV assays and mechanism of action (MOA) studies, and best-in-class drugs will be developed by chemical modifications aimed to improve drug-like properties, increase potencies, decrease toxicity, and improve pharmacokinetic properties. Aim 2 will isolate and fully characterize nanobodies broadly neutralizing NiV-B and HeV derived by immunization of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using phage display technology of cloned nanobody gene fragments. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails. Aim 3 will focus on *in vivo* efficacy evaluation of lead compounds and nanobodies in animal models. Both monotherapies and combination therapeutic modalities will be tested, along with testing antiviral nanobody delivery by aerosol. These two unique henipavirus-targeted antiviral development directions will generate novel countermeasures that may display more broad effectiveness.

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] Twenty-two cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antiviral agents approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus that is nonpathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an EC_{50} value of 3.3 µM against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will yield testable antiviral agents with potential pan-henipavirus effectiveness. Our collaborative team also has extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both viral polymerase-targeting small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays and SPR binding assay for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and later, best-in-class nanobodies will be also tested by aerosol delivery. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobody studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

For several decades, the major agents of emerged or newly identified infectious diseases in humans have been viruses of zoonotic origins.[11-13] Among these, two paramyxoviruses of importance are Nipah virus (NiV) and Hendra virus (HeV); the prototype species of the genus *Henipavirus* and are single-stranded, negative sense, enveloped RNA viruses.[14] HeV appeared in 1994, in an outbreak of severe respiratory disease in horses in Australia, with the death of 14 horses and their trainer.[15,16] NiV (Malaysia strain, NiV-M) appeared in an outbreak of encephalitis in pig farmers in Malaysia in 1998.[17,18] NiV outbreaks have since been seen in Bangladesh and India by a related strain NiV-Bangladesh (NiV-B).[19] Pteropid fruit bats are the major reservoir hosts for henipaviruses.[6,20] NiV and HeV have broad host tropism, infecting at least 18 species across 6 orders of mammals. Among these, NiV and HeV will cause a systemic and often fatal respiratory and/or neurological disease in at least 11 species including humans, with human fatality rates ranging from 40-100%.[2,21] A third henipavirus isolate, Cedar virus (CedV), was discovered in 2010.[22] However, CedV is not known to be zoonotic and has been shown to be nonpathogenic in HeV and NiV animal models.[22-24] Two additional henipaviruses are also known, Ghana bat virus and Mojiang virus, but only from sequence data.[25-27] Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and 3 outbreaks in India.[28] In 2014, an outbreak of NiV-M occurred in the Philippines with 9 fatalities of 11 human cases, with horse-to-human and human-to-human transmission.[29] The combined NiV case fatality rate is ~60% across a large region of Asia in five countries.[3] The recent 2018 NiV outbreak in Kerala, India was significant, occurring in a new geographic region, with a case fatality rate of 91% and 22 of 23 cases the result of from human-to-human transmission, underscoring both the transmissibility and the lethal nature of this virus.[4]

Human NiV/HeV infection is generally accepted to occur via the oronasal route with an incubation period of 1 to 2 weeks.[4,30] Acute NiV/HeV infection in people is systemic and likely via hematogenous viral spread from the respiratory system.[31] Disease onset is characterized by fever, myalgia, shortness of breath, and cough.[16,32] Human HeV infections have resulted in both fatal respiratory or encephalitic disease, and also recovery.[30,33] The predominant clinical feature in the Malaysia outbreak was encephalitis, but respiratory symptoms were also common.[32,34] The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease and in the 2018 NiV-B outbreak in Kerala, 83% of cases had acute respiratory distress syndrome (ARDS).[4,35] The key findings of human NiV/HeV infection are wide-spread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected.[36,37] Human NiV/HeV infection can also be prolonged following apparent recovery, and some patients can experience a late-onset or relapsed encephalitis.[33,38] Relapsed encephalitis caused by NiV appears to be a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later,[39] NiV and HeV are biosafety level-4 (BSL-4) viruses and classified as Category C priority pathogens and transboundary agents by several US agencies. Also, both NiV-M and NiV-B can cause lethal infection in nonhuman primates (NHPs), the African green monkey (AGM), when delivered by small particle aerosol.[40-42] NiV and henipaviral diseases (HeV) are included on the WHO's Blueprint List of Priority Pathogens.[43] NiV is a potential pandemic threat because of its high case-fatality rates, capacity for human-to-human transmission and potential to mutate, and a human-adapted strain in a region of high population density, could lead to rapid spread.[44] *There are presently no approved vaccines or therapeutic modalities for NiV/HeV infections in people.*

The viral RNA-dependent-RNA polymerases of paramyxoviruses are attractive targets for antiviral compound discovery, but antiviral drug discovery for NiV and HeV has been hampered by requirements of BSL-4 containment. We recently developed and optimized an authentic henipavirus replication cell-based system using a recombinant CedV (rCedV) encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications at BSL-2 and identified several inhibitory compounds that were further validated with authentic NiV-B and HeV.[7] Those leads provide the rationale for the current application. Our ability to employ rCedV provides an opportunity for anti-henipavirus drug discovery not previously available, that also allows for discovery across the whole replication cycle, not restricted to only the viral polymerase. Another well-exploited stage of virus infection is the attachment and entry process.[45] NiV/HeV possess two membrane anchored glycoproteins required for infection; one for attachment, the G glycoprotein, and the other for membrane fusion the F glycoprotein.[46] The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and all vaccine strategies.[28] Although an effective post-exposure human monoclonal antibody (mAb) therapy targeting the NiV and HeV G glycoprotein (m102.4) has been extensively examined,[47-51] its delivery must be within several days following infection in NHPs. An unexplored alternative but related antibody-based strategy are antiviral nanobodies. A nanobody, is an antibody fragment comprised of just a single monomeric variable antibody domain.[52] Nanobodies were first engineered from variable heavy chain domains of camelid heavy chain

antibodies (VHHs), and camelid VHHs retain full antigen specificity and are only a fraction of the size of a human antibody, and they have extended complementarity determining regions (CDRs) which can allow them to bind epitopes not normally accessible to conventional antibodies, such as conserved viral epitopes often masked by glycan shields.[10] Our expertise in henipavirus F and G structural and functional analyses together with anti-F and G mAb development and therapeutic application, will facilitate the development and testing of a new and understudied area of nanobody-based immunotherapeutic against NiV and HeV.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of the investigators in this proposal. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may well have activity across related members of the henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of a high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification. Finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole NiV or HeV immune sera shows cross-reactivity with CedV, however, such sera or even specific high-titer anti-CedV F or G antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins.[22] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and the largest polymerase in the order *Mononegavirale*, yet it shares high aa identity to NiV and HeV L proteins. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; aa identities of L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This overall high degree of identity, shared between CedV and that of NiV and HeV, suggests that antivirals that target the L polymerase, could be identified and also offer the possibility of identifying pan-henipavirus countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] Several small molecule inhibitors have been validated *in vitro* using infectious HeV and NiV, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support **Aim 1** to optimize our current best-in-class leads and also screen large libraries of compounds.

Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[53-55] Nanobodies can be isolated using the same phage library construction methods and phage panning procedures routinely employed for mAb discovery, which allows for *in vitro* production of various amounts and their rapid characterization. Nanobody development will be carried out by **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels.[10] Immunized mouse cohorts are in progress using recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in **Aim 2.**

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of **Aim 3**. In addition, we expect to discover, and

characterize batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in **Aim 3**. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet, Irina Gaisina, and Terry Moore, UIC and Chicago BioSolutions Inc.** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule inhibitors of henipaviruses.

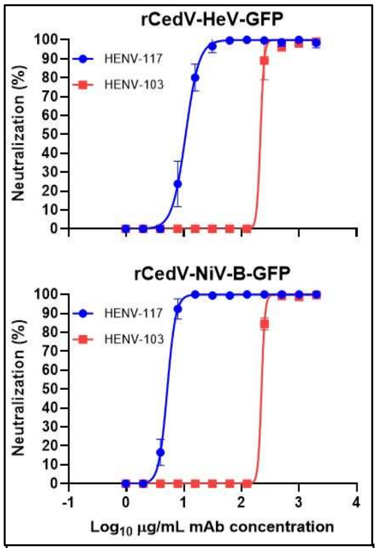


Figure 1. Neutralization curves for HENV-117 and HENV-103 mAbs against rCedV-HeV-GFP (top) and rCedV-NiV-B-GFP (bottom). Data are from a single experiment performed in technical triplicate.

C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2), cell-based HTS platform to identify small molecule antiviral agents targeting henipavirus replication without the requirement for BSL-4 containment.[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with that of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera use guidelines and procedures at the BSL-2 level were reviewed and approved by the USU, Institutional Biosafety Committee (IBC) in accordance with NIH guidelines. These replication competent rCedV chimeras create a very rapid and robust surrogate infection assay for NiV_B and HeV that can be used as a neutralization or entry inhibitor assay to measure neutralizing antibodies, or as a surrogate for generating virus escape mutants to neutralizing antibodies. Here, the F and G genes of NiV_B (2010 Faridpur isolate) and HeV (2008 Redlands isolate) were synthesized and inserted into our pOLTV5-rCedV anti-genome plasmid. For the HTS neutralization assay, a turbo-GFP gene was inserted between the CedV P and M genes. Each chimera (rCedV-NiV_B-GFP and rCedV-HeV-GFP) were rescued as previously described.[8] Other versions without reporter genes have also been prepared. All virus stocks are cloned and genomes confirmed by NGS. Typically, large stocks are prepared and frozen as single use aliquots. Show in **Figure 1** is an example of how these chimeras are used in a virus neutralization test, referred to as a fluorescent reduction neutralization test (FRNT) using two human NiV/HeV cross-reactive anti-G mAbs (HENV-103 and -117) as previously described.[56] Briefly, Vero 76 cells are used in black walled clear bottom 96-well plates, mAbs are serially diluted in medium, and equal volumes of medium containing rCedV-NiV-B or rCedV-HeV are added to each mAb dilution for a final concentration of 2000 PFU, incubated for 2 h at 37 °C. Each virus-mAb mixture (90 µL/well) is added to the pre-seeded Vero 76 cells in triplicate and the assay run for just another 24 h followed by 4% formaldehyde fixation, washing and then scanning using a CTL S6 analyzer. Fluorescent foci are counted using the CTL Basic Count™ and an IC_{50} value is determined as the antibody concentration

IC50 (ng/mL)	HeV	rCedV-HeV	NiVB	rCedV-NiVB
HENV-103	445	216	507	238
HENV-117	14	11	15	6

Table 1. IC_{50} values of cross-reactive anti-G human mAbs HENV-103 and -117) as determined by rCedV chimera FRNT assay and NiV-B and HeV conventional PRNT assay.

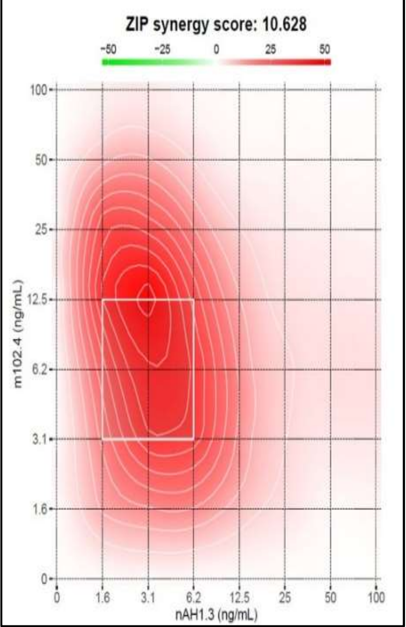


Figure 2. Synergy map with calculated synergy score using the zero interactions potency (ZIP) model generated by SynergyFinder for neutralization of rCedV-NiV-B-GFP by serially diluted m102.4 and nAH1.3 mAbs.

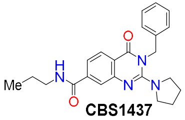
yielding 50% reduction in fluorescent foci versus control wells. **Table 1** shows the calculated IC_{50} values using rCedV chimeras vs. those determined by a conventional PRNT assay with authentic NiV-B and HeV, and they are remarkably within a 2-fold range.

In addition to conventional single agent neutralization tests, the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras can also be used in rapid HTS assays to measure synergistic activities of inhibitors, such as antibodies or drugs. The synergistic neutralizing activity of the HENV-103 and HENV-117 mAbs were described utilizing the rCedV chimera assay.[56] Shown in **Figure 2** is a neutralization synergy assay conducted with mAbs m102.4 and nAH1.3 (both NiV/HeV cross-reactive anti-G mAbs) where they are individually diluted 2-fold in a 7x7 concentration matrix. The diluted mAbs are added 1:1 to 4000 PFU per well of rCedV-NiV-B-GFP and the assay essentially carried out as above, with percent neutralization calculated by normalizing foci to a virus only control. Matrices are imported into SynergyFinder and analyzed Neutralization synergy was calculated by comparing treatment to virus only control wells. Values were imported into SynergyFinder software[57] using a Zero Interactions Potency (ZIP) statistical model; delta scores >10 indicate likely synergy as shown here.

Antiviral evaluation and combination testing of small molecules and nanobodies. These rCedV-based tools and rCedV chimera viruses will allow for rapid antiviral activity assessment and an ability to measure synergistic antiviral activities for both small molecule inhibitors derived from **Aim 1** and nanobodies derived from **Aim 2**; and will also allow the novel approach of testing small molecules and nanobody combinations in a rapid and quantitative manner, *in vitro*, to select best-in-class candidates for both monotherapeutic and combination therapeutic modalities prior to *in vivo* animal model testing.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called "SMART Set") was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 μM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 μM, and 47 hits showed only minimal cytotoxicity at 33 μM.

Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.

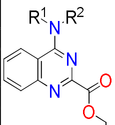


CBS1437

CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)
0.70	100	2.1	100	3.3

In **Table 2** is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437**. Note that both HEK293T and Vero cell lines were employed in these assays, and that CC_{50} values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC_{50} value of 3.3 μM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 μM). **Table 3** shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14** (**Figure 4**), bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss*

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.



N	Comp ID	R^1	R^2	% inhib at 10 μM
1	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
2	**G577-0016**	H	2,4-difluorophenyl	18
3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
4	**G577-0022**	H	3-methoxyphenyl	46
5	**G577-0028**	Me	cyclohexyl	93
6	**G577-0084**	H	4-isopropylphenyl	85
7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
8	**G577-0112**	Me	2-methylphenyl	94

*methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent, **CBS1437**, as well as specific plans for developing the SARs.*

C2.2.3. Generation of Nipah and Hendra G and F nanobodies. We recently initiated a collaborative project in nanobody development against NiV and HeV, as well as other henipavirus glycoproteins, with Dr. Jianliang Xu (**RAViD, Nanobody Core Lead**). The **Xu lab** has engineered and established transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, and has published on the power and utility of anti-SARS-CoV-2 nanobodies.[10] We have already initiated the immunizations of several nanomouse cohorts using native-like oligomeric recombinant sF (**Veesler lab**) and sG glycoproteins (**Broder lab**) of NiV-B and HeV. Immune responses are excellent and sera measured by ELISA using the immunogens. The first of these cohorts will be processed in the coming weeks, and the Xu lab will be constructing phage libraries of polyclonal VHHs from each cohort. We expect to be able to make rapid progress in **Aim 2**, in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

D. RESEARCH DESIGN AND METHODS

D1. Aim 1: Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays.

D.1.1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 μM) and low cytotoxicity (CC_{50} >100 μM).

D.1.1.1. Synthetic Chemistry. In **Figure 3** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**G577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we will optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. We will also optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 1** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) to produce 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely, 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[58-59] as well as other methods.[60,61] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible. In **Figure 4** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[62]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112**

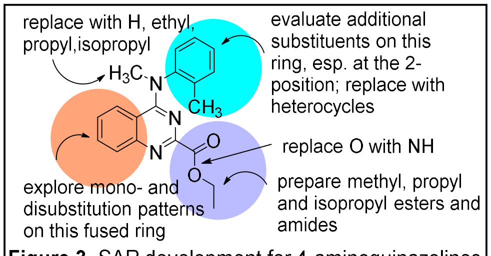
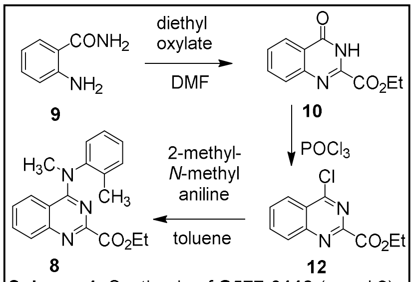
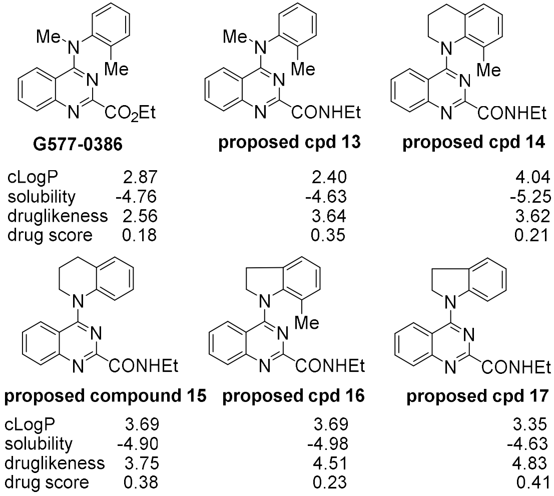


Figure 3. SAR development for 4-aminoquinazolines



Scheme 1. Synthesis of **G577-0112** (cmpd **8**).



	G577-0386	**proposed cpd 13**	**proposed cpd 14**
cLogP	2.87	2.40	4.04
solubility	-4.76	-4.63	-5.25
druglikeness	2.56	3.64	3.62
drug score	0.18	0.35	0.21

	proposed compound 15	**proposed cpd 16**	**proposed cpd 17**
cLogP	3.69	3.69	3.35
solubility	-4.90	-4.98	-4.63
druglikeness	3.75	4.51	4.83
drug score	0.38	0.23	0.41

Figure 4. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.

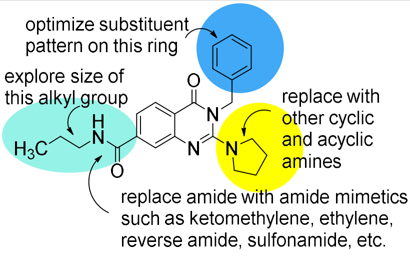


Figure 5. SAR development for **CBS1437**.

(compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester with an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scores have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding. In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. We have also researched and found additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

D.1.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5×10^4 cells per well will be treated with growth medium containing either DMSO or selected compound for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 µM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

Screening Libraries. The HTS will be performed in the **Rong Lab**. The libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of >150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including an Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions, Inc**. *Hit Selection Algorithm.* A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used as a specificity control. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (EC_{50} values less than 10 µM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. *Hit Validation, Cytotoxicity Assessment and Ranking.* We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. *Hit Quality Control and Identity Assurance.* The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds <80% pure will be purified and retested.

D.1.1.3. Evaluation of antiviral activity of advanced candidates. Inhibitors will be tested against authentic NiV and HeV as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2×10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 µM to 0.01 µM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to parallel control cultures of virus and DMSO. This work will be performed in the BSL-4 facility at UTMB (**Geisbert, Core D)**.

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Peet, Gaisina, and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy using a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or other HTS hits identified using the expanded libraries of compounds.

D.1.2. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with medium containing either DMSO or selected inhibitors at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by western blot techniques or viral RNA levels by q-RT-PCR. CedV specific antibodies against G F N and P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene, and performed using the ABI 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus.** ***Target identification for small molecules.*** The rCedV tools used in **Aim 1** will also be used for antiviral target identification. *In vitro* selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutants. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[63,64] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a “drug resistant” virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any “drug resistant” virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inhibitors using authentic NiV-B and HeV in BSL-4 by the UTMB BSL-4 Core.

D.1.2.1. Assess the binding properties by surface plasmon resonance (SPR). The NiV polymerase (L protein and a phosphoprotein P, or RdRp complex) will be prepared using a previously published protocol[65]. Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, and kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[66] RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 μM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab.**[67]

D.1.2.2. Structure determination of recombinant proteins with bound inhibitors by CryoEM. High-resolution structures of the NiV RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compounds to be defined will be obtained using CryoEM by the **Veesler Lab** at the Arnold and Mabel CryoEM Center at the UW. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[68] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including Warp[69], cryoSparc[70], and RELION[71]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed using Rosetta which is developed at the UW. As cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[72-76], to explore the conformational landscape[77] of ligand binding events, and to capture conformational variability of intermediate states[78,79], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches.

Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.1.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds.

*Milestone: Designate **advanced lead compounds** exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the **advanced lead compounds**.*

D.1.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.

Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-Tox properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[80] Compounds with IC_{50} values ≥100 μM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds.[81] Compounds with >50% recovery (1 h) will be considered for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed as previously described.[82] Compounds that do not prevent [^{3}H]dofetilide binding (IC_{50} >10 μM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method[83] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P_{app}) greater than 1×10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used.

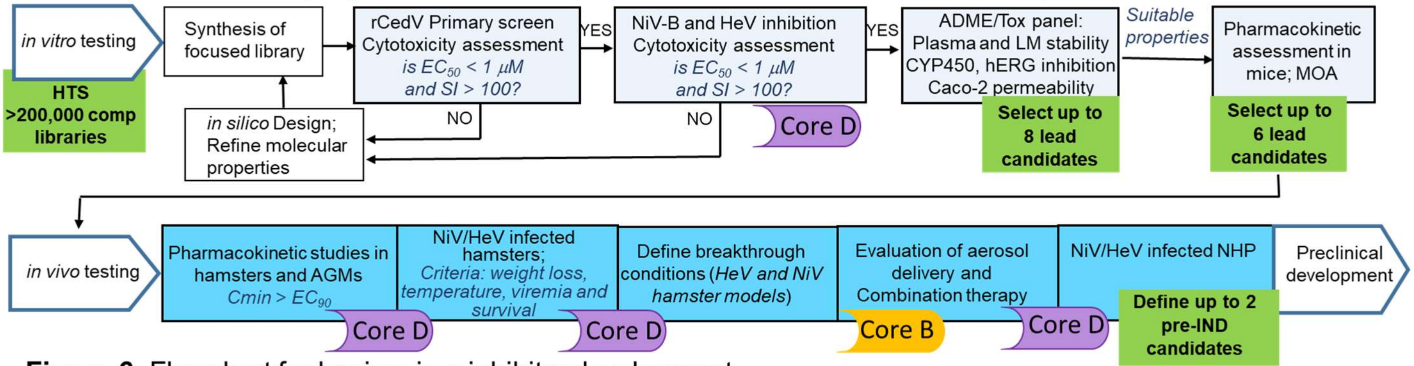


Figure 6. Flowchart for henipavirus inhibitor development.

The bacteria will be exposed to different dose levels and any reverse mutations will be determined. ***In vitro* mammalian chromosomal aberration test**: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes. **D.1.3.2. PK studies:** PK parameters including AUC, C_0, C_{max}, $T_{1/2}$, T_{max}, and F(%) will be determined. Depending on the estimation of T_{max}, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim. We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 6**).

D.2. AIM 2: Characterization of the potency and breadth of selected nanobodies.

Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. **Aim 1** will also collaborate with the **UTMB, BSL-4 Core D** to evaluate lead candidates with authentic NiV and HeV and also in vivo in **Aim 3**. Batteries of nanobodies to the F and G glycoproteins of NiV and HeV will be isolated from phage libraries constructed using cohorts of nanomice immunized with sF and sG recombinant glycoproteins (**C2.2.3. Preliminary Data).** The Core will conduct preliminary characterizations on binding and specificities and then provide purified nanobody panels to the **Veesler** and **Broder labs** for detailed studies.

D.2.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth.

D.2.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including NiV (Bangladesh and Malaysia strains) and HeV (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[84-86] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with NiV and HeV glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

D.2.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit NiV and HeV, we will use a high-throughput neutralization assay (conducted in the **Broder lab**). This assay is based on GFP-encoding, recombinant replication-competent, Cedar henipavirus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bengladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins (see: C.2.1. Preliminary Data).[7,87] This platform is safe, enabling utilization at BSL-2 compared to BSL-4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the **Broder lab** recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described HeV variant.[88] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond NiV and HeV (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[89,90]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.

Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against NiV and HeV. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.2.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition.

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The **Veesler lab** is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The **Veesler lab** has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software, which runs 24 hours per day and enables automated targeting and image acquisition, while keeping a relational database of all micrographs to expedite subsequent processing.[91] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[68,69] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[84,86,92-94] mechanical stage tilting with gold grids[95,96] or detergents to modify surface tension[97]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[95,98-102] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[103-105] The NiV attachment (G) glycoprotein engages host receptors, and is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the NiV G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3) (**Figure 7**), which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

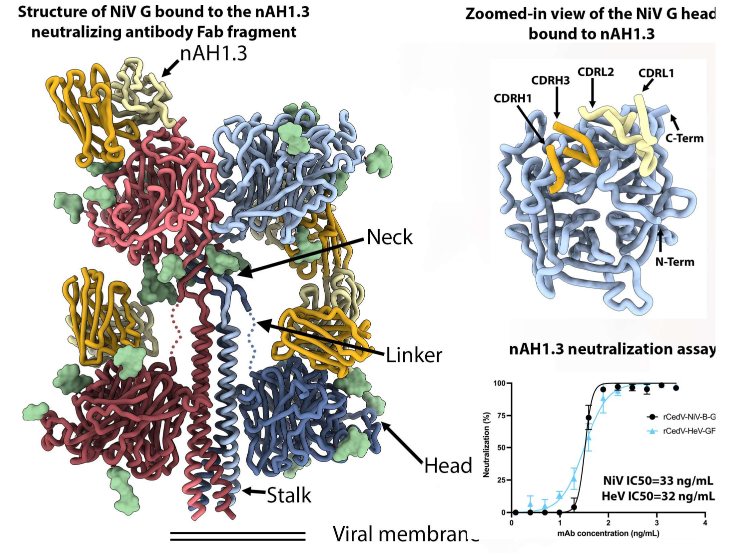


Figure 7. NiV G bound to the nAH1.3 neutralizing antibody Fab fragment. **A**) Ribbon diagram of the NiV G ectodomain bound to the broadly neutralizing nAH1.3 Fab fragment in two orthogonal orientations. Each of the four NiV G protomers is colored distinctly and resolved N-linked glycans are rendered as green surfaces. The nAH1.3 heavy and light chains are colored gold and yellow, respectively, and only the variable domains were modeled in density. The linkers connecting the neck to the two proximal head domains are shown as dashed lines due to weaker density in the cryoEM reconstruction. (**B**) Ribbon diagram of a NiV G head domain (blue) with the interacting nAH1.3 heavy and light chains CDRs rendered in gold and yellow. (**C**) nAH1.3-mediated neutralization of rCedV-NiV-B-GFP (black) and rCedV-HeV-GFP (blue).

D.2.2.1. High-resolution structural studies using X-ray crystallography. Some nanobodies might bind to regions that are intrinsically flexible, thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[106] and BUSTER-TNT.[107]

D.2.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[84-86,93,108,109]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[110-112] and high-resolution noise substitution prior to Fourier shell correlation calculations[113] to avoid overfitting. Atomic models will be assessed using Molprobity,[114] EMringer[115] and Privateer.[116] The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

D.2.3. AIM 2C. Structure-guided formulation of nanobody cocktails.

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[117-119] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected *in vivo*, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting, which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in **Aim 2A**. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.3. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with the ABSL-3 and ABSL-4 Core (**Core D**) to evaluate lead candidate drugs and nanobodies *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster and AGM.[2,120] PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV_B and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV_B and HeV infected AGM models.

D.3.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed by the **UTMB Core (Core D)**. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC_{90} in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of henipavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.3.2. *In vivo* testing of lead compounds NiV/HeV in infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 pfu of NiV_B or HeV.[87,121-123] Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.3.4. Define breakthrough conditions for anti-henipavirus antiviral agents in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antiviral agents fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^6$ PFU of NiV or HeV.[87,121-123] Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.3.5. Combination therapy of anti-henipavirus antiviral agents in hamsters. This subaim will employ the most promising antiviral agents from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^6$ PFU of NiV or HeV.[87,121-123]Treatment with at least two different antiviral agents will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

D.3.6. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ $5x10^6$ PFU of NiV_B or HeV as determined in henipavirus dose confirmation studies conducted by the ABSL-3 and ABSL-4 Core. Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by the **Aerosol Delivery Core** (see **Core B**) at various times after henipavirus infection.

D.3.7. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the LMA mucosal atomization device (MAD) as previously described[41] or by small particle aerosol as previously described[42,124] with a uniformly lethal challenge dose (~ 20,000 PFU of NiV or HeV). Importantly, the MAD system delivers atomized particles that range in size from 30 to 100 µm, which is highly consistent with the size of droplets exhaled by humans when coughing. Previous studies with AGMs infected with NiV_B using the MAD system resulted in lethality in 9/9 animals and a disease course that is highly consistent with human infection (TW Geisbert, C Broder, unpublished data). The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by the **Aerosol Delivery Core** (see **Core B**) up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

Potential Pitfalls and Alternative Strategies. It is possible that **Core B** may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antiviral agents may not improve protective efficacy or extend the therapeutic window of the agents. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and mAbs to protect animals against lethal henipavirus disease to de-risk this Aim.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues.. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the continued outbreaks of NiV across a large global region requires urgent translational countermeasure research to establish a readiness for the next NiV or related pathogenic henipavirus outbreak. We have discovered antiviral leads that show promise against NiV and HeV, and lay out a detailed plan to optimize, develop, and test these drugs. We also initiate a new plan to develop and test antiviral nanobodies. Together with the expertise, resources and experience within this proposal, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Current topics in microbiology and immunology* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *The New England journal of medicine* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *The Journal of infectious diseases* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Taylor, L. H., Latham, S. M. & Woolhouse, M. E. Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **356**, 983-989, doi:10.1098/rstb.2001.0888 (2001).

12 Desselberger, U. Emerging and re-emerging infectious diseases. *The Journal of infection* **40**, 3-15, doi:10.1053/jinf.1999.0624 (2000).

13 van Doorn, H. R. The epidemiology of emerging infectious diseases and pandemics. *Medicine* **49**, 659-662, doi:10.1016/j.mpmed.2021.07.011 (2021).

14 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. *Henipavirus.*, Vol. 1 (Lippincott Williams & Wilkins 2013).

15 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

16 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *The Medical journal of Australia* **162**, 642-645 (1995).

17 Chua, K. B. *et al.* Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**, 1432-1435, doi:10.1126/science.288.5470.1432 (2000).

18 Chua, K. B. *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**, 1257-1259, doi:10.1016/S0140-6736(99)04299-3 (1999).

19 Harcourt, B. H. *et al.* Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis* **11**, 1594-1597, doi:10.3201/eid1110.050513 (2005).

20 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

21 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

22 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

23 Schountz, T. *et al.* Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **11**, doi:10.3390/v11030291 (2019).

24 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

25 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

26 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

27 Maes, P. *et al.* Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. *Archives of virology* **163**, 2295-2310, doi:10.1007/s00705-018-3843-5 (2018).

28 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annual review of virology* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

29 Ching, P. K. *et al.* Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis* **21**, 328-331, doi:10.3201/eid2102.141433 (2015).

30 Playford, E. G. *et al.* Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis* **16**, 219-223, doi:10.3201/eid1602.090552 (2010).

31 Wong, K. T., Shieh, W. J., Zaki, S. R. & Tan, C. T. Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer seminars in immunopathology* **24**, 215-228, doi:10.1007/s00281-002-0106-y (2002).

32 Goh, K. J. *et al.* Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *The New England journal of medicine* **342**, 1229-1235, doi:10.1056/NEJM200004273421701 (2000).

33 O'Sullivan, J. D. *et al.* Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* **349**, 93-95, doi:10.1016/s0140-6736(96)06162-4 (1997).

34 Paton, N. I. *et al.* Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* **354**, 1253-1256, doi:10.1016/S0140-6736(99)04379-2 (1999).

35 Hossain, M. J. *et al.* Clinical presentation of nipah virus infection in Bangladesh. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **46**, 977-984, doi:10.1086/529147 (2008).

36 Wong, K. T. *et al.* Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *The American journal of pathology* **161**, 2153-2167, doi:10.1016/S0002-9440(10)64493-8 (2002).

37 Wong, K. T. *et al.* Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathology and applied neurobiology* **35**, 296-305, doi:10.1111/j.1365-2990.2008.00991.x (2009).

38 Tan, C. T. *et al.* Relapsed and late-onset Nipah encephalitis. *Annals of neurology* **51**, 703-708, doi:10.1002/ana.10212 (2002).

39 Abdullah, S., Chang, L. Y., Rahmat, K., Goh, K. T. & Tan, C. T. Late-onset Nipah virus encephalitis 11 years after

the initial outbreak: A case report. *Neurology Asia* **17**, 71-74 (2012).

40 Hammoud, D. A. *et al.* Aerosol exposure to intermediate size Nipah virus particles induces neurological disease in African green monkeys. *PLoS neglected tropical diseases* **12**, e0006978, doi:10.1371/journal.pntd.0006978 (2018).

41 Geisbert, J. B. *et al.* An Intranasal Exposure Model of Lethal Nipah Virus Infection in African Green Monkeys. *The Journal of infectious diseases* **221**, S414-S418, doi:10.1093/infdis/jiz391 (2020).

42 Prasad, A. N. *et al.* A Lethal Aerosol Exposure Model of Nipah Virus Strain Bangladesh in African Green Monkeys. *The Journal of infectious diseases* **221**, S431-S435, doi:10.1093/infdis/jiz469 (2020).

43 Mehand, M. S., Al-Shorbaji, F., Millett, P. & Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research* **159**, 63-67, doi:10.1016/j.antiviral.2018.09.009 (2018).

44 Luby, S. P. The pandemic potential of Nipah virus. *Antiviral research* **100**, 38-43, doi:10.1016/j.antiviral.2013.07.011 (2013).

45 Broder, C. C. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. *Current opinion in virology* **2**, 176-187, doi:10.1016/j.coviro.2012.02.016 (2012).

46 Azarm, K. D. & Lee, B. Differential Features of Fusion Activation within the Paramyxoviridae. *Viruses* **12**, doi:10.3390/v12020161 (2020).

47 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).

48 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

49 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

50 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

51 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

52 Harmsen, M. M. & De Haard, H. J. Properties, production, and applications of camelid single-domain antibody fragments. *Applied microbiology and biotechnology* **77**, 13-22, doi:10.1007/s00253-007-1142-2 (2007).

53 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

54 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

55 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

56 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell reports* **36**, 109628, doi:10.1016/j.celrep.2021.109628 (2021).

57 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493, doi:10.1093/nar/gkaa216 (2020).

58 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

59 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

60 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

61 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

62 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

63 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

64 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

65 Jordan, P. C. *et al.* Initiation, extension, and termination of RNA synthesis by a paramyxovirus polymerase. *PLoS Pathog* **14**, e1006889, doi:10.1371/journal.ppat.1006889 (2018).

66 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Analytical Biochemistry* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

67 Schafer, A. *et al.* Evidence for distinct mechanisms of small molecule inhibitors of filovirus entry. *PLoS pathogens* **17**, e1009312, doi:10.1371/journal.ppat.1009312 (2021).

68 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *J Struct Biol* **166**, 95-102 (2009).

69 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

70 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

71 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

72 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).

73 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).

74 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).

75 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).

76 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).

77 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).

78 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).

79 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).

80 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).

81 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

82 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

83 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

84 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).

85 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

86 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).

87 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).

88 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).

89 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).

90 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).

91 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

92 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

93 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

94 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

95 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

96 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).

97 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).

98 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).

99 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).

100 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).

101 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).

102 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).

103 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).

104 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).

105 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).

106 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).

107 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).

108 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).

109 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).

110 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

111 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

112 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).

113 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).

114 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).

115 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).

116 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).

117 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).

118 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).

119 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).

120 de Wit, E. & Munster, V. J. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *The Journal of pathology* **235**, 196-205, doi:10.1002/path.4444 (2015).

121 Baseler, L. *et al.* Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. *PLoS neglected tropical diseases* **10**, e0005120, doi:10.1371/journal.pntd.0005120 (2016).

122 Wong, K. T. *et al.* A golden hamster model for human acute Nipah virus infection. *The American journal of pathology* **163**, 2127-2137, doi:10.1016/S0002-9440(10)63569-9 (2003).

123 Rockx, B. *et al.* Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *Journal of virology* **85**, 7658-7671, doi:10.1128/JVI.00473-11 (2011).

124 Cong, Y. *et al.* Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS neglected tropical diseases* **11**, e0005532, doi:10.1371/journal.pntd.0005532 (2017).

The pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV) cause severe respiratory illness and encephalitis in both animals and humans with human case fatality rates ranging from 40-100%. The NiV-Bangladesh strain (NiV-B) is associated with multiple rounds of human-to-human transmission and is a pathogen of pandemic concern for which there are no vaccines or antivirals approved for human use. We have recently shown that the nonpathogenic Cedar henipavirus (CedV) is a useful surrogate platform for high throughput screening (HTS) for antiviral drug discovery, and for virus neutralization assays. This proposal focuses on developing antiviral small molecule and nanobody countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach. We created reporter gene-encoding recombinant CedVs and discovered a series of fused heterocycles, e.g., 4-aminoquinazoline-based, antivirals, and showed that a representative quinazoline **CBS1435** was an effective inhibitor of rCedV replication and also both NiV-B and HeV, with EC_{50} values in the low μM range and minimal cytotoxicity. Neutralizing antibodies to the henipavirus F and G glycoproteins have also shown therapeutic promise, but antiviral nanobodies as potential immunotherapeutics represent a novel area of NiV and HeV countermeasure development. This proposal will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Aim 1 will focus on optimizing lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. Viral polymerase-targeting lead molecules will be selected using NiV-B and HeV assays and mechanism of action (MOA) studies, and best-in-class drugs will be developed by chemical modifications aimed to improve drug-like properties, increase potencies, decrease toxicity, and improve pharmacokinetic properties. Aim 2 will isolate and fully characterize nanobodies broadly neutralizing NiV-B and HeV derived by immunization of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using phage display technology of cloned nanobody gene fragments. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails. Aim 3 will focus on *in vivo* efficacy evaluation of lead compounds and nanobodies in animal models. Both monotherapies and combination therapeutic modalities will be tested, along with testing antiviral nanobody delivery by aerosol. These two unique henipavirus-targeted antiviral development directions will generate novel countermeasures that may display more broad effectiveness.

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] Twenty-two cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antiviral agents approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus that is nonpathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will yield testable antiviral agents with potential pan-henipavirus effectiveness. Our collaborative team also has extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both viral polymerase-targeting small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays and SPR binding assay for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and later, best-in-class nanobodies will be also tested by aerosol delivery. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobody studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

For several decades, the major agents of emerged or newly identified infectious diseases in humans have been viruses of zoonotic origins.[11-13] Among these, two paramyxoviruses of importance are Nipah virus (NiV) and Hendra virus (HeV); the prototype species of the genus *Henipavirus* and are single-stranded, negative sense, enveloped RNA viruses.[14] HeV appeared in 1994, in an outbreak of severe respiratory disease in horses in Australia, with the death of 14 horses and their trainer.[15,16] NiV (Malaysia strain, NiV-M) appeared in an outbreak of encephalitis in pig farmers in Malaysia in 1998.[17,18] NiV outbreaks have since been seen in Bangladesh and India by a related strain NiV-Bangladesh (NiV-B).[19] Pteropid fruit bats are the major reservoir hosts for henipaviruses.[6,20] NiV and HeV have broad host tropism, infecting at least 18 species across 6 orders of mammals. Among these, NiV and HeV will cause a systemic and often fatal respiratory and/or neurological disease in at least 11 species including humans, with human fatality rates ranging from 40-100%.[2,21] A third henipavirus isolate, Cedar virus (CedV), was discovered in 2010.[22] However, CedV is not known to be zoonotic and has been shown to be nonpathogenic in HeV and NiV animal models.[22-24] Two additional henipaviruses are also known, Ghana bat virus and Mojiang virus, but only from sequence data.[25-27] Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and 3 outbreaks in India.[28] In 2014, an outbreak of NiV-M occurred in the Philippines with 9 fatalities of 11 human cases, with horse-to-human and human-to-human transmission.[29] The combined NiV case fatality rate is ~60% across a large region of Asia in five countries.[3] The recent 2018 NiV outbreak in Kerala, India was significant, occurring in a new geographic region, with a case fatality rate of 91% and 22 of 23 cases the result of from human-to-human transmission, underscoring both the transmissibility and the lethal nature of this virus.[4]

Human NiV/HeV infection is generally accepted to occur via the oronasal route with an incubation period of 1 to 2 weeks.[4,30] Acute NiV/HeV infection in people is systemic and likely via hematogenous viral spread from the respiratory system.[31] Disease onset is characterized by fever, myalgia, shortness of breath, and cough.[16,32] Human HeV infections have resulted in both fatal respiratory or encephalitic disease, and also recovery.[30,33] The predominant clinical feature in the Malaysia outbreak was encephalitis, but respiratory symptoms were also common.[32,34] The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease and in the 2018 NiV-B outbreak in Kerala, 83% of cases had acute respiratory distress syndrome (ARDS).[4,35] The key findings of human NiV/HeV infection are wide-spread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected.[36,37] Human NiV/HeV infection can also be prolonged following apparent recovery, and some patients can experience a late-onset or relapsed encephalitis.[33,38] Relapsed encephalitis caused by NiV appears to be a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later,[39] NiV and HeV are biosafety level-4 (BSL-4) viruses and classified as Category C priority pathogens and transboundary agents by several US agencies. Also, both NiV-M and NiV-B can cause lethal infection in nonhuman primates (NHPs), the African green monkey (AGM), when delivered by small particle aerosol.[40-42] NiV and henipaviral diseases (HeV) are included on the WHO's Blueprint List of Priority Pathogens.[43] NiV is a potential pandemic threat because of its high case-fatality rates, capacity for human-to-human transmission and potential to mutate, and a human-adapted strain in a region of high population density, could lead to rapid spread.[44] *There are presently no approved vaccines or therapeutic modalities for NiV/HeV infections in people.*

The viral RNA-dependent-RNA polymerases of paramyxoviruses are attractive targets for antiviral compound discovery, but antiviral drug discovery for NiV and HeV has been hampered by requirements of BSL-4 containment. We recently developed and optimized an authentic henipavirus replication cell-based system using a recombinant CedV (rCedV) encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications at BSL-2 and identified several inhibitory compounds that were further validated with authentic NiV-B and HeV.[7] Those leads provide the rationale for the current application. Our ability to employ rCedV provides an opportunity for anti-henipavirus drug discovery not previously available, that also allows for discovery across the whole replication cycle, not restricted to only the viral polymerase. Another well-exploited stage of virus infection is the attachment and entry process.[45] NiV/HeV possess two membrane anchored glycoproteins required for infection; one for attachment, the G glycoprotein, and the other for membrane fusion the F glycoprotein.[46] The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and all vaccine strategies.[28] Although an effective post-exposure human monoclonal antibody (mAb) therapy targeting the NiV and HeV G glycoprotein (m102.4) has been extensively examined,[47-51] its delivery must be within several days following infection in NHPs. An unexplored alternative but related antibody-based strategy are antiviral nanobodies. A nanobody, is an antibody fragment comprised of just a single monomeric variable antibody domain.[52] Nanobodies were first engineered from variable heavy chain domains of camelid heavy chain

antibodies (VHHs), and camelid VHHs retain full antigen specificity and are only a fraction of the size of a human antibody, and they have extended complementarity determining regions (CDRs) which can allow them to bind epitopes not normally accessible to conventional antibodies, such as conserved viral epitopes often masked by glycan shields.[10] Our expertise in henipavirus F and G structural and functional analyses together with anti-F and G mAb development and therapeutic application, will facilitate the development and testing of a new and understudied area of nanobody-based immunotherapeutic against NiV and HeV.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of the investigators in this proposal. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may well have activity across related members of the henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of a high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification. Finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole NiV or HeV immune sera shows cross-reactivity with CedV, however, such sera or even specific high-titer anti-CedV F or G antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins.[22] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and the largest polymerase in the order *Mononegavirale*, yet it shares high aa identity to NiV and HeV L proteins. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; aa identities of L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This overall high degree of identity, shared between CedV and that of NiV and HeV, suggests that antivirals that target the L polymerase, could be identified and also offer the possibility of identifying pan-henipavirus countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] Several small molecule inhibitors have been validated *in vitro* using infectious HeV and NiV, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support **Aim 1** to optimize our current best-in-class leads and also screen large libraries of compounds.

Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[53-55] Nanobodies can be isolated using the same phage library construction methods and phage panning procedures routinely employed for mAb discovery, which allows for *in vitro* production of various amounts and their rapid characterization. Nanobody development will be carried out by **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels.[10] Immunized mouse cohorts are in progress using recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in **Aim 2.**

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of **Aim 3**. In addition, we expect to discover, and

characterize batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in **Aim 3**. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet, Irina Gaisina, and Terry Moore, UIC and Chicago BioSolutions Inc.** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule inhibitors of henipaviruses.

C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2), cell-based HTS platform to identify small molecule antiviral agents targeting henipavirus replication without the requirement for BSL-4 containment.[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with that of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera use guidelines and procedures at the BSL-2 level were reviewed and approved by the USU, Institutional Biosafety Committee (IBC) in accordance with NIH guidelines. These replication competent rCedV chimeras create a very rapid and robust surrogate infection assay for NiV_B and HeV that can be used as a neutralization or entry inhibitor assay to measure neutralizing antibodies, or as a surrogate for generating virus escape mutants to neutralizing antibodies. Here, the F and G genes of NiV_B (2010 Faridpur isolate) and HeV (2008 Redlands isolate) were synthesized and inserted into our pOLTV5-rCedV anti-genome plasmid. For the HTS neutralization assay, a turbo-GFP gene was inserted between the CedV P and M genes. Each chimera (rCedV-NiV_B-GFP and rCedV-HeV-GFP) were rescued as previously described.[8] Other versions without reporter genes have also been prepared. All virus stocks are cloned and genomes confirmed by NGS. Typically, large stocks are prepared and frozen as single use aliquots. Show in **Figure 1** is an example of how these chimeras are used in a virus neutralization test, referred to as a fluorescent reduction neutralization test (FRNT) using two human NiV/HeV cross-reactive anti-G mAbs (HENV-103 and -117) as previously described.[56] Briefly, Vero 76 cells are used in black walled clear bottom 96-well plates, mAbs are serially diluted in medium, and equal volumes of medium containing rCedV-NiV-B or rCedV-HeV are added to each mAb dilution for a final concentration of 2000 PFU, incubated for 2 h at 37 °C. Each virus-mAb mixture (90 µL/well) is added to the pre-seeded Vero 76 cells in triplicate and the assay run for just another 24 h followed by 4% formaldehyde fixation, washing and then scanning using a CTL S6 analyzer. Fluorescent foci are counted using the CTL Basic Count™ and an IC$_{50}$ value is determined as the antibody concentration

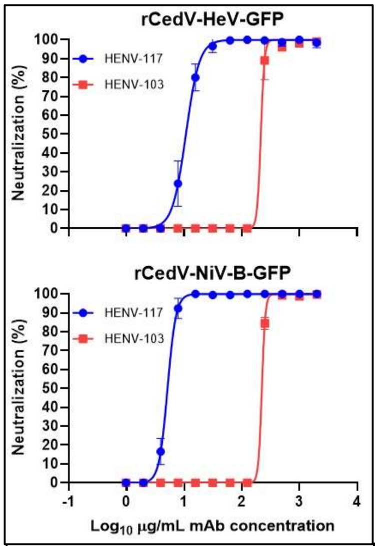


Figure 1. Neutralization curves for HENV-117 and HENV-103 mAbs against rCedV-HeV-GFP (top) and rCedV-NiV-B-GFP (bottom). Data are from a single experiment performed in technical triplicate.

IC50 (ng/mL)	HeV	rCedV-HeV	NiVB	rCedV-NiVB
HENV-103	445	216	507	238
HENV-117	14	11	15	6

Table 1. IC$_{50}$ values of cross-reactive anti-G human mAbs HENV-103 and -117) as determined by rCedV chimera FRNT assay and NiV-B and HeV conventional PRNT assay.

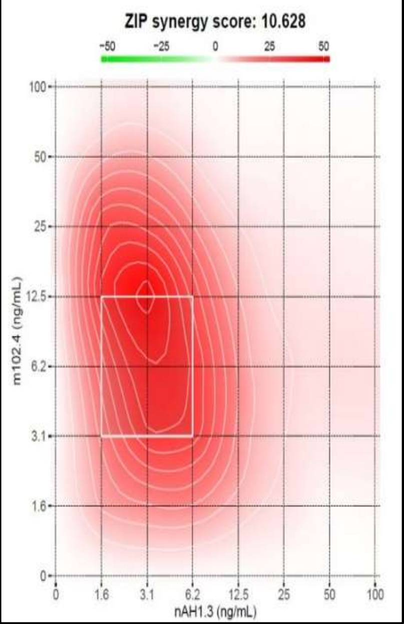


Figure 2. Synergy map with calculated synergy score using the zero interactions potency (ZIP) model generated by SynergyFinder for neutralization of rCedV-NiV-B-GFP by serially diluted m102.4 and nAH1.3 mAbs.

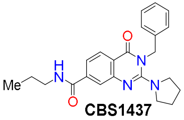
yielding 50% reduction in fluorescent foci versus control wells. **Table 1** shows the calculated IC_{50} values using rCedV chimeras vs. those determined by a conventional PRNT assay with authentic NiV-B and HeV, and they are remarkably within a 2-fold range.

In addition to conventional single agent neutralization tests, the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras can also be used in rapid HTS assays to measure synergistic activities of inhibitors, such as antibodies or drugs. The synergistic neutralizing activity of the HENV-103 and HENV-117 mAbs were described utilizing the rCedV chimera assay.[56] Shown in **Figure 2** is a neutralization synergy assay conducted with mAbs m102.4 and nAH1.3 (both NiV/HeV cross-reactive anti-G mAbs) where they are individually diluted 2-fold in a 7x7 concentration matrix. The diluted mAbs are added 1:1 to 4000 PFU per well of rCedV-NiV-B-GFP and the assay essentially carried out as above, with percent neutralization calculated by normalizing foci to a virus only control. Matrices are imported into SynergyFinder and analyzed Neutralization synergy was calculated by comparing treatment to virus only control wells. Values were imported into SynergyFinder software[57] using a Zero Interactions Potency (ZIP) statistical model; delta scores >10 indicate likely synergy as shown here.

Antiviral evaluation and combination testing of small molecules and nanobodies. These rCedV-based tools and rCedV chimera viruses will allow for rapid antiviral activity assessment and an ability to measure synergistic antiviral activities for both small molecule inhibitors derived from **Aim 1** and nanobodies derived from **Aim 2**; and will also allow the novel approach of testing small molecules and nanobody combinations in a rapid and quantitative manner, *in vitro*, to select best-in-class candidates for both monotherapeutic and combination therapeutic modalities prior to *in vivo* animal model testing.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called "SMART Set") was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 μM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 μM, and 47 hits showed only minimal cytotoxicity at 33 μM.

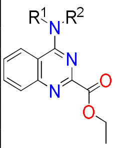
Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.



CBS1437

CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)
0.70	100	2.1	100	3.3

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.

	N	Comp ID	R^1	R^2	% inhib at 10 μM
	1	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
	2	**G577-0016**	H	2,4-difluorophenyl	18
	3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
	4	**G577-0022**	H	3-methoxyphenyl	46
	5	**G577-0028**	Me	cyclohexyl	93
	6	**G577-0084**	H	4-isopropylphenyl	85
	7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
	8	**G577-0112**	Me	2-methylphenyl	94

In **Table 2** is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437**. Note that both HEK293T and Vero cell lines were employed in these assays, and that CC_{50} values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC_{50} value of 3.3 μM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 μM). **Table 3** shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14** (**Figure 4**), bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss* *methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent, **CBS1437**, as well as specific plans for developing the SARs.*

C2.2.3. Generation of Nipah and Hendra G and F nanobodies. We recently initiated a collaborative project in nanobody development against NiV and HeV, as well as other henipavirus glycoproteins, with Dr. Jianliang Xu (**RAViD, Nanobody Core Lead**). The **Xu lab** has engineered and established transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, and has published on the power and utility of anti-SARS-CoV-2 nanobodies.[10] We have already initiated the immunizations of several nanomouse cohorts using native-like oligomeric recombinant sF (**Veesler lab**) and sG glycoproteins (**Broder lab**) of NiV-B and HeV. Immune responses are excellent and sera measured by ELISA using the immunogens. The first of these cohorts will be processed in the coming weeks, and the Xu lab will be constructing phage libraries of polyclonal VHHs from each cohort. We expect to be able to make rapid progress in **Aim 2**, in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

D. RESEARCH DESIGN AND METHODS

D1. Aim 1: Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays.

D.1.1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 μM) and low cytotoxicity (CC_{50} >100 μM).

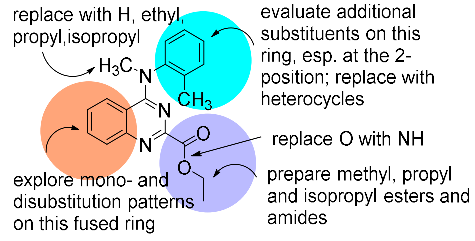
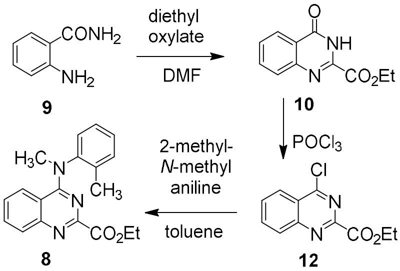
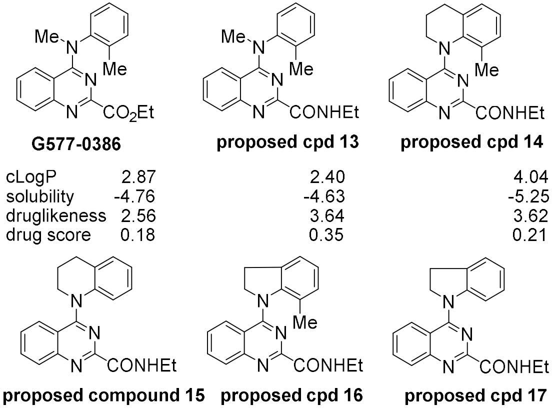


Figure 3. SAR development for 4-aminoquinazolines



Scheme 1. Synthesis of **G577-0112** (cmpd **8**).

D.1.1.1. Synthetic Chemistry. In **Figure 3** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**G577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we will optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. We will also optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 1** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) to produce 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely, 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[58-59] as well as other methods.[60,61] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible. In **Figure 4** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[62]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112**



	G577-0386	**proposed cpd 13**	**proposed cpd 14**
cLogP	2.87	2.40	4.04
solubility	-4.76	-4.63	-5.25
druglikeness	2.56	3.64	3.62
drug score	0.18	0.35	0.21

	proposed compound 15	**proposed cpd 16**	**proposed cpd 17**
cLogP	3.69	3.69	3.35
solubility	-4.90	-4.98	-4.63
druglikeness	3.75	4.51	4.83
drug score	0.38	0.23	0.41

Figure 4. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.

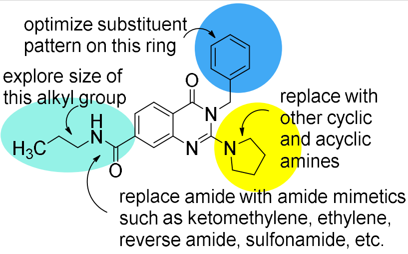


Figure 5. SAR development for **CBS1437**.

(compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester with an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scores have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding. In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. We have also researched and found additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

D.1.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of $2.5x10^4$ cells per well will be treated with growth medium containing either DMSO or selected compound for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 μM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

Screening Libraries. The HTS will be performed in the **Rong Lab**. The libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of >150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including an Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions, Inc**. *Hit Selection Algorithm.* A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used as a specificity control. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (EC_{50} values less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. *Hit Validation, Cytotoxicity Assessment and Ranking.* We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. *Hit Quality Control and Identity Assurance.* The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds <80% pure will be purified and retested.

D.1.1.3. Evaluation of antiviral activity of advanced candidates. Inhibitors will be tested against authentic NiV and HeV as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2×10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to parallel control cultures of virus and DMSO. This work will be performed in the BSL-4 facility at UTMB (**Geisbert, Core D)**.

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Peet, Gaisina, and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy using a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or other HTS hits identified using the expanded libraries of compounds.

D.1.2. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with medium containing either DMSO or selected inhibitors at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by western blot techniques or viral RNA levels by q-RT-PCR. CedV specific antibodies against G F N and P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene, and performed using the ABI 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus.** ***Target identification for small molecules.*** The rCedV tools used in **Aim 1** will also be used for antiviral target identification. *In vitro* selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutants. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[63,64] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a “drug resistant” virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any “drug resistant” virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inhibitors using authentic NiV-B and HeV in BSL-4 by the UTMB BSL-4 Core.

D.1.2.1. Assess the binding properties by surface plasmon resonance (SPR). The NiV polymerase (L protein and a phosphoprotein P, or RdRp complex) will be prepared using a previously published protocol[65]. Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, and kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[66] RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 μM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab.**[67]

D.1.2.2. Structure determination of recombinant proteins with bound inhibitors by CryoEM. High-resolution structures of the NiV RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compounds to be defined will be obtained using CryoEM by the **Veesler Lab** at the Arnold and Mabel CryoEM Center at the UW. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[68] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including Warp[69], cryoSparc[70], and RELION[71]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed using Rosetta which is developed at the UW. As cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[72-76], to explore the conformational landscape[77] of ligand binding events, and to capture conformational variability of intermediate states[78,79], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches.

Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.1.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds. *Milestone: Designate* ***advanced lead compounds*** *exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the* ***advanced lead compounds****.*

D.1.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.
Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-Tox properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[80] Compounds with IC_{50} values ≥100 μM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds.[81] Compounds with >50% recovery (1 h) will be considered for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed as previously described.[82] Compounds that do not prevent [^{3}H]dofetilide binding (IC_{50} >10 μM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method[83] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P_{app}) greater than 1 × 10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella*

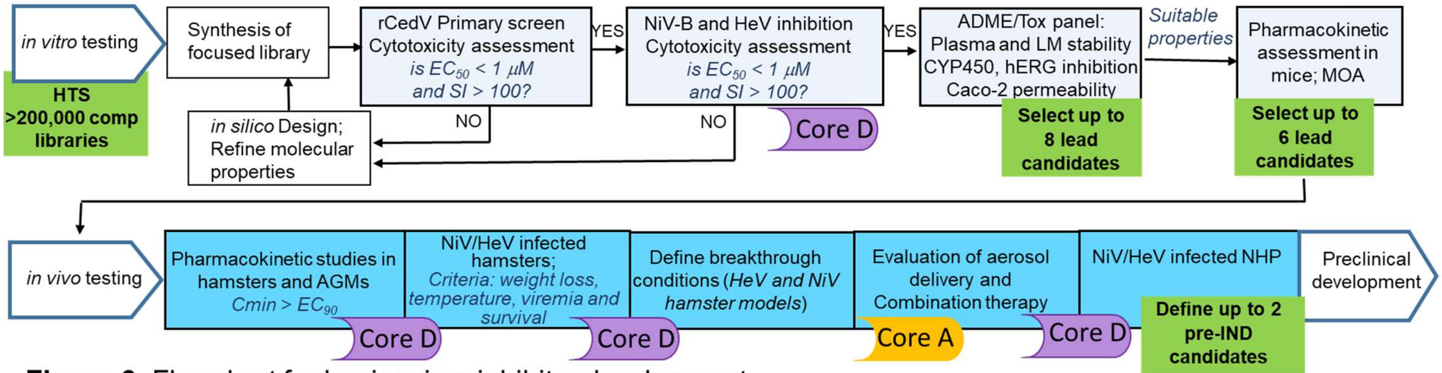


Figure 6. Flowchart for henipavirus inhibitor development.

typhimurium strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels and any reverse mutations will be determined. ***In vitro* mammalian chromosomal aberration test**: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes. **D.1.3.2. PK studies:** PK parameters including AUC, C_0, C_{max}, $T_{1/2}$, T_{max}, and F(%) will be determined. Depending on the estimation of T_{max}, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim. We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 6**).

D.2. AIM 2: Characterization of the potency and breadth of selected nanobodies.

Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. **Aim 1** will also collaborate with the **UTMB, BSL-4 Core D** to evaluate lead candidates with authentic NiV and HeV and also in vivo in **Aim 3**. Batteries of nanobodies to the F and G glycoproteins of NiV and HeV will be isolated from phage libraries constructed using cohorts of nanomice immunized with sF and sG recombinant glycoproteins (**C2.2.3. Preliminary Data).** The Core will conduct preliminary characterizations on binding and specificities and then provide purified nanobody panels to the **Veesler** and **Broder labs** for detailed studies.

D.2.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth.

D.2.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including NiV (Bengladesh and Malaysia strains) and HeV (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[84-86] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with NiV and HeV glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

D.2.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit NiV and HeV, we will use a high-throughput neutralization assay (conducted in the **Broder lab**). This assay is based on GFP-encoding, recombinant replication-competent, Cedar henipavirus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bengladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins (see: C.2.1. Preliminary Data).[7,87] This platform is safe, enabling utilization at BSL-2 compared to BSL-4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the **Broder lab** recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described HeV variant.[88] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond NiV and HeV (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[89,90]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.

Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against NiV and HeV. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.2.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition.

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The **Veesler lab** is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The **Veesler lab** has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software, which runs 24 hours per day and enables automated targeting and image acquisition, while keeping a relational database of all micrographs to expedite subsequent processing.[91] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[68,69] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[84,86,92-94] mechanical stage tilting with gold grids[95,96] or detergents to modify surface tension[97]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[95,98-102] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[103-105] The NiV attachment (G) glycoprotein engages host receptors, and is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the NiV G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3) (**Figure 7**), which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

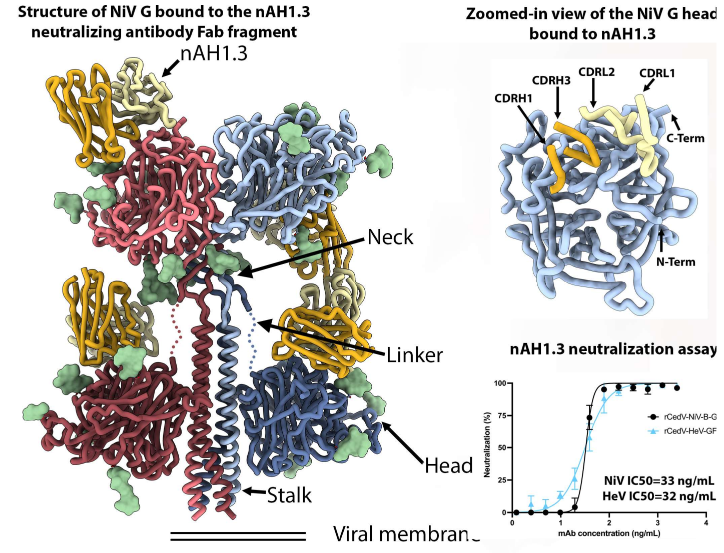


Figure 7. NiV G bound to the nAH1.3 neutralizing antibody Fab fragment. **A**) Ribbon diagram of the NiV G ectodomain bound to the broadly neutralizing nAH1.3 Fab fragment in two orthogonal orientations. Each of the four NiV G protomers is colored distinctly and resolved N-linked glycans are rendered as green surfaces. The nAH1.3 heavy and light chains are colored gold and yellow, respectively, and only the variable domains were modeled in density. The linkers connecting the neck to the two proximal head domains are shown as dashed lines due to weaker density in the cryoEM reconstruction. (**B**) Ribbon diagram of a NiV G head domain (blue) with the interacting nAH1.3 heavy and light chains CDRs rendered in gold and yellow. (**C**) nAH1.3-mediated neutralization of rCedV-NiV-B-GFP (black) and rCedV-HeV-GFP (blue).

D.2.2.1. High-resolution structural studies using X-ray crystallography. Some nanobodies might bind to regions that are intrinsically flexible, thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[106] and BUSTER-TNT.[107]

D.2.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[84-86,93,108,109]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[110-112] and high-resolution noise substitution prior to Fourier shell correlation calculations[113] to avoid overfitting. Atomic models will be assessed using Molprobity,[114] EMringer[115] and Privateer.[116] The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

D.2.3. AIM 2C. Structure-guided formulation of nanobody cocktails.

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[117-119] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected *in vivo*, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting, which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in **Aim 2A**. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.3. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with the ABSL-3 and ABSL-4 Core (**Core D**) to evaluate lead candidate drugs and nanobodies *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster and AGM.[2,120] PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV_B and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV_B and HeV infected AGM models.

D.3.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed by the **UTMB Core (Core D)**. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC_{90} in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of henipavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.3.2. *In vivo* testing of lead compounds NiV/HeV in infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 pfu of NiV_B or HeV.[87,121-123] Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.3.4. Define breakthrough conditions for anti-henipavirus antiviral agents in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antiviral agents fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^6$ PFU of NiV or HeV.[87,121-123] Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.3.5. Combination therapy of anti-henipavirus antiviral agents in hamsters. This subaim will employ the most promising antiviral agents from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^6$ PFU of NiV or HeV.[87,121-123]Treatment with at least two different antiviral agents will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

D.3.6. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ $5x10^6$ PFU of NiV_B or HeV as determined in henipavirus dose confirmation studies conducted by the ABSL-3 and ABSL-4 Core. Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by the **Aerosol Delivery Core** (see **Core A**) at various times after henipavirus infection.

D.3.7. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the LMA mucosal atomization device (MAD) as previously described[41] or by small particle aerosol as previously described[42,124] with a uniformly lethal challenge dose (~ 20,000 PFU of NiV or HeV). Importantly, the MAD system delivers atomized particles that range in size from 30 to 100 µm, which is highly consistent with the size of droplets exhaled by humans when coughing. Previous studies with AGMs infected with NiV_B using the MAD system resulted in lethality in 9/9 animals and a disease course that is highly consistent with human infection (TW Geisbert, C Broder, unpublished data). The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by the **Aerosol Delivery Core** (see **Core A**) up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

Potential Pitfalls and Alternative Strategies. It is possible that **Core A** may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antiviral agents may not improve protective efficacy or extend the therapeutic window of the agents. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and mAbs to protect animals against lethal henipavirus disease to de-risk this Aim.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues.. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the continued outbreaks of NiV across a large global region requires urgent translational countermeasure research to establish a readiness for the next NiV or related pathogenic henipavirus outbreak. We have discovered antiviral leads that show promise against NiV and HeV, and lay out a detailed plan to optimize, develop, and test these drugs. We also initiate a new plan to develop and test antiviral nanobodies. Together with the expertise, resources and experience within this proposal, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Current topics in microbiology and immunology* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *The New England journal of medicine* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *The Journal of infectious diseases* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Taylor, L. H., Latham, S. M. & Woolhouse, M. E. Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **356**, 983-989, doi:10.1098/rstb.2001.0888 (2001).

12 Desselberger, U. Emerging and re-emerging infectious diseases. *The Journal of infection* **40**, 3-15, doi:10.1053/jinf.1999.0624 (2000).

13 van Doorn, H. R. The epidemiology of emerging infectious diseases and pandemics. *Medicine* **49**, 659-662, doi:10.1016/j.mpmed.2021.07.011 (2021).

14 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. *Henipavirus.*, Vol. 1 (Lippincott Williams & Wilkins 2013).

15 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

16 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *The Medical journal of Australia* **162**, 642-645 (1995).

17 Chua, K. B. *et al.* Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**, 1432-1435, doi:10.1126/science.288.5470.1432 (2000).

18 Chua, K. B. *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**, 1257-1259, doi:10.1016/S0140-6736(99)04299-3 (1999).

19 Harcourt, B. H. *et al.* Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis* **11**, 1594-1597, doi:10.3201/eid1110.050513 (2005).

20 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

21 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

22 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

23 Schountz, T. *et al.* Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **11**, doi:10.3390/v11030291 (2019).

24 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

25 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

26 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

27 Maes, P. *et al.* Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. *Archives of virology* **163**, 2295-2310, doi:10.1007/s00705-018-3843-5 (2018).

28 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annual review of virology* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

29 Ching, P. K. *et al.* Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis* **21**, 328-331, doi:10.3201/eid2102.141433 (2015).

30 Playford, E. G. *et al.* Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis* **16**, 219-223, doi:10.3201/eid1602.090552 (2010).

31 Wong, K. T., Shieh, W. J., Zaki, S. R. & Tan, C. T. Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer seminars in immunopathology* **24**, 215-228, doi:10.1007/s00281-002-0106-y (2002).

32 Goh, K. J. *et al.* Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *The New England journal of medicine* **342**, 1229-1235, doi:10.1056/NEJM200004273421701 (2000).

33 O'Sullivan, J. D. *et al.* Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* **349**, 93-95, doi:10.1016/s0140-6736(96)06162-4 (1997).

34 Paton, N. I. *et al.* Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* **354**, 1253-1256, doi:10.1016/S0140-6736(99)04379-2 (1999).

35 Hossain, M. J. *et al.* Clinical presentation of nipah virus infection in Bangladesh. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **46**, 977-984, doi:10.1086/529147 (2008).

36 Wong, K. T. *et al.* Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *The American journal of pathology* **161**, 2153-2167, doi:10.1016/S0002-9440(10)64493-8 (2002).

37 Wong, K. T. *et al.* Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathology and applied neurobiology* **35**, 296-305, doi:10.1111/j.1365-2990.2008.00991.x (2009).

38 Tan, C. T. *et al.* Relapsed and late-onset Nipah encephalitis. *Annals of neurology* **51**, 703-708, doi:10.1002/ana.10212 (2002).

39 Abdullah, S., Chang, L. Y., Rahmat, K., Goh, K. T. & Tan, C. T. Late-onset Nipah virus encephalitis 11 years after the initial outbreak: A case report. *Neurology Asia* **17**, 71-74 (2012).

40 Hammoud, D. A. *et al.* Aerosol exposure to intermediate size Nipah virus particles induces neurological disease in African green monkeys. *PLoS neglected tropical diseases* **12**, e0006978, doi:10.1371/journal.pntd.0006978 (2018).

41 Geisbert, J. B. *et al.* An Intranasal Exposure Model of Lethal Nipah Virus Infection in African Green Monkeys. *The Journal of infectious diseases* **221**, S414-S418, doi:10.1093/infdis/jiz391 (2020).

42 Prasad, A. N. *et al.* A Lethal Aerosol Exposure Model of Nipah Virus Strain Bangladesh in African Green Monkeys. *The Journal of infectious diseases* **221**, S431-S435, doi:10.1093/infdis/jiz469 (2020).

43 Mehand, M. S., Al-Shorbaji, F., Millett, P. & Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research* **159**, 63-67, doi:10.1016/j.antiviral.2018.09.009 (2018).

44 Luby, S. P. The pandemic potential of Nipah virus. *Antiviral research* **100**, 38-43, doi:10.1016/j.antiviral.2013.07.011 (2013).

45 Broder, C. C. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. *Current opinion in virology* **2**, 176-187, doi:10.1016/j.coviro.2012.02.016 (2012).

46 Azarm, K. D. & Lee, B. Differential Features of Fusion Activation within the Paramyxoviridae. *Viruses* **12**, doi:10.3390/v12020161 (2020).

47 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).

48 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

49 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

50 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

51 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

52 Harmsen, M. M. & De Haard, H. J. Properties, production, and applications of camelid single-domain antibody fragments. *Applied microbiology and biotechnology* **77**, 13-22, doi:10.1007/s00253-007-1142-2 (2007).

53 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

54 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

55 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

56 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell reports* **36**, 109628, doi:10.1016/j.celrep.2021.109628 (2021).

57 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493, doi:10.1093/nar/gkaa216 (2020).

58 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

59 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

60 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

61 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

62 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

63 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

64 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

65 Jordan, P. C. *et al.* Initiation, extension, and termination of RNA synthesis by a paramyxovirus polymerase. *PLoS Pathog* **14**, e1006889, doi:10.1371/journal.ppat.1006889 (2018).

66 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Analytical Biochemistry* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

67 Schafer, A. *et al.* Evidence for distinct mechanisms of small molecule inhibitors of filovirus entry. *PLoS pathogens* **17**, e1009312, doi:10.1371/journal.ppat.1009312 (2021).

68 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *J Struct Biol* **166**, 95-102 (2009).

69 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

70 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

71 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

72 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).

73 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).

74 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).

75 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).

76 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).

77 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).

78 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).

79 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).

80 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).

81 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

82 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

83 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

84 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).

85 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

86 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).

87 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).

88 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).

89 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).

90 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).

91 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

92 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

93 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

94 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

95 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

96 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).

97 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).

98 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).

99 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).

100 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).

101 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).

102 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).

103 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).

104 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).

105 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).

106 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).

107 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).

108 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).

109 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).

110 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

111 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

112 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).

113 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).

114 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).

115 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).

116 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).

117 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).

118 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).

119 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).

120 de Wit, E. & Munster, V. J. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *The Journal of pathology* **235**, 196-205, doi:10.1002/path.4444 (2015).

121 Baseler, L. *et al.* Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. *PLoS neglected tropical diseases* **10**, e0005120, doi:10.1371/journal.pntd.0005120 (2016).

122 Wong, K. T. *et al.* A golden hamster model for human acute Nipah virus infection. *The American journal of pathology* **163**, 2127-2137, doi:10.1016/S0002-9440(10)63569-9 (2003).

123 Rockx, B. *et al.* Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *Journal of virology* **85**, 7658-7671, doi:10.1128/JVI.00473-11 (2011).

124 Cong, Y. *et al.* Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS neglected tropical diseases* **11**, e0005532, doi:10.1371/journal.pntd.0005532 (2017).

The pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV) cause severe respiratory illness and encephalitis in both animals and humans with human case fatality rates ranging from 40-100%. The NiV-Bangladesh strain (NiV-B) is associated with multiple rounds of human-to-human transmission and is a pathogen of pandemic concern for which there are no vaccines or antivirals approved for human use. We have recently shown that the nonpathogenic Cedar henipavirus (CedV) is a useful surrogate platform for high throughput screening (HTS) for antiviral drug discovery, and for virus neutralization assays. This proposal focuses on developing antiviral small molecule and nanobody countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach. We created reporter gene-encoding recombinant CedVs and discovered a series of fused heterocycles, e.g., 4-aminoquinazoline-based, antivirals, and showed that a representative quinazoline **CBS1435** was an effective inhibitor of rCedV replication and also both NiV-B and HeV, with EC_{50} values in the low μM range and minimal cytotoxicity. Neutralizing antibodies to the henipavirus F and G glycoproteins have also shown therapeutic promise, but antiviral nanobodies as potential immunotherapeutics represent a novel area of NiV and HeV countermeasure development. This proposal will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Aim 1 will focus on optimizing lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. Viral polymerase-targeting l~~L~~ead molecules will be selected using NiV-B and HeV assays and~~,~~ mechanism of action (MOA) studies, and best-in-class drugs will be developed by chemical modifications aimed to improve drug-like properties, increase potencies, decrease toxicity, and improve pharmacokinetic properties. Aim 2 will isolate and fully characterize nanobodies broadly neutralizing NiV-B and HeV derived by immunization of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using phage display technology of cloned nanobody gene fragments. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails. Aim 3 will focus on *in vivo* efficacy evaluation of lead compounds and nanobodies in animal models. Both monotherapies and combination therapeutic modalities will be tested, along with testing antiviral nanobody delivery by aerosol. These two unique henipavirus-targeted antiviral development directions will generate novel countermeasures that may display more broad effectiveness.

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] Twenty-two cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antiviral agents approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus that is nonpathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will ~~potentially~~ yield testable antiviral agents with potential pan-henipavirus effectiveness. Our collaborative team also has extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both viral polymerase-targeting small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays and SPR binding assay for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and later, best-in-class nanobodies will be also tested by aerosol delivery. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobody studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

For several decades, the major agents of emerged or newly identified infectious diseases in humans have been viruses of zoonotic origins.[11-13] Among these, two paramyxoviruses of importance are Nipah virus (NiV) and Hendra virus (HeV); the prototype species of the genus *Henipavirus* and are single-stranded, negative sense, enveloped RNA viruses.[14] HeV appeared in 1994, in an outbreak of severe respiratory disease in horses in Australia, with the death of 14 horses and their trainer.[15,16] NiV (Malaysia strain, NiV-M) appeared in an outbreak of encephalitis in pig farmers in Malaysia in 1998.[17,18] NiV outbreaks have since been seen in Bangladesh and India by a related strain NiV-Bangladesh (NiV-B).[19] Pteropid fruit bats are the major reservoir hosts for henipaviruses.[6,20] NiV and HeV have broad host tropism, infecting at least 18 species across 6 orders of mammals. Among these, NiV and HeV will cause a systemic and often fatal respiratory and/or neurological disease in at least 11 species including humans, with human fatality rates ranging from 40-100%.[2,21] A third henipavirus isolate, Cedar virus (CedV), was discovered in 2010.[22] However, CedV is not known to be zoonotic and has been shown to be nonpathogenic in HeV and NiV animal models.[22-24] Two additional henipaviruses are also known, Ghana bat virus and Mojiang virus, but only from sequence data.[25-27] Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and 3 outbreaks in India.[28] In 2014, an outbreak of NiV-M occurred in the Philippines with 9 fatalities of 11 human cases, with horse-to-human and human-to-human transmission.[29] The combined NiV case fatality rate is ~60% across a large region of Asia in five countries.[3] The recent 2018 NiV outbreak in Kerala, India was significant, occurring in a new geographic region, with a case fatality rate of 91% and 22 of 23 cases the result of from human-to-human transmission, underscoring both the transmissibility and the lethal nature of this virus.[4]

Human NiV/HeV infection is generally accepted to occur via the oronasal route with an incubation period of 1 to 2 weeks.[4,30] Acute NiV/HeV infection in people is systemic and likely via hematogenous viral spread from the respiratory system.[31] Disease onset is characterized by fever, myalgia, shortness of breath, and cough.[16,32] Human HeV infections have resulted in both fatal respiratory or encephalitic disease, and also recovery.[30,33] The predominant clinical feature in the Malaysia outbreak was encephalitis, but respiratory symptoms were also common.[32,34] The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease and in the 2018 NiV-B outbreak in Kerala, 83% of cases had acute respiratory distress syndrome (ARDS).[4,35] The key findings of human NiV/HeV infection are wide-spread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected.[36,37] Human NiV/HeV infection can also be prolonged following apparent recovery, and some patients can experience a late-onset or relapsed encephalitis.[33,38] Relapsed encephalitis caused by NiV appears to be a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later,[39] NiV and HeV are biosafety level-4 (BSL-4) viruses and classified as Category C priority pathogens and transboundary agents by several US agencies. Also, both NiV-M and NiV-B can cause lethal infection in nonhuman primates (NHPs), the African green monkey (AGM), when delivered by small particle aerosol.[40-42] NiV and henipaviral diseases (HeV) are included on the WHO's Blueprint List of Priority Pathogens.[43] NiV is a potential pandemic threat because of its high case-fatality rates, capacity for human-to-human transmission and potential to mutate, and a human-adapted strain in a region of high population density, could lead to rapid spread.[44] ***There are presently no approved vaccines or therapeutic modalities for NiV/HeV infections in people.***

The viral RNA-dependent-RNA polymerases of paramyxoviruses are attractive targets for antiviral compound discovery, but antiviral drug discovery for NiV and HeV has been hampered by requirements of BSL-4 containment. We recently developed and optimized an authentic henipavirus replication cell-based system using a recombinant CedV (rCedV) encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications at BSL-2 and identified several inhibitory compounds that were further validated with authentic NiV-B and HeV.[7] Those leads provide the rationale for the current application. Our ability to employ rCedV provides an opportunity for anti-henipavirus drug discovery not previously available, that also allows for discovery across the whole replication cycle, not restricted to only the viral polymerase. Another well-exploited stage of virus infection is the attachment and entry process.[45] NiV/HeV possess two membrane anchored glycoproteins required for infection; one for attachment, the G glycoprotein, and the other for membrane fusion the F glycoprotein.[46] The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and all vaccine strategies.[28] Although an effective post-exposure human monoclonal antibody (mAb) therapy targeting the NiV and HeV G glycoprotein (m102.4) has been extensively examined,[47-51] its delivery must be within several days following infection in NHPs. An unexplored alternative but related antibody-based strategy are antiviral nanobodies. A nanobody, is an antibody fragment comprised of just a single monomeric variable antibody domain.[52] Nanobodies were first engineered from variable heavy chain domains of camelid heavy chain

antibodies (VHHs), and camelid VHHs retain full antigen specificity and are only a fraction of the size of a human antibody, and they have extended complementarity determining regions (CDRs) which can allow them to bind epitopes not normally accessible to conventional antibodies, such as conserved viral epitopes often masked by glycan shields.[10] Our expertise in henipavirus F and G structural and functional analyses together with anti-F and G mAb development and therapeutic application, will facilitate the development and testing of a new and understudied area of nanobody-based immunotherapeutic ~~for~~ against NiV and HeV.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of the investigators in this proposal. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may well have activity across related members of the henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of a high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification. Finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole NiV or HeV immune sera shows cross-reactivity with CedV, however, such sera or even specific high-titer anti-CedV F or G antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins.[22] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and the largest polymerase in the order *Mononegavirale*, yet it shares high aa identity to NiV and HeV L proteins. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; aa identities of L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This overall high degree of identity, shared between CedV and that of NiV and HeV, suggests that antivirals that target the L polymerase, could be identified and ~~perhaps~~ also offer the possibility of identifying pan-henipavirus countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] Several small molecule inhibitors have been validated *in vitro* using infectious HeV and NiV, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support **Aim 1** to optimize our current best-in-class leads and also screen large libraries of compounds.

Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[53-55] Nanobodies can be isolated using the same phage library construction methods and phage panning procedures routinely employed for mAb discovery, which allows for *in vitro* production of various amounts and their rapid characterization. Nanobody development will be carried out by **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels.[10] Immunized mouse cohorts are in progress using recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in **Aim 2.**

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of **Aim 3**. In addition, we expect to discover, and

characterize batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in **Aim 3**. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet, Irina Gaisina, and Terry Moore, UIC and Chicago BioSolutions Inc.** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule inhibitors of henipaviruses.

C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2), cell-based HTS platform to identify small molecule antiviral agents targeting henipavirus replication without the requirement for BSL-4 containment.[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with that of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera use guidelines and procedures at the BSL-2 level were reviewed and approved by the USU, Institutional Biosafety Committee (IBC) in accordance with NIH guidelines. These replication competent rCedV chimeras create a very rapid and robust surrogate infection assay for NiV_B and HeV that can be used as a neutralization or entry inhibitor assay to measure neutralizing antibodies, or as a surrogate for generating virus escape mutants to neutralizing antibodies. Here, the F and G genes of NiV_B (2010 Faridpur isolate) and HeV (2008 Redlands isolate) were synthesized and inserted into our pOLTV5-rCedV anti-genome plasmid. For the HTS neutralization assay, a turbo-GFP gene was inserted between the CedV P and M genes. Each chimera (rCedV-NiV_B-GFP and rCedV-HeV-GFP) were rescued as previously described.[8] Other versions without reporter genes have also been prepared. All virus stocks are cloned and genomes confirmed by NGS. Typically, large stocks are prepared and frozen as single use aliquots. Show in **Figure 1** is an example of how these chimeras are used in a virus neutralization test, referred to as a fluorescent reduction neutralization test (FRNT) using two human NiV/HeV cross-reactive anti-G mAbs (HENV-103 and -117) as previously described.[56] Briefly, Vero 76 cells are used in black walled clear bottom 96-well plates, mAbs are serially diluted in medium, and equal volumes of medium containing rCedV-NiV-B or rCedV-HeV are added to each mAb dilution for a final concentration of 2000 PFU, incubated for 2 h at 37 °C. Each virus-mAb mixture (90 µL/well) is added to the pre-seeded Vero 76 cells in triplicate and the assay run for just another 24 h followed by 4% formaldehyde fixation, washing and then scanning using a CTL S6 analyzer. Fluorescent foci are counted using the CTL Basic Count™ and an IC_{50} value is determined

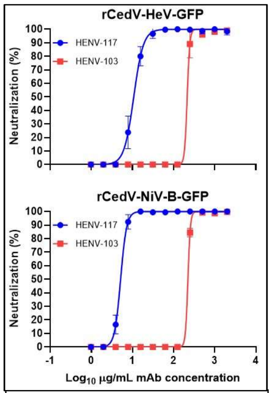


Figure 1. Neutralization curves for HENV-117 and HENV-103 mAbs against rCedV-HeV-GFP (top) and rCedV-NiV-B-GFP (bottom). Data are from a single experiment performed in technical triplicate.

IC50 (ng/mL)	HeV	rCedV-HeV	NiVB	rCedV-NiVB
HENV-103	445	216	507	238
HENV-117	14	11	15	6

Table 1. IC_{50} values of cross-reactive anti-G human mAbs HENV-103 and -117) as determined by rCedV chimera FRNT assay and NiV-B and HeV conventional PRNT assay.

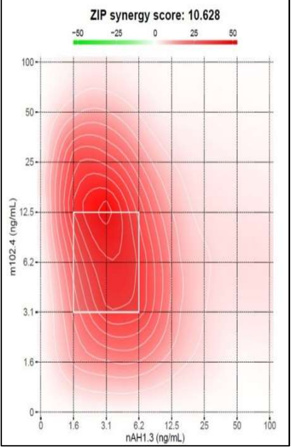


Figure 2. Synergy map with calculated synergy score using the zero interactions potency (ZIP) model generated by SynergyFinder for neutralization of rCedV-NiV-B-GFP by serially diluted m102.4 and nAH1.3 mAbs.

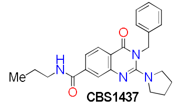
as the antibody concentration yielding 50% reduction in fluorescent foci versus control wells. **Table 1** shows the calculated IC$_{50}$ values using rCedV chimeras vs. those determined by a conventional PRNT assay with authentic NiV-B and HeV, and they are remarkably within a 2-fold range.

In addition to conventional single agent neutralization tests, the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras can also be used in rapid HTS assays to measure synergistic activities of inhibitors, such as antibodies or drugs. The synergistic neutralizing activity of the HENV-103 and HENV-117 mAbs were described utilizing the rCedV chimera assay.[56] Shown in **Figure 2** is a neutralization synergy assay conducted with mAbs m102.4 and nAH1.3 (both NiV/HeV cross-reactive anti-G mAbs) where they are individually diluted 2-fold in a 7x7 concentration matrix. The diluted mAbs are added 1:1 to 4000 PFU per well of rCedV-NiV-B-GFP and the assay essentially carried out as above, with percent neutralization calculated by normalizing foci to a virus only control. Matrices are imported into SynergyFinder and analyzed Neutralization synergy was calculated by comparing treatment to virus only control wells. Values were imported into SynergyFinder software[57] using a Zero Interactions Potency (ZIP) statistical model; delta scores >10 indicate likely synergy as shown here.

Antiviral evaluation and combination testing of small molecules and nanobodies. These rCedV-based tools and rCedV chimera viruses will allow for rapid antiviral activity assessment and an ability to measure synergistic antiviral activities for both small molecule inhibitors derived from **Aim 1** and nanobodies derived from **Aim 2**; and will also allow the novel approach of testing small molecules and nanobody combinations in a rapid and quantitative manner, *in vitro*, to select best-in-class candidates for both monotherapeutic and combination therapeutic modalities prior to *in vivo* animal model testing.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called "SMART Set") was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 µM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 µM, and 47 hits showed only minimal cytotoxicity at 33 µM.

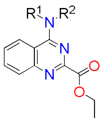
Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.



CBS1437

CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC$_{50}$ (µM)	CC$_{50}$ (µM)	EC$_{50}$ (µM)	CC$_{50}$ (µM)	EC$_{50}$ (µM)
0.70	100	2.1	100	3.3

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.

	N	Compound ID	R^1	R^2	% inhib at 10 µM
	**1**	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
	2	**G577-0016**	H	2,4-difluorophenyl	18
	3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
	4	**G577-0022**	H	3-methoxyphenyl	46
	5	**G577-0028**	Me	cyclohexyl	93
	6	**G577-0084**	H	4-isopropylphenyl	85
	7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
	8	**G577-0112**	Me	2-methylphenyl	94

In **Table 2** is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437**. Note that both HEK293T and Vero cell lines were employed in these assays, and that CC$_{50}$ values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC$_{50}$ value of 3.3 µM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 µM). **Table 3** shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14** (**Figure 4**), bearing an unsubstituted cyclohexyl

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group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent,* ***CBS1437****, as well as specific plans for developing the SARs.*

C2.2.3. Generation of Nipah and Hendra G and F nanobodies. We recently initiated a collaborative project in nanobody development against NiV and HeV, as well as other henipavirus glycoproteins, with Dr. Jianliang Xu (**RAViD, Nanobody Core Lead**). The Xu lab has engineered and established transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, and has published on the power and utility of anti-SARS-CoV-2 nanobodies.[10] We have already initiated the immunizations of several nanomouse cohorts using native-like oligomeric recombinant sF (**Veesler lab**) and sG glycoproteins (**Broder lab**) of NiV-B and HeV. Immune responses are excellent and sera measured by ELISA using the immunogens. The first of these cohorts will be processed in the coming weeks, and the Xu lab will be constructing phage libraries of polyclonal VHHs from each cohort. We expec~~cep~~t to be able to make rapid progress in **Aim 2**, in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

D. RESEARCH DESIGN AND METHODS

D1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC$_{50}$ <1 μM) and low cytotoxicity (CC$_{50}$ >100 μM).

D1.1. Synthetic Chemistry. In **Figure 3** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**G577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as “ADME handles” to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we will optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. We will also optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc.

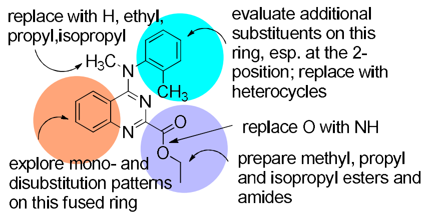
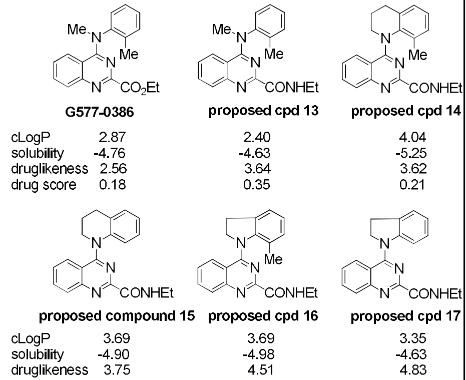


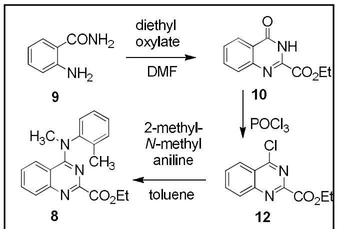
Figure 3. SAR development for 4-aminoquinazolines



	G577-0386	proposed cpd 13	proposed cpd 14
cLogP	2.87	2.40	4.04
solubility	-4.76	-4.63	-5.25
druglikeness	2.56	3.64	3.62
drug score	0.18	0.35	0.21

	proposed compound 15	proposed cpd 16	proposed cpd 17
cLogP	3.69	3.69	3.35
solubility	-4.90	-4.98	-4.63
druglikeness	3.75	4.51	4.83
drug score	0.38	0.23	0.41

Figure 4. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.



Scheme 1. Synthesis of **G577-0112** (cmpd **8**).

In **Scheme 1** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) to produce 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely, 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[58-59] as well as other methods.[60,61] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible.

In **Figure 4** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[62]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112** (compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester with an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scores have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding. In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. We have also researched and found additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

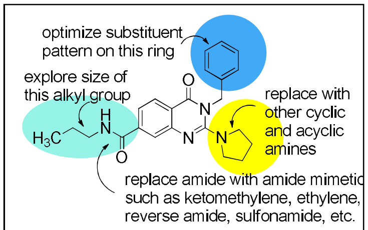


Figure 5. SAR development for **CBS1437**.

D.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5×10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at predetermined nontoxic concentrations for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 μM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

Screening Libraries. The HTS will be performed in the **Rong Lab**.These libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of >150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including an Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions, Inc**. *Hit Selection Algorithm.* A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used ~~again a~~as a specificity control. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (EC_{50} values less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. *Hit Validation.*

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Cytotoxicity Assessment and Ranking. We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" as described previously[97] in a 96-well format. The ratio of CC$_{50}$/EC$_{50}$ is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. *Hit Quality Control and Identity Assurance.* The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds <80% pure will be purified and retested.

D.1.3. Evaluation of antiviral activity of advanced candidates. Inhibitors will be tested against authentic NiV and HeV as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2×10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to parallel control cultures of virus and DMSO. This work will be performed in the BSL-4 facility at UTMB (**Geisbert, Core D**).

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Peet, Gaisina, and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy using a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or other HTS hits identified using the expanded libraries of compounds.

D.2.1. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with medium containing either DMSO or selected inhibitors at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by western blot techniques or viral RNA levels by q-RT-PCR. CedV specific antibodies against G F N and P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene, and performed using the ABI 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus.** ***Target identification for small molecules.*** The rCedV tools used in **Aim 1** will~~can~~ also be used~~exploited~~ for antiviral target identification. *In vitro* selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutants. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[63,64] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a "drug resistant" virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any "drug resistant" virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inhibitors using authentic NiV-B and HeV in BSL-4 by the UTMB BSL-4 Core.

D.2.2. Assess the binding properties by surface plasmon resonance (SPR). The NiV polymerase (L protein and a phosphoprotein P, or RdRp complex) will be prepared using a previously published protocol(**ref**). Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_{D}) to the RdRp protein, and kinetics [association rate constants (k_{a}), and dissociation rate constants (k_{d})] as previously described.[65] ~~Prefusion-stabilized~~ RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 µM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_{D}) will be calculated as $K_{\mathrm{D}} = k_{\mathrm{d}}/k_{\mathrm{a}}$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab (ref)**.

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D.2.3. Structure~~tructure~~ determination of recombinant proteins with bound inhibitors by ~~by X-Ray crystallography and~~ CryoEM. High-resolution structures of the NiV RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compounds to be defined will be obtained using ~~both x-ray crystallography and~~ CryoEM by ~~Dr.~~the **Veesler Lab** at the Arnold and Mabel CryoEM Center at the UW. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands~~.~~ ~~Biophysical techniques such as native mass spectrometry and thermal shift assays, light scattering, and fluorophore analysis to measure protein stability and folding are routine and will be used to ensure that bound compounds are not destabilizing domain structures to prevent crystallization~~. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[66] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including Warp[67], cryoSparc[68], and RELION[69]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed using Rosetta which is developed at the UW. As cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[70-74], to explore the conformational landscape[75] of ligand binding events, and to capture conformational variability of intermediate states[76,77], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches.

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Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds. *Milestone: Designate **advanced lead compounds** exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the **advanced lead compounds**.*

D.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.
Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-T properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out using the CYP3A4, and CYP2D6 Human Cytochrome P450 High Throughput Inhibitor Screening Kits (BD Gentest Corp.) to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[78] Compounds with IC_{50} values ≥100 µM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds by the method of Kuhnz *et al*.[79] Compounds with >30% recovery (1 h) will be considered for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed

in a [^{3}H] dofetilide-binding assay as previously described.[80] using SHSY5Y cells. Compounds that do not prevent [^{3}H]dofetilide binding (IC_{50} >10 μM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method[81] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P_{app}) greater than 1×10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels of the compound up to a solubility-limiting level in the presence or absence of rat liver enzymes (rat S9 liver fraction) acting in the normal process of metabolism and any reverse mutations will be determined. ***In vitro* mammalian chromosomal aberration test**: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes.

D.3.2. PK studies: Naïve male/female mice (equal number, 3 groups of 36 animals total) will be treated with a single dose via i.v., i.p., or oral gavage administration and blood will be collected and processed to obtain plasma and serum from three animals/group/time point at up to 12 time points through 24 h post dose. Two doses will be used to establish the drug exposure-dose range relationship: the estimated efficacious dose (ED), based upon preliminary data at 25 mg/kg i.p. or p.o. and an observable adverse effect dose (OAED) (to be determined). Compounds of interest will be extracted from biological specimens by protein precipitation, analyzed on a triple-quadruple mass spectrometer (LC-MS/MS) and quantified using an internal standard method with a minimum of a five-point calibration curve. PK parameters including AUC, C_0, C_{max}, $T_{1/2}$, T_{max}, and F(%) will be determined. Depending on the estimation of T_{max}, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

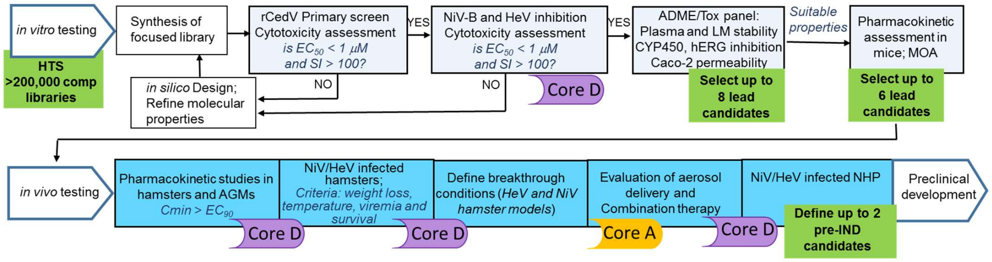

Figure 6. Flowchart for henipavirus inhibitor development. NOT FINAL

Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc. and Reaction Biology, Corp., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim.

We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 6**).

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D.4. AIM 2: Characterization of the potency and breadth of selected nanobodies.

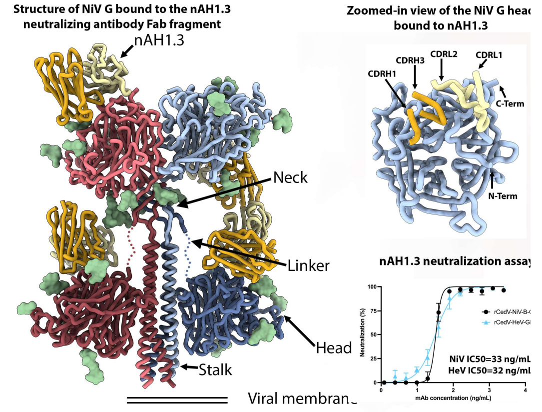
Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. **Aim 1** will also collaborate with the **UTMB, BSL-4 Core D** to evaluate lead candidates with authentic NiV and HeV and also in vivo in **Aim 3**. Batteries of nanobodies to the F and G glycoproteins of NiV and HeV will be isolated from phage libraries constructed using cohorts of nanomice immunized with sF and sG recombinant glycoproteins (**C2.2.3. Preliminary Data).** The Core will conduct preliminary characterizations on binding and specificities and then provide purified nanobody panels to the **Veesler** and **Broder labs** for detailed studies.

D.4.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth.

D.4.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including NiV (Bengladesh and Malaysia strains) and HeV (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[82-84] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with NiV and HeV glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

D.4.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit NiV and HeV, we will use a high-throughput neutralization assay (conducted in the **Broder lab**). This assay is based on GFP-encoding, recombinant replication-competent, Cedar henipavirus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bangladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins (see: C.2.1. Preliminary Data).[7,85] This platform is safe, enabling utilization at BSL-2 compared to BSL-4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the **Broder lab** recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described HeV variant.[86] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond NiV and HeV (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[87,88]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.



A) Ribbon diagram of the NiV G ectodomain bound to the broadly neutralizing nAH1.3 Fab fragment in two orthogonal orientations. Each of the four NiV G protomers is colored distinctly and resolved N-linked glycans are rendered as green surfaces. The nAH1.3 heavy and light chains are colored gold and yellow, respectively, and only the variable domains were modeled in density. The linkers connecting the neck to the two proximal head domains are shown as dashed lines due to weaker density in the cryoEM reconstruction. (**B**) Ribbon diagram of a NiV G head domain (blue) with the interacting nAH1.3 heavy and light chains CDRs rendered in gold and yellow. (**C**) nAH1.3-mediated neutralization of rCedV-NiV-B-GFP (black) and rCedV-HeV-GFP (blue).

Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against NiV and HeV. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.4.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition.

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The **Veesler lab** is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The **Veesler lab** has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software, which runs 24 hours per day and enables automated targeting and image acquisition, while keeping a relational database of all micrographs to expedite subsequent processing.[89] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[66,67] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the

epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[82,84,90-92] mechanical stage tilting with gold grids[93,94] or detergents to modify surface tension[95]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[93,96-100] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[101-103]
The NiV attachment (G) glycoprotein engages host receptors, and is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the NiV G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3), which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

D.4.2.1. High-resolution structural studies using X-ray crystallography. Some nanobodies might bind to regions that are intrinsically flexible, thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[104] and BUSTER-TNT.[105]

D.4.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[82-84,91,106,107]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the formulation of cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[108-110] and high-resolution noise substitution prior to Fourier shell correlation calculations[111] to avoid overfitting. Atomic models will be assessed using Molprobity,[112] EMringer[113] and Privateer.[114] The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails.

D.4.3. AIM 2C. Structure-guided formulation of nanobody cocktails.

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[115-117] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected *in vivo*, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting, which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in **Aim 2A**. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.5. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with the ABSL-3 and ABSL-4 Core (**Core D**) to evaluate lead candidate drugs and nanobodies *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster and AGM.[2,118] PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV$_B$ and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV$_B$ and HeV infected AGM models.

D.5.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed by the **UTMB Core (Core D)**. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC$_{90}$ in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of henipavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.5.2. *In vivo* testing of lead compounds NiV/HeV in infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 pfu of NiV$_B$ or HeV.[85,119-121] Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.5.3. Define breakthrough conditions for anti-henipavirus antiviral agents in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antiviral agents fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^6 PFU of NiV or HeV.[85,119-121] Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.5.4. Combination therapy of anti-henipavirus antiviral agents in hamsters. This subaim will employ the most promising antiviral agents from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^6 PFU of NiV or HeV.[85,119-121]Treatment with at least two different antiviral agents will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

D.5.5. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ 5×10^6 PFU of NiV_B or HeV as determined in henipavirus dose confirmation studies conducted by the ABSL-3 and ABSL-4 Core. Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by the **Aerosol Delivery Core** (see **Core A**) at various times after henipavirus infection.

D.5.6. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the LMA mucosal atomization device (MAD) as previously described[41] or by small particle aerosol as previously described[42,122] with a uniformly lethal challenge dose (~ 20,000 PFU of NiV or HeV). Importantly, the MAD system delivers atomized particles that range in size from 30 to 100 µm, which is highly consistent with the size of droplets exhaled by humans when coughing. Previous studies with AGMs infected with NiV_B using the MAD system resulted in lethality in 9/9 animals and a disease course that is highly consistent with human infection (TW Geisbert, C Broder, unpublished data). The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by the **Aerosol Delivery Core** (see **Core A**) up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

Potential Pitfalls and Alternative Strategies. It is possible that **Aerosol Delivery Core (Core A)** may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antiviral agents may not improve protective efficacy or extend the therapeutic window of the agents. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and mAbs to protect animals against lethal henipavirus disease to de-risk this Aim.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues, i.e., antiviral drugs will be coded so that researchers will not know which compounds were given to which animals until experiments and analysis are completed. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the emergence and re-emergence of NiV infections throughout the world, coupled with its high fatality rate, provide very significant motivation for the preparation of small molecule inhibitors of this virus to create a readiness for the next NiV or other related pathogenic henipavirus outbreaks. We have discovered two differentiated series of compounds that show early promise as agents that have potential for treating NiV infections, and we have designed a series of optimized druglike candidates for future preparation and development.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Current topics in microbiology and immunology* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *The New England journal of medicine* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *The Journal of infectious diseases* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral research* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Taylor, L. H., Latham, S. M. & Woolhouse, M. E. Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **356**, 983-989, doi:10.1098/rstb.2001.0888 (2001).

12 Desselberger, U. Emerging and re-emerging infectious diseases. *The Journal of infection* **40**, 3-15, doi:10.1053/jinf.1999.0624 (2000).

13 van Doorn, H. R. The epidemiology of emerging infectious diseases and pandemics. *Medicine* **49**, 659-662, doi:10.1016/j.mpmed.2021.07.011 (2021).

14 Basu, A. *et al.* New small molecule entry inhibitors targeting hemagglutinin-mediated influenza a virus fusion. *Journal of virology* **88**, 1447-1460, doi:10.1128/JVI.01225-13 (2014).

15 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

16 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *The Medical journal of Australia* **162**, 642-645 (1995).

17 Chua, K. B. *et al.* Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**, 1432-1435, doi:10.1126/science.288.5470.1432 (2000).

18 Chua, K. B. *et al.* Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* **354**, 1257-1259, doi:10.1016/S0140-6736(99)04299-3 (1999).

19 Harcourt, B. H. *et al.* Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis* **11**, 1594-1597, doi:10.3201/eid1110.050513 (2005).

20 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

21 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

22 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

23 Schountz, T. *et al.* Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters. *Viruses* **11**, doi:10.3390/v11030291 (2019).

24 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

25 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).
26 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).
27 Maes, P. *et al.* Taxonomy of the family Arenaviridae and the order Bunyavirales: update 2018. *Archives of virology* **163**, 2295-2310, doi:10.1007/s00705-018-3843-5 (2018).
28 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annual review of virology* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).
29 Ching, P. K. *et al.* Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis* **21**, 328-331, doi:10.3201/eid2102.141433 (2015).
30 Playford, E. G. *et al.* Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis* **16**, 219-223, doi:10.3201/eid1602.090552 (2010).
31 Wong, K. T., Shieh, W. J., Zaki, S. R. & Tan, C. T. Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer seminars in immunopathology* **24**, 215-228, doi:10.1007/s00281-002-0106-y (2002).
32 Goh, K. J. *et al.* Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *The New England journal of medicine* **342**, 1229-1235, doi:10.1056/NEJM200004273421701 (2000).
33 O'Sullivan, J. D. *et al.* Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* **349**, 93-95, doi:10.1016/s0140-6736(96)06162-4 (1997).
34 Paton, N. I. *et al.* Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet* **354**, 1253-1256, doi:10.1016/S0140-6736(99)04379-2 (1999).
35 Hossain, M. J. *et al.* Clinical presentation of nipah virus infection in Bangladesh. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **46**, 977-984, doi:10.1086/529147 (2008).
36 Wong, K. T. *et al.* Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *The American journal of pathology* **161**, 2153-2167, doi:10.1016/S0002-9440(10)64493-8 (2002).
37 Wong, K. T. *et al.* Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathology and applied neurobiology* **35**, 296-305, doi:10.1111/j.1365-2990.2008.00991.x (2009).
38 Tan, C. T. *et al.* Relapsed and late-onset Nipah encephalitis. *Annals of neurology* **51**, 703-708, doi:10.1002/ana.10212 (2002).
39 Abdullah, S., Chang, L. Y., Rahmat, K., Goh, K. T. & Tan, C. T. Late-onset Nipah virus encephalitis 11 years after

the initial outbreak: A case report. *Neurology Asia* **17**, 71-74 (2012).
40 Hammoud, D. A. *et al.* Aerosol exposure to intermediate size Nipah virus particles induces neurological disease in African green monkeys. *PLoS neglected tropical diseases* **12**, e0006978, doi:10.1371/journal.pntd.0006978 (2018).
41 Geisbert, J. B. *et al.* An Intranasal Exposure Model of Lethal Nipah Virus Infection in African Green Monkeys. *The Journal of infectious diseases* **221**, S414-S418, doi:10.1093/infdis/jiz391 (2020).
42 Prasad, A. N. *et al.* A Lethal Aerosol Exposure Model of Nipah Virus Strain Bangladesh in African Green Monkeys. *The Journal of infectious diseases* **221**, S431-S435, doi:10.1093/infdis/jiz469 (2020).
43 Mehand, M. S., Al-Shorbaji, F., Millett, P. & Murgue, B. The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts. *Antiviral research* **159**, 63-67, doi:10.1016/j.antiviral.2018.09.009 (2018).
44 Luby, S. P. The pandemic potential of Nipah virus. *Antiviral research* **100**, 38-43, doi:10.1016/j.antiviral.2013.07.011 (2013).
45 Broder, C. C. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. *Current opinion in virology* **2**, 176-187, doi:10.1016/j.coviro.2012.02.016 (2012).
46 Azarm, K. D. & Lee, B. Differential Features of Fusion Activation within the Paramyxoviridae. *Viruses* **12**, doi:10.3390/v12020161 (2020).
47 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).
48 Geisbert, T. W. *et al*. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

49 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

50 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

51 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

52 Harmsen, M. M. & De Haard, H. J. Properties, production, and applications of camelid single-domain antibody fragments. *Applied microbiology and biotechnology* **77**, 13-22, doi:10.1007/s00253-007-1142-2 (2007).

53 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

54 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

55 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

56 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell reports* **36**, 109628, doi:10.1016/j.celrep.2021.109628 (2021).

57 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493, doi:10.1093/nar/gkaa216 (2020).

58 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

59 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

60 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

61 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

62 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

63 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

64 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

65 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Analytical Biochemistry* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

66 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *J Struct Biol* **166**, 95-102 (2009).

67 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

68 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

69 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

70 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).

71 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).

72 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).

73 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).

74 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).

75 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).

76 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).

77 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).

78 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).

79 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

80 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

81 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

82 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).

83 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

84 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).

85 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).

86 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).

87 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).

88 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).

89 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

90 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

91 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

92 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

93 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

94 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).
95 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).
96 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).
97 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).
98 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).
99 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).
100 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).
101 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).
102 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).
103 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).
104 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).
105 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).
106 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).
107 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).
108 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).
109 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).
110 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).
111 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).
112 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).
113 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).
114 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).
115 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).
116 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).
117 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).
118 de Wit, E. & Munster, V. J. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *The Journal of pathology* **235**, 196-205, doi:10.1002/path.4444 (2015).

119 Baseler, L. *et al.* Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. *PLoS neglected tropical diseases* **10**, e0005120, doi:10.1371/journal.pntd.0005120 (2016).

120 Wong, K. T. *et al.* A golden hamster model for human acute Nipah virus infection. *The American journal of pathology* **163**, 2127-2137, doi:10.1016/S0002-9440(10)63569-9 (2003).

121 Rockx, B. *et al.* Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *Journal of virology* **85**, 7658-7671, doi:10.1128/JVI.00473-11 (2011).

122 Cong, Y. *et al.* Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS neglected tropical diseases* **11**, e0005532, doi:10.1371/journal.pntd.0005532 (2017).

The pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV) cause severe respiratory illness and encephalitis in both animals and humans with human case fatality rates ranging from 40-100%. The NiV-Bangladesh strain (NiV-B) is associated with multiple rounds of human-to-human transmission and is a pathogen of pandemic concern for which there are no vaccines or antivirals approved for human use. We have recently shown that the nonpathogenic Cedar henipavirus (CedV) is a useful surrogate platform for high throughput screening (HTS) for antiviral drug discovery, and for virus neutralization assays. This proposal focuses on developing antiviral small molecule and nanobody countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach. We created reporter gene-encoding recombinant CedVs and discovered a series of fused heterocycles, e.g., 4-aminoquinazoline-based, antivirals, and showed that a representative quinazoline **CBS1435** was an effective inhibitor of rCedV replication and also both NiV-B and HeV, with EC_{50} values in the low µM range and minimal cytotoxicity. Neutralizing antibodies to the henipavirus F and G glycoproteins have also shown therapeutic promise, but antiviral nanobodies as potential immunotherapeutics represent a novel area of NiV and HeV countermeasure development. This proposal will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Aim 1 will focus on optimizing lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. Lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed by chemical modifications aimed to improve drug-like properties, increase potencies, decrease toxicity, and improve pharmacokinetic properties. Aim 2 will isolate and fully characterize nanobodies broadly neutralizing NiV-B and HeV derived by immunization of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using phage display technology of cloned nanobody gene fragments. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails. Aim 3 will focus on *in vivo* efficacy evaluation of lead compounds and nanobodies in animal models. Both monotherapies and combination therapeutic modalities will be tested, along with testing antiviral nanobody delivery by aerosol. These two unique henipavirus-targeted antiviral development directions will generate novel countermeasures that may display more broad effectiveness.

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] Twenty-two cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antiviral agents approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus that is nonpathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will potentially yield testable antiviral agents with potential pan-henipavirus effectiveness. Our collaborative team also has extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and later, best-in-class nanobodies will be also tested by aerosol delivery. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobody studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

Over the past 40 years the major etiological agents of emerged or identified infectious diseases in humans have been viruses.[11] Among these, the paramyxoviruses, which include many important human and animal pathogens, also include two emerged, zoonotic viral pathogens of importance: the henipaviruses; Hendra virus (HeV) and Nipah virus (NiV).[12,13] In 1994, HeV caused an outbreak of severe respiratory disease in horses in Brisbane, Australia, resulting in the deaths of 14 horses and their trainer.[14,15] NiV was the causative agent of a significant outbreak of severe encephalitis in pig farmers in Malaysia in 1998. Spread of NiV infections typically requires direct contact with an infected source, and transmission can occur between people and from animals to people. Symptoms may culminate in a severe respiratory and/or neurological disease that is often fatal. Complications of the infection can result in relapsed encephalitis following recovery. On September 5, 2021, a twelve-year-old boy in the Indian state of Kerala died after contracting NiV infection. During the process of transporting him to medical centers for a diagnosis, twenty-three health care workers exposed to this boy also died, which underscores both the transmissability and the lethal nature of this virus. *There is presently no vaccine or small molecule therapeutics that have been developed for NiV infections.* Pteropid bats appear to be the major reservoir hosts for henipaviruses.[6,16] HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bat hosts. Among these animals, HeV and NiV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with human fatality rates ranging from 40-100%.[2,17]

NiV and HeV are classified as biosafety level-4 (BSL-4) restricted pathogens and both remain transboundary threats to livestock and people throughout South Asia and Australia. NiV and henipaviral diseases have been categorized by the World Health Organization (WHO) as an epidemic threat needing urgent research and countermeasure development and are included in the WHO R&D Blueprint list of priority pathogens with epidemic potential.[18] Presently, there are no prophylactic or therapeutic treatment options for henipavirus infections approved for use in humans. A subunit vaccine, Equivac® HeV, has been available for use in horses in Australia since 2012[19] for the prevention of HeV infection. This equine vaccine received full registration by the Australian Pesticides and Veterinary Medicines Authority in 2015, with all vaccinated horses receiving a microchip with a database being maintained and is the first commercially deployed vaccine against a BSL-4 agent.[20] Although a human monoclonal antibody (mAb) m102.4 has successfully completed a phase I safety trial in Australia,[21] developing vaccines or therapeutics against henipaviruses for use in people remains a high priority. Henipaviruses are single-stranded, negative sense, enveloped RNA viruses with two membrane anchored glycoproteins involved in virus entry; one mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and also the main focus of several vaccine strategies.[2] Although an effective post-exposure human monoclonal antibody (mAb) therapy (anti-NiV/HeV G glycoprotein mAb m102.4) has been extensively examined,[22-26] it must be administered within several days following lethal infection in animal challenge models. In contrast, however, antiviral drug discovery for HeV and NiV has been significantly hampered due to requirements of biosafety level-4 (BSL-4) containment. Recently, a third isolate, namely, Cedar virus (CedV) has been added to the *Henipavirus* genus.[27] However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease and resides in nature in the same bat host species as HeV.[27,28] Nucleic acid based detection studies have identified related henipavirus species, including complete genomic sequences,[29,30] but HeV, NiV, and CedV are the only virus isolates reported.

We have recently optimized a recombinant CedV encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications and have identified several inhibitory compounds that were further validated with the infectious NiV-Bangladesh (NiV-B) strain. The validated compounds provide the foundation for the current application.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of the investigators in this proposal. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may well have activity across related members of the henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice

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that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of a high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification. Finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole NiV or HeV immune sera shows cross-reactivity with CedV, however, such sera or even specific high-titer anti-CedV F or G antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins. [27] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and the largest polymerase in the order *Mononegavirale*, yet it shares high aa identity to NiV and HeV L proteins. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; aa identities of L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This overall high degree of identity, shared between CedV and that of NiV and HeV, suggests that antivirals that target the L polymerase, could be identified and perhaps also offer the possibility of identifying pan-henipavirus countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] Several small molecule inhibitors have been validated *in vitro* using infectious HeV and NiV, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support **Aim 1** to optimize our current best-in-class leads and also screen large libraries of compounds.

Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[31-33] Nanobodies can be isolated using the same phage library construction methods and phage panning procedures routinely employed for mAb discovery, which allows for *in vitro* production of various amounts and their rapid characterization. Nanobody development will be carried out by **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels.[10] Immunized mouse cohorts are in progress using recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in **Aim 2.**

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of **Aim 3**. In addition, we expect to discover, and characterize batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in **Aim 3**. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet, Irina Gaisina, and Terry Moore, UIC and Chicago BioSolutions Inc.** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule inhibitors of henipaviruses.

C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2), cell-based HTS platform to identify small molecule antiviral agents targeting henipavirus replication without the requirement for BSL-4 containment.[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with that of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera use guidelines and procedures at the BSL-2 level were reviewed and approved by the USU, Institutional Biosafety Committee (IBC) in accordance with NIH guidelines. These replication competent rCedV chimeras create a very rapid and robust surrogate infection assay for NiV$_B$ and HeV that can be used as a neutralization or entry inhibitor assay to measure neutralizing antibodies, or as a surrogate for generating virus escape mutants to neutralizing antibodies. Here, the F and G genes of NiV$_B$ (2010 Faridpur isolate) and HeV (2008 Redlands isolate) were synthesized and inserted into our pOLTV5-rCedV anti-genome plasmid. For the HTS neutralization assay, a turbo-GFP gene was inserted between the CedV P and M genes. Each chimera (rCedV-NiV$_B$-GFP and rCedV-HeV-GFP) were rescued as previously described.[8] Other versions without reporter genes have also been prepared. All virus stocks are cloned and genomes confirmed by NGS. Typically, large stocks are prepared and frozen as single use aliquots. Show in **Figure 1** is an example of how these chimeras are used in a virus neutralization test, referred to as a fluorescent reduction neutralization test (FRNT) using two human NiV/HeV cross-reactive anti-G mAbs (HENV-103 and -117) as previously described.[34] Briefly, Vero 76 cells are used in black walled clear bottom 96-well plates, mAbs are serially diluted in medium, and equal volumes of medium containing rCedV-NiV-B or rCedV-HeV are added to each mAb dilution for a final concentration of 2000 PFU, incubated for 2 h at 37 °C. Each virus-mAb mixture (90 µL/well) is added to the pre-seeded Vero 76 cells in triplicate and the assay run for just another 24 h followed by 4% formaldehyde fixation, washing and then scanning using a CTL S6 analyzer. Fluorescent foci are counted using the CTL Basic Count™ and an IC$_{50}$ value is determined as the antibody concentration yielding 50% reduction in fluorescent foci versus control wells. **Table 1** shows the calculated IC$_{50}$ values using rCedV chimeras vs. those determined by a conventional PRNT assay with authentic NiV-B and HeV, and they are remarkably within a 2-fold range.

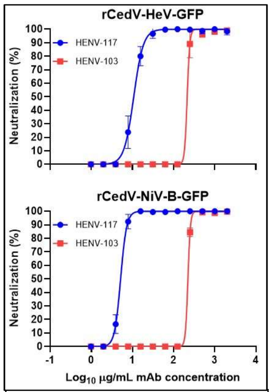


Figure 1. Neutralization curves for HENV-117 and HENV-103 mAbs against rCedV-HeV-GFP (top) and rCedV-NiV-B-GFP (bottom). Data are from a single experiment performed in technical triplicate.

IC50 (ng/mL)	HeV	rCedV-HeV	NiVB	rCedV-NiVB
HENV-103	445	216	507	238
HENV-117	14	11	15	6

Table 1. IC$_{50}$ values of cross-reactive anti-G human mAbs HENV-103 and -117) as determined by rCedV chimera FRNT assay and NiV-B and HeV conventional PRNT assay.

In addition to conventional single agent neutralization tests, the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras can also be used in rapid HTS assays to measure synergistic activities of inhibitors, such as antibodies or drugs. The synergistic neutralizing activity of the HENV-103 and HENV-117 mAbs were described utilizing the rCedV chimera assay.[34] Shown in **Figure 2** is a neutralization synergy assay conducted with mAbs m102.4 and nAH1.3 (both NiV/HeV cross-reactive anti-G mAbs) where they are individually diluted 2-fold in a 7x7 concentration matrix. The diluted mAbs are added 1:1 to 4000 PFU per well of rCedV-NiV-B-GFP and the assay essentially carried out as above, with percent neutralization calculated by normalizing foci to a virus only control. Matrices are imported into SynergyFinder and analyzed Neutralization synergy was calculated by comparing treatment to virus only control wells. Values were imported into SynergyFinder software[35] using a Zero Interactions Potency (ZIP) statistical model; delta scores >10 indicate likely synergy as shown here.

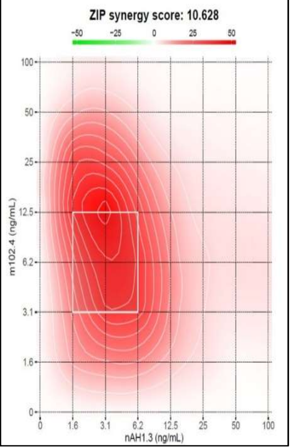


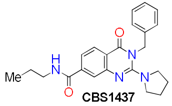
Figure 2. Synergy map with calculated synergy score using the zero interactions potency (ZIP) model generated by SynergyFinder for neutralization of rCedV-NiV-B-GFP by serially diluted m102.4 and nAH1.3 mAbs.

Antiviral evaluation and combination testing of small molecules and nanobodies. These rCedV-based tools and rCedV chimera viruses will allow for rapid antiviral activity assessment and an ability to measure synergistic antiviral activities for both small molecule inhibitors derived from **Aim 1** and nanobodies derived from

Aim 2; and will also allow the novel approach of testing small molecules and nanobody combinations in a rapid and quantitative manner, *in vitro*, to select best-in-class candidates for both monotherapeutic and combination therapeutic modalities prior to *in vivo* animal model testing.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called "SMART Set") was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 µM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 µM, and 47 hits showed only minimal cytotoxicity at 33 µM.

Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.



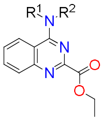
CBS1437

CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)
0.70	100	2.1	100	3.3

In Table 2 is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437**. Note that both HEK293T and Vero cell lines were employed in these assays, and that CC_{50} values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC_{50} value of 3.3 µM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 µM).

Table 3 shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14,** bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent, **CBS1437**, as well as specific plans for developing the SARs.*

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.

	N	Compound ID	R^1	R^2	% inhib at 10 µM
	**1**	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
	2	**G577-0016**	H	2,4-difluorophenyl	18
	3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
	4	**G577-0022**	H	3-methoxyphenyl	46
	5	**G577-0028**	Me	cyclohexyl	93
	6	**G577-0084**	H	4-isopropylphenyl	85
	7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
	8	**G577-0112**	Me	2-methylphenyl	94

C2.2.3. Generation of Nipah and Hendra G and F nanobodies. We recently initiated a collaborative project in nanobody development against NiV and HeV, as well as other henipavirus glycoproteins, with Dr. Jianliang Xu (**RAViD, Nanobody Core Lead**). The Xu lab has engineered and established transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, and has published on the power and utility of anti-SARS-CoV-2 nanobodies.[10] We have already initiated the immunizations of several nanomouse cohorts using native-like oligomeric recombinant sF (**Veesler lab**) and sG glycoproteins (**Broder lab**) of NiV-B and HeV. Immune responses are excellent and sera measured by ELISA using the immunogens. The first of these cohorts will be processed in the coming weeks, and the Xu lab will be constructing phage libraries of polyclonal VHHs

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from each cohort. We except to be able to make rapid progress in **Aim 2**, in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

D. RESEARCH DESIGN AND METHODS

D1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 µM) and low cytotoxicity (CC_{50} >100 µM).

D1.1. Synthetic Chemistry. In **Figure 3** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**G577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we will optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. We will also optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 1** is shown a brief synopsis of the general method used for the synthesis of 4-aminoquinazolines. The specific procedures for the three steps vary, but all initiate with anthranilamides. This general route will allow us to prepare compounds with varied R_1, R_2 and R_3 groups as well as compounds with substituents that replace the carboethoxy functionality at the 2-position. Several anthranilamides are commercially available, which will allow us to initially access starting materials with a variety of R_3 groups. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[36-37] as well as other methods.[38,39] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible.

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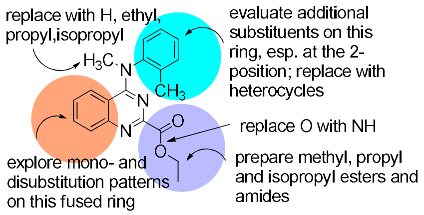
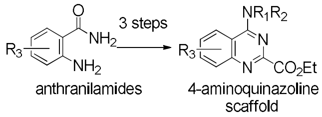
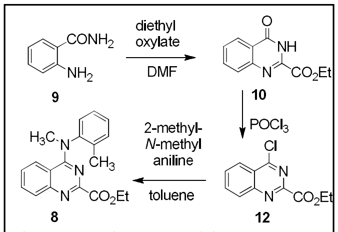


Figure 3. SAR development for 4-aminoquinazolines



Scheme 1. General synthesis for 4-aminoquinazolines.



Scheme 2. Synthesis of **G577-0112** (cmpd **8**).

In **Scheme 2** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) to produce 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely, 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline.

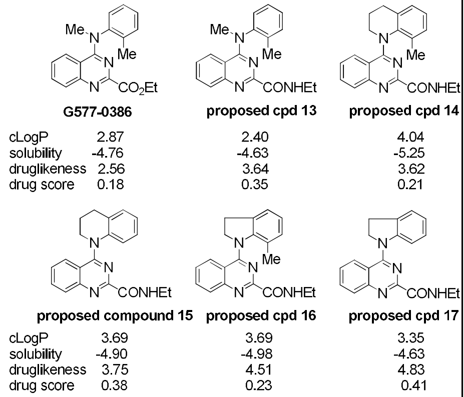


Figure 4. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.

In **Figure 4** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[40]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112** (compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester with an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scores have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding. In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. We have also researched and found additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

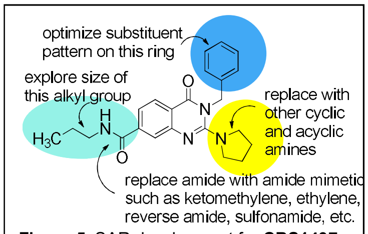


Figure 5. SAR development for **CBS1437**.

D.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5×10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at predetermined nontoxic concentrations for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 µM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

Screening Libraries. These libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of >150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including an Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions, Inc**. *Hit Selection Algorithm.* A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 µM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used again as a specificity control. We expect that the majority of the "hit" compounds will not inhibit the VSV-G-mediated viral entry. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (EC_{50} values less than 10 µM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. *Hit Validation, Cytotoxicity Assessment and Ranking.* We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. *Hit Quality Control and Identity Assurance.* The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds <80% pure will be purified and retested.

D.1.3. Evaluation of antiviral activity of advanced candidates. Inhibitors will be tested against authentic NiV and HeV as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2×10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to parallel control cultures of virus and DMSO. This work will be performed in the BSL-4 facility at UTMB (**Geisbert, Core D)**.

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Peet, Gaisina, and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy using a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or other HTS hits identified using the expanded libraries of compounds.

D.2.1. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with medium containing either DMSO or selected inhibitors at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by western blot techniques or viral RNA levels by q-RT-PCR. CedV specific antibodies against G F N and P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene, and performed using the ABI 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus.** ***Target identification for small molecules.*** The rCedV tools used in **Aim 1** can also be exploited for antiviral target identification. *In vitro* selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutants. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[41,42] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a “drug resistant” virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any “drug resistant” virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inhibitors using authentic NiV-B and HeV in BSL-4 by the UTMB BSL-4 Core.

D.2.2. Assess the binding properties by surface plasmon resonance (SPR). The NiV polymerase (L protein and a phosphoprotein P) will be prepared using a previously published protocol. Biacore T200 and Biacore

8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, and kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[43] Prefusion-stabilized RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 µM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab**.

D.2.3. Structure determination of recombinant proteins with bound inhibitors by X-Ray crystallography and CryoEM. High-resolution structures of the NiV RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compounds to be defined will be obtained using both x-ray crystallography and CryoEM by the **Dr. Vessler Lab**. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands. Initial crystallization screens will be set up robotically using in-house Gryphon crystallization robotics. Biophysical techniques such as native mass spectrometry and thermal shift assays, light scattering, and fluorophore analysis to measure protein stability and folding are routine and will be used to ensure that bound compounds are not destabilizing domain structures to prevent crystallization. X-ray data collection will be done where? Crystal structures will be determined by molecular replacement using existing structures as search models. For inhibitors binding to regions other than above or if co-crystals cannot be obtained, cryo-EM will be used to determine the complex structure. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[44] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including cryoSparc[45], EMAN[46], and RELION[47]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed by molecular dynamics (MD) studies. While cryo-EM can provide access to the near-atomic resolution structures of large biomolecular systems, in their near native environment, MD can be used to reveal its dynamic behavior and refine low-resolution regions of cryo-EM reconstructions[48,49]. Furthermore, as X-ray/cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional inhibitors as they become available), MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[50-54], to explore the conformational landscape[55] of ligand binding events, and to capture conformational variability of intermediate states[48,56], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches. David, please look at this section. Make necessary corrections.

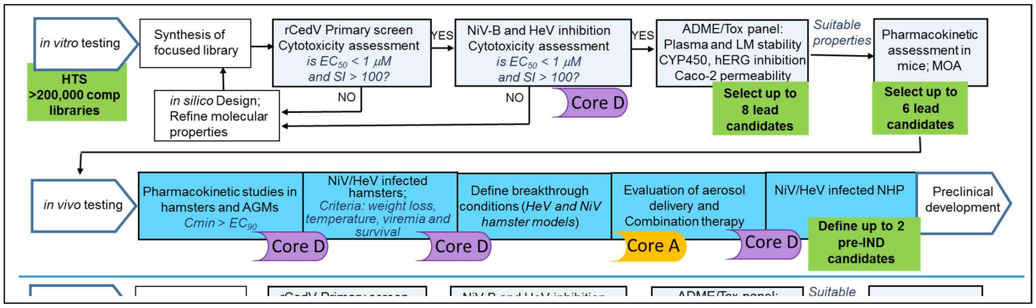
Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds. *Milestone: Designate* ***advanced lead compounds*** *exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the* ***advanced lead compounds****.*

D.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.
Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-T properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out using the CYP3A4, and CYP2D6 Human Cytochrome P450 High Throughput Inhibitor Screening Kits (BD Gentest Corp.) to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[57] Compounds with IC_{50} values ≥100 µM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds by the method of Kuhnz *et al*.[58] Compounds with >30% recovery (1 h) will be considered for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed in a [^{3}H] dofetilide-binding assay as previously described.[59] using SHSY5Y cells. Compounds that do not prevent

[^{3}H]dofetilide binding (IC_{50} >10 µM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method[60] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P_{app}) greater than 1×10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels of the compound up to a solubility-limiting level in the presence or absence of rat liver enzymes (rat S9 liver fraction) acting in the normal process of metabolism and any reverse mutations will be determined. ***In vitro* mammalian chromosomal aberration test**: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes.

D.3.2. PK studies: Naïve male/female mice (equal number, 3 groups of 36 animals total) will be treated with a single dose via i.v., i.p., or oral gavage administration and blood will be collected and processed to obtain plasma and serum from three animals/group/time point at up to 12 time points through 24 h post dose. Two doses will be used to establish the drug exposure-dose range relationship: the estimated efficacious dose (ED), based upon preliminary data at 25 mg/kg i.p. or p.o. and an observable adverse effect dose (OAED) (to be determined). Compounds of interest will be extracted from biological specimens by protein precipitation, analyzed on a triple-quadruple mass spectrometer (LC-MS/MS) and quantified using an internal standard method with a minimum of a five-point calibration curve. PK parameters including AUC, C_0, C_{max}, $T_{1/2}$, T_{max}, and F(%) will be determined. Depending on the estimation of T_{max}, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.



Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc. and Reaction Biology, Corp., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim.

We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 6**).

D.4. AIM 2: Characterization of the potency and breadth of selected nanobodies.

Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. **Aim 1** will also collaborate with the **UTMB, BSL-4 Core D** to evaluate lead candidates with authentic NiV and HeV and also in vivo in **Aim 3**. Batteries of nanobodies to the F and G glycoproteins of NiV and HeV will be isolated from phage libraries constructed using cohorts of nanomice immunized with sF and sG recombinant glycoproteins (**C2.2.3. Preliminary Data).** The Core will conduct preliminary characterizations on binding and specificities and then provide purified nanobody panels to the **Veesler** and **Broder labs** for detailed studies.

D.4.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth.

D.4.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including NiV (Bengladesh and Malaysia strains) and HeV (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[61-63] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with NiV and HeV glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

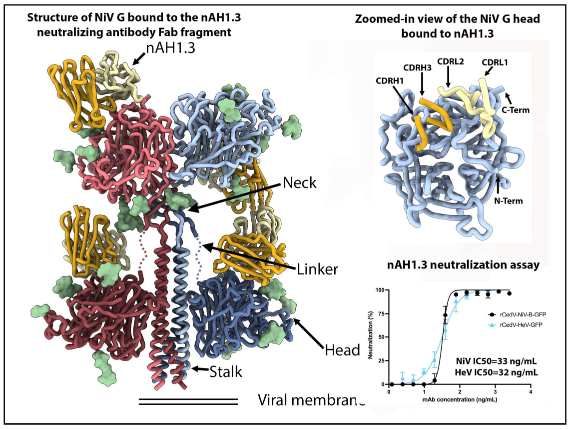
D.4.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit NiV and HeV, we will use a high-throughput neutralization assay (conducted in the **Broder lab**). This assay is based on GFP-encoding, recombinant replication-competent, Cedar henipavirus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bangladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins (see: C.2.1. Preliminary Data).[7,64] This platform is safe, enabling utilization at BSL-2 compared to BSL-4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the **Broder lab** recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described HeV variant.[65] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond NiV and HeV (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[66,67]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.

Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against NiV and HeV. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.4.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition.

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The **Veesler lab** is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The **Veesler lab** has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software, which runs 24 hours per day and enables automated targeting and image acquisition, while keeping a relational database of all micrographs to expedite subsequent processing.[68] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[44,69] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[61,63,70-72] mechanical stage tilting with gold grids[73,74] or detergents to modify surface tension[75]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened



resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[73,76-80] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[81-83]

The NiV attachment (G) glycoprotein engages host receptors, and is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the NiV G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3), which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

D.4.2.1. High-resolution structural studies using X-ray crystallography. Some nanobodies might bind to regions that are intrinsically flexible, thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[84] and BUSTER-TNT.[85]

D.4.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[61-63,71,86,87]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the formulation of cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[88-90] and high-resolution noise substitution prior to Fourier shell correlation calculations[91] to avoid overfitting. Atomic models will be assessed using Molprobity,[92] EMringer[93] and Privateer.[94] The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails.

D.4.3. AIM 2C. Structure-guided formulation of nanobody cocktails.

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[95-97] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected *in vivo*, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting, which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in **Aim 2A**. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.5. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with the ABSL-3 and ABSL-4 Core (**Core D**) to evaluate lead candidate drugs and nanobodies *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster and AGM.[2,98] PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV$_B$ and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV$_B$ and HeV infected AGM models.

D.5.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed by the **UTMB Core (Core D)**. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC$_{90}$ in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of henipavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.5.2. *In vivo* testing of lead compounds NiV/HeV in infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 pfu of NiV$_B$ or HeV.[64,99-101] Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.5.3. Define breakthrough conditions for anti-henipavirus antiviral agents in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antiviral agents fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^6 PFU of NiV or HeV.[64,99-101] Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.5.4. Combination therapy of anti-henipavirus antiviral agents in hamsters. This subaim will employ the most promising antiviral agents from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^6 PFU of NiV or HeV.[64,99-101]Treatment with at least two different antiviral agents will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

D.5.5. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ 5x10^6 PFU of NiV_B or HeV as determined in henipavirus dose confirmation studies conducted by the ABSL-3 and ABSL-4 Core. Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by the **Aerosol Delivery Core** (see **Core A**) at various times after henipavirus infection.

D.5.6. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the LMA mucosal atomization device (MAD) as previously described[102] or by small particle aerosol as previously described[103,104] with a uniformly lethal challenge dose (~ 20,000 PFU of NiV or HeV). Importantly, the MAD system delivers atomized particles that range in size from 30 to 100 µm, which is highly consistent with the size of droplets exhaled by humans when coughing. Previous studies with AGMs infected with NiV_B using the MAD system resulted in lethality in 9/9 animals and a disease course that is highly consistent with human infection (TW Geisbert, C Broder, unpublished data). The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by the **Aerosol Delivery Core** (see **Core A**) up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

Potential Pitfalls and Alternative Strategies. It is possible that **Aerosol Delivery Core (Core A)** may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antiviral agents may not improve protective efficacy or extend the therapeutic window of the agents. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and mAbs to protect animals against lethal henipavirus disease to de-risk this Aim.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues, i.e., antiviral drugs will be coded so that researchers will not know which compounds were given to which animals until experiments and analysis are completed. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the emergence and re-emergence of NiV infections throughout the world, coupled with its high fatality rate, provide very significant motivation for the preparation of small molecule inhibitors of this virus to create a readiness for the next NiV or other related pathogenic henipavirus outbreaks. We have discovered two differentiated series of compounds that show early promise as agents that have potential for treating NiV infections, and we have designed a series of optimized druglike candidates for future preparation and development.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *N Engl J Med* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Desselberger, U. Emerging and re-emerging infectious diseases. *J Infect* **40**, 3-15 (2000).

12 Luby, S. P. & Broder, C. C. in *Viral Infections of Humans, Epidemiology and Control* (eds R. A. Kaslow, L. R. Stanberry, & J. W. Le Duc) Ch. 22, 519-536 (Springer Science+Business Media, 2014).

13 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

14 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

15 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *Med J Aust* **162**, 642-645 (1995).

16 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

17 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

18 Sweileh, W. M. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health* **13**, 9, doi:10.1186/s12992-017-0233-9 (2017).

19 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379, doi:10.3201/eid2003.131159 (2014).

20 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

21 Playford, E. G. *et al.* Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*, doi:10.1016/s1473-3099(19)30634-6 (2020).

22 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).

23 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

24 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

25 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

26 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

27 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

28 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

29 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

30 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

31 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

32 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

33 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

34 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell reports* **36**, 109628, doi:10.1016/j.celrep.2021.109628 (2021).

35 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493, doi:10.1093/nar/gkaa216 (2020).

36 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

37 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

38 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

39 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

40 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

41 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

42 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

43 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Analytical Biochemistry* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

44 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *J Struct Biol* **166**, 95-102 (2009).

45 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

46 Ludtke, S. J. 3-D structures of macromolecules using single-particle analysis in EMAN. *Methods Mol Biol* **673**, 157-173, doi:10.1007/978-1-60761-842-3_9 (2010).

47 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

48 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).

49 Hughes, T. E. T. *et al.* Structural insights on TRPV5 gating by endogenous modulators. *Nature communications* **9**, 4198, doi:10.1038/s41467-018-06753-6 (2018).

50 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).

51 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).

52 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).

53 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).

54 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).

55 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).

56 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).

57 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).

58 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

59 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

60 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

61 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).

62 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

63 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).

64 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).

65 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).

66 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).

67 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).

68 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

69 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

70 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

71 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

72 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

73 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

74 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).

75 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).

76 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).

77 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).

78 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).

79 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).

80 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).

81 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).

82 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).

83 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).

84 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).

85 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).

86 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).

87 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).

88 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

89 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

90 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).

91 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).

92 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).

93 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).

94 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).

95 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).

96 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).

97 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).

98 de Wit, E. & Munster, V. J. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *The Journal of pathology* **235**, 196-205, doi:10.1002/path.4444 (2015).

99 Baseler, L. *et al.* Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. *PLoS neglected tropical diseases* **10**, e0005120, doi:10.1371/journal.pntd.0005120 (2016).

100 Wong, K. T. *et al.* A golden hamster model for human acute Nipah virus infection. *The American journal of pathology* **163**, 2127-2137, doi:10.1016/S0002-9440(10)63569-9 (2003).

101 Rockx, B. *et al.* Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *Journal of virology* **85**, 7658-7671, doi:10.1128/JVI.00473-11 (2011).

102 Geisbert, J. B. *et al.* An Intranasal Exposure Model of Lethal Nipah Virus Infection in African Green Monkeys. *The Journal of infectious diseases* **221**, S414-S418, doi:10.1093/infdis/jiz391 (2020).

103 Cong, Y. *et al.* Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS neglected tropical diseases* **11**, e0005532, doi:10.1371/journal.pntd.0005532 (2017).

104 Prasad, A. N. *et al.* A Lethal Aerosol Exposure Model of Nipah Virus Strain Bangladesh in African Green Monkeys. *The Journal of infectious diseases* **221**, S431-S435, doi:10.1093/infdis/jiz469 (2020).

The pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV) cause severe respiratory illness and encephalitis in both animals and humans with human case fatality rates ranging from 40-100%. The NiV-Bangladesh strain (NiV-B) is associated with multiple rounds of human-to-human transmission and is a pathogen of pandemic concern for which there are no vaccines or antivirals approved for human use. We have recently shown that the non-pathogenic Cedar henipavirus (CedV) is useful surrogate platform for high throughput screening (HTS) for antiviral drug discovery, and for virus neutralization assays. This proposal focuses on developing antiviral small molecule and nanobody countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach. We created reporter gene-encoding recombinant CedVs and discovered a series of fused heterocycles, e.g., 4-aminoquinazoline-based, antivirals, and showed that a representative quinazoline CBS1435 was an effective inhibitor of rCedV replication and also both NiV-B and HeV, with EC_{50}s in the low µM range and minimal cytotoxicity. Neutralizing antibodies to the henipavirus F and G glycoproteins have also shown therapeutic promise, but antiviral nanobodies as potential immunotherapeutics represent a novel area of NiV and HeV countermeasure development. This proposal will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Aim 1 will focus on optimizing lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. Lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed by chemical modifications aimed to improve drug-like properties, increase potencies, decrease toxicity, and improve pharmacokinetic properties. Aim 2 will isolate and fully characterize nanobodies broadly neutralizing NiV-B and HeV derived by immunization of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using phage display technology of cloned nanobody gene fragments. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails. Aim 3 will focus on *in vivo* efficacy evaluation of lead compounds and nanobodies in animal models. Both monotherapies and combination therapeutic modalities will be tested, along with testing antiviral nanobody delivery by aerosol. These two unique henipavirus-targeted antiviral development directions will generate novel countermeasures that may display more broad effectiveness.

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] 22 cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antivirals approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus non-pathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will potentially yield testable antivirals with potential pan-henipavirus effectiveness. Our collaborative team also has an extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and later, best-in-class ~~also aerosol delivery of~~ nanobodies will be also tested by aerosol delivery. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobodies studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

Over the past 40 years the major etiological agents of emerged or identified infectious diseases in humans have been viruses.[11] Among these, the paramyxoviruses, which include many important human and animal pathogens, also include two emerged, zoonotic viral pathogens of importance: the henipaviruses; Hendra virus (HeV) and Nipah virus (NiV).[12,13]~~.~~ In 1994, HeV caused an outbreak of severe respiratory disease in horses in Brisbane, Australia, resulting in the deaths of 14 horses and their trainer.[14,15] NiV was the causative agent of a significant outbreak of severe encephalitis in pig farmers in Malaysia in 1998. Spread of NiV infections typically requires direct contact with an infected source, and transmission can occur between people and from animals to people. Symptoms may culminate in a severe respiratory and/or neurological disease that is often fatal. Complications of the infection can result in relapsed encephalitis following recovery. On September 5, 2021, a twelve-year-old boy in the Indian state of Kerala died after contracting ~~the Nipah virus~~NiV infection. During the process of transporting him to medical centers for a diagnosis, twenty-three health care workers exposed to this boy also died, which underscores both the transmissibility and the lethal nature of this virus. *There is presently no vaccine or small molecule therapeutics that have been developed for NiV infections.* Pteropid bats appear to be the major reservoir hosts for henipaviruses.[6,16]~~.~~ HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bat hosts. Among these animals, HeV and NiV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with human fatality rates ranging from 40-100%.[2,17]~~.~~

NiV and HeV are classified as biosafety level-4 (BSL-4) restricted pathogens and both remain transboundary threats to livestock and people throughout South Asia and Australia. NiV and henipaviral diseases have been categorized by the World Health Organization (WHO) as an epidemic threat needing urgent research and countermeasure development and are included in the WHO R&D Blueprint list of priority pathogens with epidemic potential.[18]~~.~~ Presently, there are no prophylactic or therapeutic treatment options for henipavirus infections approved for use in humans. A subunit vaccine, Equivac® HeV, has been available for use in horses in Australia since 2012[19] for the prevention of HeV infection. This equine vaccine received full registration by the Australian Pesticides and Veterinary Medicines Authority in 2015, with all vaccinated horses receiving a microchip with a database being maintained and is the first commercially deployed vaccine against a BSL-4 agent.[20]~~.~~ Although a human monoclonal antibody (mAb) m102.4 has successfully completed a phase I safety trial in Australia,[21]~~,~~ developing vaccines or therapeutics against henipaviruses for use in people remains a high priority. Henipaviruses are single-stranded, negative sense, enveloped RNA viruses with two membrane anchored glycoproteins involved in virus entry; one mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and also the main focus of several vaccine strategies.[2]~~.~~ Although an effective post-exposure human monoclonal antibody (mAb) therapy (anti-NiV/HeV G glycoprotein mAb m102.4) has been extensively examined,[22-26]~~,~~ it must be administered within several days following lethal infection in animal challenge models. In contrast, however, antiviral drug discovery for HeV and NiV has been significantly hampered due to requirements of biosafety level-4 (BSL-4) containment. Recently, a third isolate, namely, Cedar virus (CedV) has been added to the *Henipavirus* genus.[27]~~.~~ However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease and resides in nature in the same bat host species as HeV.[27,28]~~.~~ Nucleic acid based detection studies have identified related henipavirus species, including complete genomic sequences,[29,30]~~,~~ but HeV, NiV, and CedV are the only virus isolates reported.

We have recently optimized a recombinant CedV encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications and have identified several inhibitory compounds that were further validated with the infectious NiV-Bangladesh (NiV-B) strain. The validated compounds provide the foundation for the current application.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of this proposal's investigators. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may will have activity across related members of henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice

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that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification, and finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole ~~inactivated~~ NiV or HeV immune sera ~~will~~ shows cross-reactivity with CedV, however such sera or even specific high-titer anti-CedV F or G ~~glycoprotein~~ antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins. [27] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and ~~it is also~~ the largest polymerase in the order *Mononegavirale*, yet it ~~still~~ shares high aa identity to NiV and HeV L protein. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; ~~the~~ aa identities of ~~the important~~ L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This overall high degree of ~~protein~~ identity, ~~overall,~~ shared between CedV~~,~~ and that of NiV and HeV, suggests that antiviral~~ agent~~s that target ~~for example,~~ the L polymerase, could be identified and perhaps also offer the possibility of pan-henipavirus ~~antiviral~~ countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] ~~Indeed, s~~Several small molecule inhibitors have been validated in vitro using infectious HeV and NiV~~ in BSL-4~~, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support **Aim 1** to optimize our current best-in-class leads and also screen large libraries of antiviral compounds.

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Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[31-33] Nanobodies can be isolated using the same phage library construction methods and phage panning procedures routinely employed for mAb discovery, which allows for in vitro production of various amounts and their rapid characterization. ~~In collaboration~~Nanobody development will be carried out by~~ with~~ **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels~~,~~.[10] ~~we have several I~~Iimmunized mouse cohorts are in progress ~~now underway using native-like oligomeric recombinant~~using recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in **Aim 2**. ~~in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.~~

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By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of **Aim 3**. In addition, we expect to discover, and well-characterize, batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in **Aim 3**. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

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C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet and Irina Gaisina, Chicago BioSolutions** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core Lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company

engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule ~~entry~~ inhibitors of henipaviruses~~Nipah~~.

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C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2 ~~containment~~), cell-based HTS platform to identify small molecule antivirals targeting henipavirus replication without the requirement for BSL-4 containment.[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with that of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera use guidelines and procedures at BSL-2 were reviewed and approved by the USU, Institutional Biosafety Committee (IBC) in accordance with NIH guidelines. These replication competent rCedV chimeras create a very rapid and robust surrogate infection assay for NiV-B and HeV that can be used as a neutralization ~~tool~~ or entry inhibitor assay to measure neutralizing antibodies ~~or sera~~, or as a surrogate for generating virus escape mutants to ~~F or G~~ neutralizing antibodies. Here, the F and G genes of NiV-B (2010 Faridpur isolate) and HeV (2008 Redlands isolate) were synthesized and inserted into our pOLTV5-rCedV anti-genome plasmid. For the HTS neutralization assay, a turbo-GFP gene was inserted between the CedV P and M genes. Each chimera (rCedV-NiV-B-GFP and rCedV-HeV-GFP) were rescued as previously described.[8] Other versions without reporter genes have also been prepared ~~(rCedV-NiV-B and rCedV-HeV)~~. All virus stocks are cloned and genomes confirmed by NGS. Typically, large stocks are prepared and frozen as single use aliquots. Show in **Figure 1** is an example of how these chimeras are used in a virus neutralization test, referred to as a fluorescent reduction neutralization test (FRNT) using two human NiV/HeV cross-reactive anti-G mAbs (HENV-103 and -117) as previously described.[34] Briefly, Vero 76 cells are used in black walled clear bottom 96-well plates, mAbs are serially diluted in medium, and equal volumes of medium containing rCedV-NiV-B or rCedV-HeV are added to each mAb dilution for a final concentration of 2000 PFU, incubated for 2 hrs at 37~~-~~°C. Each virus-mAb mixture (90 µl/well) is added to the pre-seeded Vero 76 cells in triplicate and the assay run for just another 24 h followed by 4% formaldehyde fixation, washing and then scanning using a CTL S6 analyzer. Fluorescent foci ~~were~~ are counted using the CTL Basic Count™ and an IC$_{50}$ is determined as the antibody concentration ~~at which there was a~~yielding 50% reduction in fluorescent foci versus ~~untreated~~ control wells. **Table 1** shows the calculated ~~EC$_{50}$~~ IC$_{50}$ values using rCedV chimeras vs. those determined by conventional PRNT assay with authentic NiV-B and HeV ~~in BSL-4~~, and they are remarkably within a 2-fold range.

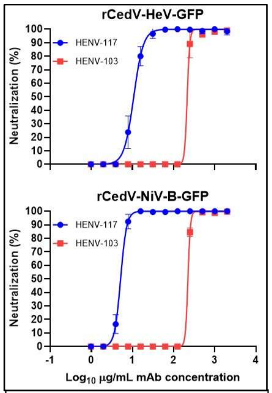


Figure 1. Neutralization curves for HENV-117 and HENV-103 mAbs against rCedV-HeV-GFP (top) and rCedV-NiV-B-GFP (bottom). Data are from a single experiment performed in technical triplicate.

IC50 (ng/mL)	HeV	rCedV-HeV	NiVB	rCedV-NiVB
HENV-103	445	216	507	238
HENV-117	14	11	15	6

Table 1. IC$_{50}$ values of cross-reactive anti-G human mAbs HENV-103 and -117) as determined by rCedV chimera FRNT assay and NiV-B and HeV conventional PRNT assay.

In addition to conventional single agent neutralization tests, the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras can also be used in rapid HTS ~~neutralization~~ assays to measure synergistic activities of inhibitors, such as antibodies or drugs. The synergistic neutralizing activity of the HENV-103 and HENV-117 mAbs were described utilizing the rCedV chimera assay.[34] Shown in **Figure 2** is a neutralization synergy assay conducted with mAbs m102.4 and nAH1.3 (both NiV/HeV cross-reactive anti-G mAbs) where they are individually diluted 2-fold in a 7x7 concentration matrix. The diluted mAbs are added 1:1 to 4000 PFU per well of rCedV-NiV-B-GFP and the assay essentially carried out as above, with percent neutralization calculated by normalizing foci to a virus only control. Matrices are imported into SynergyFinder and analyzed Neutralization synergy was calculated by comparing treatment to virus only control wells. Values were imported into SynergyFinder software[35] using a

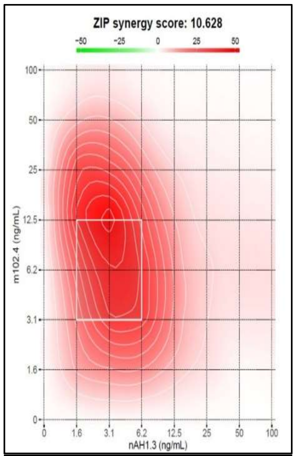


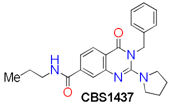
Figure 2. Synergy map with calculated synergy score using the zero interactions potency (ZIP) model generated by SynergyFinder for neutralization of rCedV-NiV-B-GFP by serially diluted m102.4 and nAH1.3 mAbs.

Zero Interactions Potency (ZIP) statistical model; delta scores >10 indicate likely synergy as shown here. ~~m102.4 and nAH1.3 indicate here.~~

Antiviral evaluation and combination testing of small molecules and nanobodies. These rCedV-based tools and rCedV chimera viruses will allow for rapid antiviral activity assessment and an ability to measure synergistic antiviral activities for both small molecule inhibitors derived from **Aim 1** and nanobodies derived from **Aim 2**; and will also allow the novel approach of testing small molecules and nanobody combinations in a rapid and quantitative manner, in vitro, to select best-in-class candidates for both monotherapeutic and combination therapeutic modalities prior to in vivo animal model testing.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called "SMART Set") was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 µM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 µM, and 47 hits showed only minimal cytotoxicity at 33 µM.

Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.

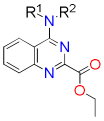

CBS1437

CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC_{50} (µM)	**CC_{50} (µM)**	**EC_{50} (µM)**	**CC_{50} (µM)**	**EC_{50} (µM)**
0.70	100	2.1	100	3.3

In **Table 2** is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437**. Note that both HEK293T and Vero cell lines were employed in these assays, and that CC_{50} values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC_{50} value of 3.3 µM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 µM).

Table 3 shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14,** bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent, **CBS1437**, as well as specific plans for developing the SARs.*

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.

	N	Compound ID	R^1	R^2	% inhib at 10 µM
	**1**	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
	2	**G577-0016**	H	2,4-difluorophenyl	18
	3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
	4	**G577-0022**	H	3-methoxyphenyl	46
	5	**G577-0028**	Me	cyclohexyl	93
	6	**G577-0084**	H	4-isopropylphenyl	85
	7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
	8	**G577-0112**	Me	2-methylphenyl	94

C2.2.3. Generation of Nipah and Hendra G and F nanobodies. We recently initiated a collaborative project in nanobody development against NiV and HeV, as well as other henipavirus glycoproteins, with Dr. Jianliang Xu (**RAViD, Nanobody Core Lead**). The Xu lab has engineered and established transgenic mice that produce

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VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, and has published on the power and utility of anti-SARS-CoV-2 nanobodies(REF). We have already initiated the immunizations of several nanomouse cohorts using native-like oligomeric recombinant sF (**Veesler lab**) and sG glycoproteins (**Broder lab**) of NiV-B and HeV. Immune responses are excellent and sera measured by ELISA using the immunogens. The first of these cohorts will be processed in the coming weeks, and the Xu lab will be constructing phage libraries of polyclonal VHHs from each cohort. We except to be able to make rapid progress in **Aim 2**, in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

Commented [CB5]: Xu, J. et al. Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. Nature 595, 278-282, doi:10.1038/s41586-021-03676-z (2021).

D. RESEARCH DESIGN AND METHODS

D1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 µM) and low cytotoxicity (CC_{50} >100 µM).

D1.1. Synthetic Chemistry. In **Figure 3** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**F577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as “ADME handles” to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. In addition, we will optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be more chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 1** is shown a brief synopsis of the general method used for the synthesis of 4-aminoquinazolines. The specific procedures for the three steps vary, but all initiate with anthranilamides. This general route will allow us to prepare compounds with varied R_1, R_2 and R_3 groups as well as compounds with substituents that replace the carboethoxy functionality at the 2-position. Several anthranilamides are commercially available, which will allow us to initially access starting materials with a variety of R_3 groups. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[36-37] as well as other methods.[38,39] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible.

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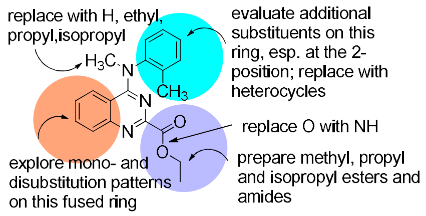
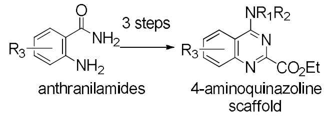
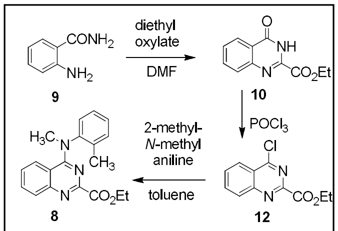


Figure 3. SAR development for 4-aminoquinazolines

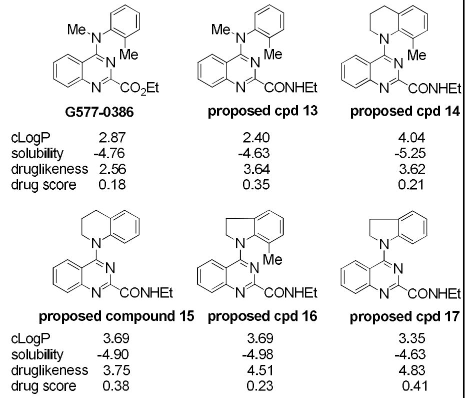


Scheme 1. General synthesis for 4-aminoquinazolines.



Scheme 2. Synthesis of **G577-0112** (cmpd **8**).

In **Scheme 2** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) produces 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline.



	G577-0386	proposed cpd 13	proposed cpd 14
cLogP	2.87	2.40	4.04
solubility	-4.76	-4.63	-5.25
druglikeness	2.56	3.64	3.62
drug score	0.18	0.35	0.21

	proposed compound 15	proposed cpd 16	proposed cpd 17
cLogP	3.69	3.69	3.35
solubility	-4.90	-4.98	-4.63
druglikeness	3.75	4.51	4.83
drug score	0.38	0.23	0.41

Figure 4. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.

In **Figure 4** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[40]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112** (compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester to an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scres have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding.

In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. Additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

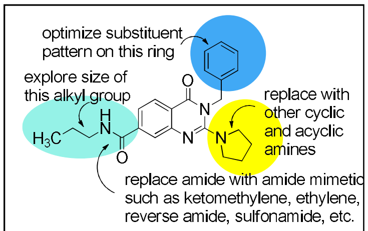


Figure 5. SAR development for **CBS1437**.

D.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5 x 10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at predetermined nontoxic concentrations for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation ~~(PerkinElmer, Waltham, MA) as per the manufacturer's instructions~~. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T ~~cells~~ and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 μM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

<u>*Screening Libraries.*</u> These libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of >150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions,**

Inc. *Hit Selection Algorithm.* A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used again as a specificity control. We expect that the majority of the "hit" compounds will not inhibit the VSV-G-mediated viral entry. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (potent inhibitor, EC_{50} less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. *Hit Validation, Cytotoxicity Assessment and Ranking.* We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" ~~(Promega, Madison, WI)~~ as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. *Hit Quality Control and Identity Assurance.* The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds ~~found to be~~ <80% pure will be purified and retested.

D.1.3. Evaluation of ~~protective properties~~antiviral activity of ~~the~~ advanced candidates. ~~in the henipavirus infectious assays~~ Inhibitors will be ~~done~~ tested against authentic NiV and HeV as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2 × 10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to ~~that from~~ parallel control cultures ~~with~~ of virus and DMSO ~~vehicle only~~. This work will be performed in the BSL-4 facility at UTMB ~~(by the~~ **Geisbert ~~Lab (Core ?~~BSL-4 Core lead)** .

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Gaisina and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy ~~based on a~~using a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or ~~the additional~~other HTS hits identified ~~with the HTS of the~~using the expanded libraries of compounds ~~for defining lead compounds for advancement~~.

D.2.1. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with ~~cell growth~~ medium containing either DMSO or selected furopyrimidines at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by ~~standard~~ western blot techniques or viral RNA levels by ~~quantitative real time polymerase chain reaction assays (~~q-RT-PCR~~)~~. CedV specific antibodies against ~~the attachment (~~G~~) and fusion (~~F~~) that were generated in the~~ ~~**Broder Lab**~~ ~~and antibodies to CedV~~ N and ~~CedV~~ P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene, and ~~. The one-step reaction will be~~ performed using the ~~Applied Biosystems (~~ABI~~)~~ 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies,

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HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus.** ***Target identification for small molecules.*** The rCedV tools used in ~~the small molecule inhibitor identification assays (~~**Aim 1**~~)~~ can also be exploited for antiviral target identification. In vitro selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutants~~ viruses~~. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[41,42] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a "drug resistant" virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any "drug resistant" virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L ~~polymerase~~ gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inibitors using authentic NiV-B and HeV in BSL-4 by the UTMB BSL-4 Core.

D.2.2. Assess the binding properties by surface plasmon resonance (SPR). The ~~Nipah~~ NiV polymerase (L protein and a phosphoprotein P) will be prepared using a previously published protocol. Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[43] Prefusion-stabilized RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 μM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab**.

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D.2.3. Structure determination of recombinant proteins with bound inhibitors by X-Ray crystallography and CryoEM. High-resolution structures of the ~~Nipah~~ NiV RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compound to be developed will be obtained using both x-ray crystallography and CryoEM by Dr. Vessler Lab. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands. Initial crystallization screens will be set up robotically using in-house Gryphon crystallization robotics. Biophysical techniques such as native mass spectrometry and thermal shift assays, light scattering, and fluorophore analysis to measure protein stability and folding are routine and will be used to ensure that bound compounds are not destabilizing domain structures to prevent crystallization. X-ray data collection will be done where? Crystal structures will be determined by molecular replacement using existing structures as search models. For inhibitors binding to regions other than above or if co-crystals cannot be obtained, cryo-EM will be used to determine the complex structure. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[44] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including cryoSparc[45], EMAN[46], and RELION[47]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed by molecular dynamics (MD) studies. While cryo-EM can provide access to the near-atomic resolution structures of large biomolecular systems--in their near native environment, MD can be used to reveal its dynamic behavior and refine low-resolution regions of cryo-EM reconstructions[48,49]. Furthermore, as X-ray/cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[50-54], to explore the conformational landscape[55] of ligand binding events, and to capture conformational variability of intermediate states[48,56], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches. David, please look at this section. Make necessary corrections.

Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds. *Milestone: Designate **advanced lead compounds** exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the **advanced lead compounds**.*

D.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.
Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-T properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out using the CYP3A4, and CYP2D6 Human Cytochrome P450 High Throughput Inhibitor Screening Kits (BD Gentest Corp.)~~, Woburn, MA)~~ to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[57] Compounds with IC$_{50}$ values ≥100 µM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds by the method of Kuhnz *et al*.[58] Compounds with >30% recovery (1 h) will be considered for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed in a $[^3H]$ dofetilide-binding assay as previously described~~ by Finlayson *et al*~~.[59] using SHSY5Y cells. Compounds that do not prevent $[^3H]$dofetilide binding (IC$_{50}$ >10 µM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method ~~as described by Gres *et al*.~~[60] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P_{app}) greater than 1 × 10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels of the compound up to a solubility-limiting level in the presence or absence of rat liver enzymes (rat S9 liver fraction) acting in the normal process of metabolism and any reverse mutations will be determined. ***In vitro* mammalian chromosomal aberration test**: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes.

D.3.2. PK studies: Naïve male/female mice (equal number, 3 groups of 36 animals total) will be treated with a single dose via i.v., i.p., or oral gavage administration and blood will be collected and processed to obtain plasma and serum from three animals/group/time point at up to 12 time points through 24 h post dose. Two doses will

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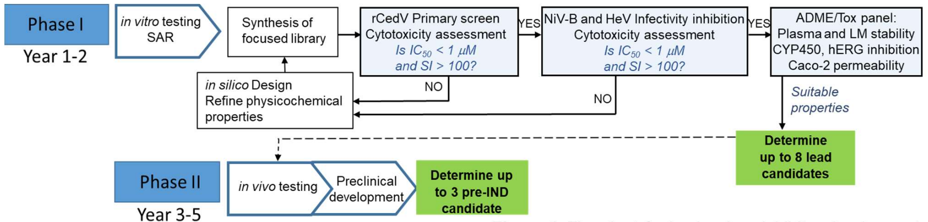

Figure 4. Flowchart for henipavirus inhibitor development

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be used to establish the drug exposure-dose range relationship: the estimated efficacious dose (ED), based upon preliminary data at 25 mg/kg i.p. or p.o. and an observable adverse effect dose (OAED) (to be determined). Compounds of interest will be extracted from biological specimens by protein precipitation, analyzed on a triple-quadruple mass spectrometer (LC-MS/MS) and quantified using an internal standard method with a minimum of a five-point calibration curve. PK parameters including AUC, C_0, C_{max}, $T_{1/2}$, T_{max}, and F(%) will be determined. Depending on the estimation of T_{max}, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc. and Reaction Biology, Corp., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim.

We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 5**).

D.4. AIM 2: Characterization of the potency and breadth of selected nanobodies.

Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. **Aim 1** will also collaborate with the ~~core???~~ UTMB, BSL-4 Core to evaluate lead candidates with authentic NiV and HeV and also in vivo in **Aim 3**. Batteries of nanobodies to the F and G glycoproteins of NiV and HeV will be isolated from phage libraries constructed using cohorts of nanomice immunized with sF and sG recombinant glycoproteins (**C2.2.3. Preliminary Data**). The Core will conduct preliminary characterizations on binding and specificities and then provide purified nanobody panels to the **Veesler** and **Broder labs** for detailed studies.

D.4.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth.

D.4.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including NiV ~~pah virus~~ (Bangladesh and Malaysia strains) and ~~Hendra~~ HeV ~~virus~~ (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[61-63] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with ~~Nipah and Hendra virus~~NiV and HeV glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

D.4.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit ~~Nipah virus and Hendra virus~~NiV and HeV, we will use a high-throughput neutralization assay ~~developed in the~~ (conducted in the **Broder lab**). This assay is based on ~~green fluorescent protein (~~GFP~~)~~-encoding, recombinant replication-competent, Cedar ~~(~~henipa~~)~~virus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bangladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins (see: C.2.1. Preliminary Data).[7,64] This platform is safe, enabling utilization at BSL-2 compared to BSL-4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the **Broder lab** recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described ~~Hendra virus~~HeV variant.[65] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond ~~Nipah and Hendra viruses~~NiV and HeV (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[66,67]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.

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Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against ~~Nipah virus and Hendra virus~~NiV and HeV. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.4.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition.

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The **Veesler lab** is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The **Veesler lab** has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software which runs 24 hours per day and enables automated targeting and image acquisition while keeping a relational database of all micrographs to expedite subsequent processing.[68] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[44,69] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[61,63,70-72] mechanical stage tilting with gold grids[73,74] or detergents to modify surface tension[75]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[73,76-80] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[81-83]

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The ~~Nipah virus~~NiV attachment (G) glycoprotein engages host receptors, is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the ~~Nipah virus~~NiV G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3) which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

D.4.2.1. High-resolution structural studies using X-ray crystallography. Some ~~of the~~ nanobodies might bind to regions that are intrinsically flexible thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[84] and BUSTER-TNT.[85]~~.~~

D.4.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[61-63,71,86,87]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the formulation of cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[88-90] and high-resolution noise substitution prior to Fourier shell correlation calculations[91] to avoid overfitting. Atomic models will be assessed using Molprobity,[92]~~,~~ EMringer[93] and Privateer.[94]~~.~~ The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails.

D.4.3. AIM 2C. Structure-guided formulation of nanobody cocktails.

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[95-97] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected in vivo, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in Aim 2A. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.5. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with the **ABSL-3 and ABSL-4 Core** (Core D) to evaluate lead candidate drugs and nanobodies~~s~~ *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in ~~a~~ well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster ~~(refs)~~ and AGM (refs 1, 2~~s~~). PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV_B and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV_B and HeV infected AGM models.

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D.5.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed ~~by Core D~~by the UTMB Core. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC_{90} in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of heniavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.5.2. *In vivo* testing of lead compounds NiV/HeV infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ $5x10^6$ pfu of NiV_B or HeV (refs 3-6). Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight

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loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.5.3. Define breakthrough conditions for anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antivirals fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^6 PFU of NiV or HeV (refs 3-6). Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

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D.5.4. Combination therapy of anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising antivirals from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^6~~5x10^5~~ PFU of NiV or HeV (refs 3-6). Treatment with at least two different antivirals will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

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D.5.5. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ 5x10^6 PFU of NiV$_B$~~-B~~ or HeV as determined in henipavirus dose confirmation studies conducted by the **ABSL-3 and ABSL-4 Core** ~~Core D~~ (see Core D). Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by the **Aerosol Delivery Core**~~Core X~~ (see Core A~~X~~) at various times after henipavirus infection.

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D.5.6. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the LMA mucosal atomization device (MAD)~~MAD system~~ as previously described (ref 7) or by small particle aerosol as previously described (refs 8, 9) with a uniformly lethal challenge dose (~ ~~-~~20,000 PFU of NiV or HeV). Importantly, the MAD system delivers atomized particles that range in size from 30 to 100 µm, which is highly consistent with the size of droplets exhaled by humans when coughing. Previous studies with AGMs infected with NiV$_B$ using the MAD system resulted in lethality in 9/9 animals and a disease course that is highly consistent with human infection (TW Geisbert, C Broder, unpublished data). The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by the **Aerosol Delivery Core**~~Core X~~ (see Core A)up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

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Potential Pitfalls and Alternative Strategies. It is possible that **Aerosol Delivery Core (Core A)** ~~X~~ may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antivirals may not improve protective efficacy or extend the therapeutic window of antivirals. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and ~~monoclonal antibodies~~mAbs to protect animals against lethal henipavirus disease to de-risk this Aim.~~.~~

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General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues, i.e., antiviral drugs will be coded so that researchers will not know which compounds were given to which animals until experiments and analysis are completed. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the emergence and re-emergence of ~~Nipah virus~~NiV infections throughout the world, coupled with its high fatality rate, provide very significant motivation for the preparation of small molecule inhibitors of this virus to create a readiness for the next ~~Nipah virus~~NiV or other related pathogenic henipavirus outbreaks. We *have discovered two differentiated series of compounds that show early promise as agents that have potential for treating ~~Nipah virus~~NiV infections, and we have designed a series of optimized druglike candidates for future preparation and development.*

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *N Engl J Med* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Desselberger, U. Emerging and re-emerging infectious diseases. *J Infect* **40**, 3-15 (2000).

12 Luby, S. P. & Broder, C. C. in *Viral Infections of Humans, Epidemiology and Control* (eds R. A. Kaslow, L. R. Stanberry, & J. W. Le Duc) Ch. 22, 519-536 (Springer Science+Business Media, 2014).

13 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

14 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

15 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *Med J Aust* **162**, 642-645 (1995).

16 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

17 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

18 Sweileh, W. M. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health* **13**, 9, doi:10.1186/s12992-017-0233-9 (2017).

19 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379, doi:10.3201/eid2003.131159 (2014).

20 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

21 Playford, E. G. *et al.* Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*, doi:10.1016/s1473-3099(19)30634-6 (2020).

22 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).

23 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

24 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

25 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

26 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

27 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

28 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

29 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

30 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

31 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

32 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

33 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

34 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell reports* **36**, 109628, doi:10.1016/j.celrep.2021.109628 (2021).

35 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493, doi:10.1093/nar/gkaa216 (2020).

36 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

37 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

38 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

39 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

40 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

41 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

42 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

43 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Anal Biochem* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

44 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *Journal of structural biology* **166**, 95-102 (2009).

45 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

46 Ludtke, S. J. 3-D structures of macromolecules using single-particle analysis in EMAN. *Methods in molecular biology* **673**, 157-173, doi:10.1007/978-1-60761-842-3_9 (2010).

47 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *Journal of structural biology* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

48 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).

49 Hughes, T. E. T. *et al.* Structural insights on TRPV5 gating by endogenous modulators. *Nature communications* **9**, 4198, doi:10.1038/s41467-018-06753-6 (2018).

50 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).

51 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).

52 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).

53 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).

54 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).

55 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).

56 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).

57 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).

58 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

59 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

60 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

61 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).

62 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

63 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).

64 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).

65 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).

66 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).

67 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).

68 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

69 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

70 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

71 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

72 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

73 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

74 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).

75 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).

76 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).

77 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).

78 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).

79 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).

80 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).

81 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).

82 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).

83 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).

84 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).

85 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).

86 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).

87 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).

88 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

89 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

90 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).

91 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).

92 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).

93 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).

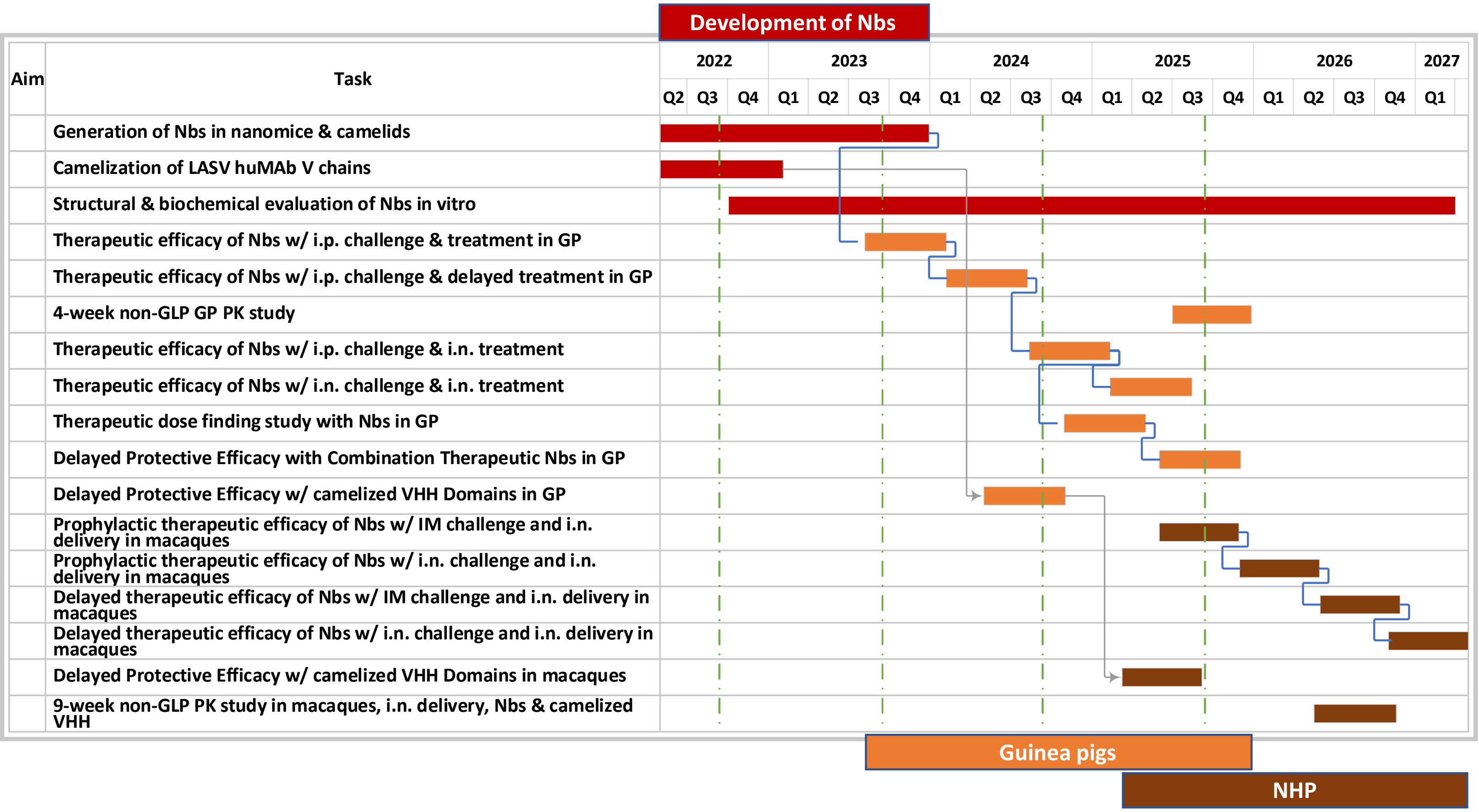
94 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).

95 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).

96 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).

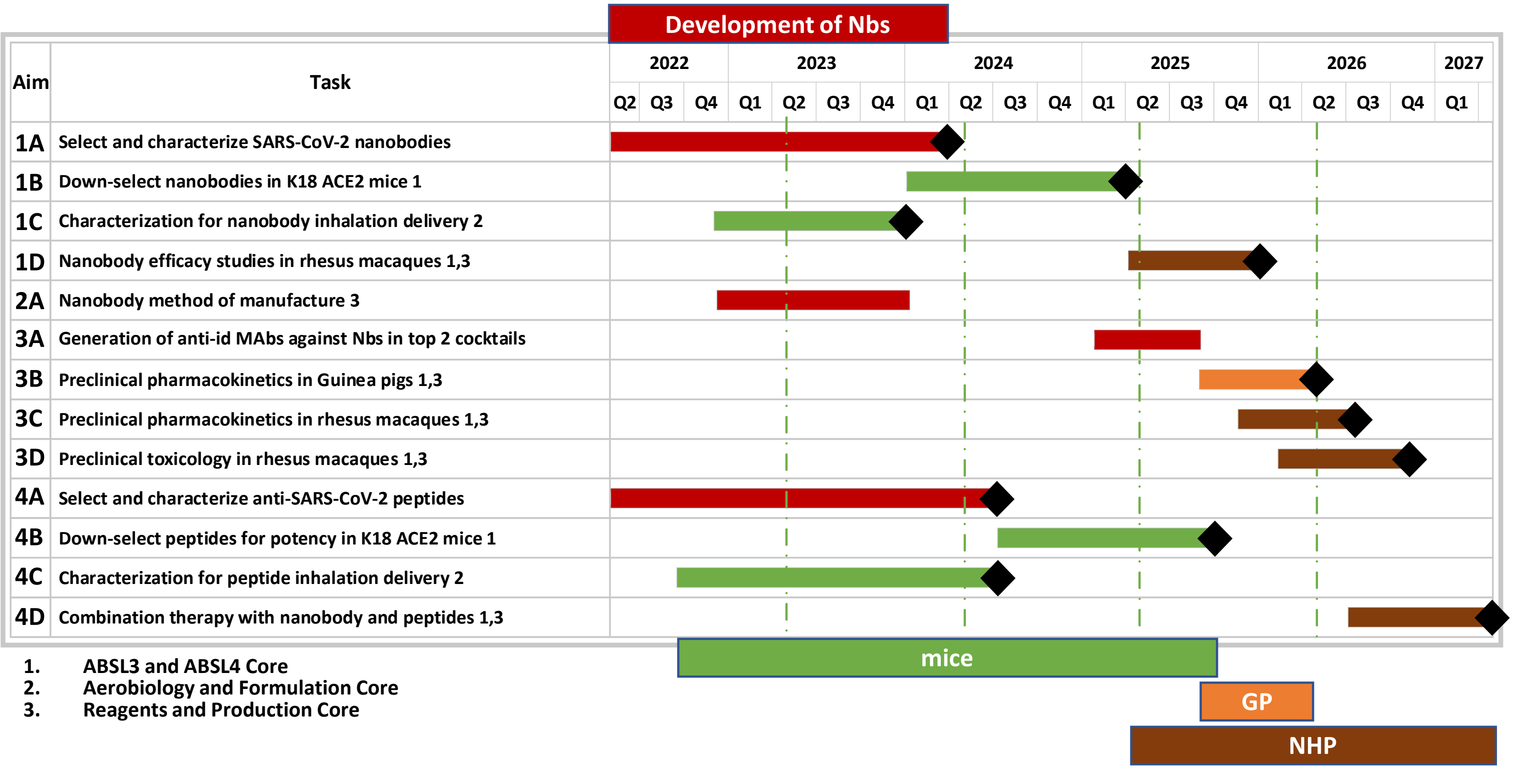
97 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).

RAViD Center Project 2: Structure-guided development of nanobody therapeutics against Arenaviruses



Aim	Task
	Generation of Nbs in nanomice & camelids
	Camelization of LASV huMAb V chains
	Structural & biochemical evaluation of Nbs in vitro
	Therapeutic efficacy of Nbs w/ i.p. challenge & treatment in GP
	Therapeutic efficacy of Nbs w/ i.p. challenge & delayed treatment in GP
	4-week non-GLP GP PK study
	Therapeutic efficacy of Nbs w/ i.p. challenge & i.n. treatment
	Therapeutic efficacy of Nbs w/ i.n. challenge & i.n. treatment
	Therapeutic dose finding study with Nbs in GP
	Delayed Protective Efficacy with Combination Therapeutic Nbs in GP
	Delayed Protective Efficacy w/ camelized VHH Domains in GP
	Prophylactic therapeutic efficacy of Nbs w/ IM challenge and i.n. delivery in macaques
	Prophylactic therapeutic efficacy of Nbs w/ i.n. challenge and i.n. delivery in macaques
	Delayed therapeutic efficacy of Nbs w/ IM challenge and i.n. delivery in macaques
	Delayed therapeutic efficacy of Nbs w/ i.n. challenge and i.n. delivery in macaques
	Delayed Protective Efficacy w/ camelized VHH Domains in macaques
	9-week non-GLP PK study in macaques, i.n. delivery, Nbs & camelized VHH

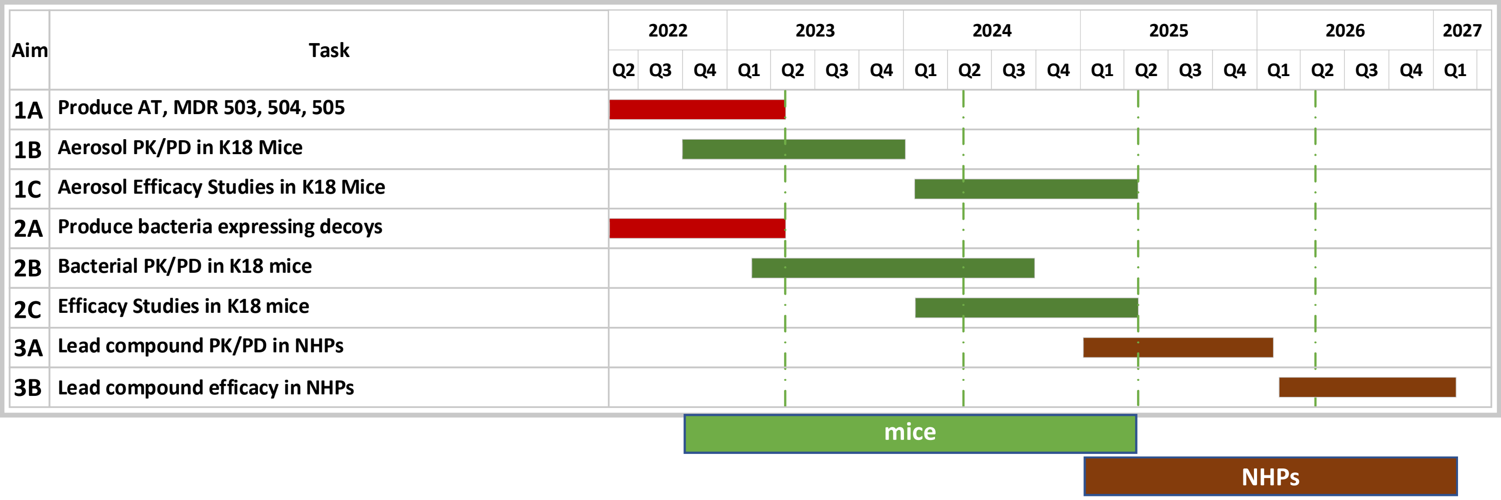
RAViD Center Project 3: Aerosol delivery of nanobodies and peptides for coronaviruses



Aim	Task
1A	Select and characterize SARS-CoV-2 nanobodies
1B	Down-select nanobodies in K18 ACE2 mice 1
1C	Characterization for nanobody inhalation delivery 2
1D	Nanobody efficacy studies in rhesus macaques 1,3
2A	Nanobody method of manufacture 3
3A	Generation of anti-id MAbs against Nbs in top 2 cocktails
3B	Preclinical pharmacokinetics in Guinea pigs 1,3
3C	Preclinical pharmacokinetics in rhesus macaques 1,3
3D	Preclinical toxicology in rhesus macaques 1,3
4A	Select and characterize anti-SARS-CoV-2 peptides
4B	Down-select peptides for potency in K18 ACE2 mice 1
4C	Characterization for peptide inhalation delivery 2
4D	Combination therapy with nanobody and peptides 1,3

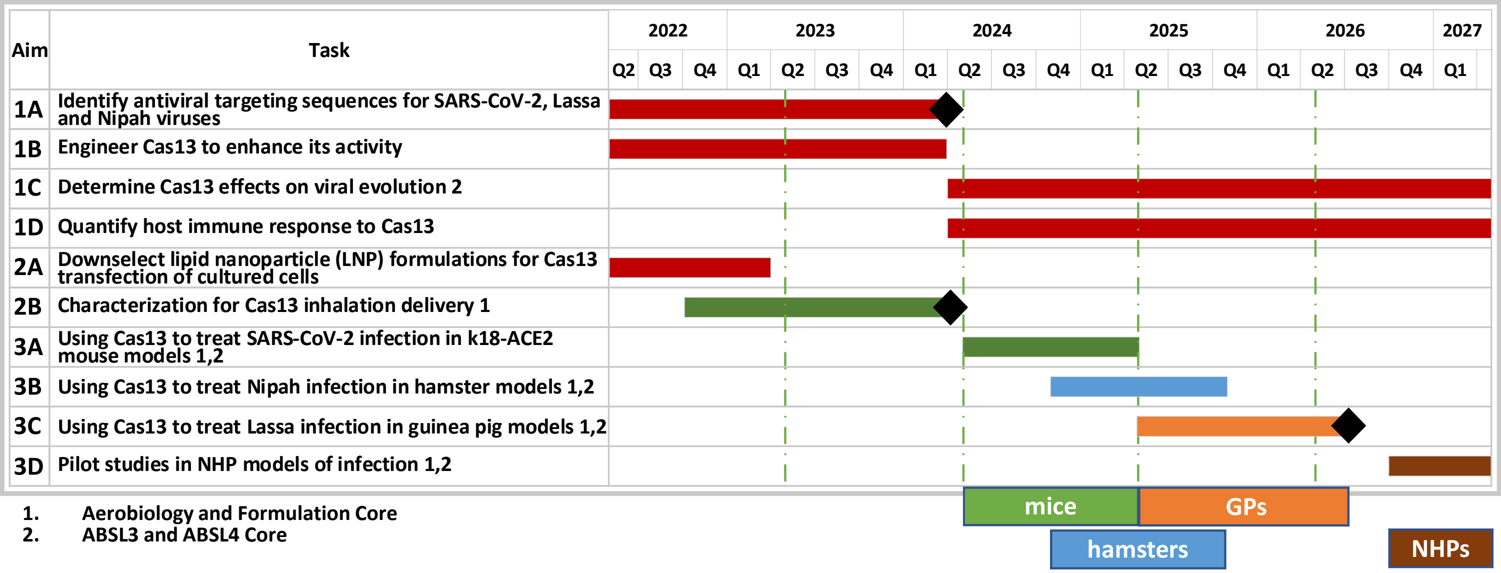
1. ABSL3 and ABSL4 Core
2. Aerobiology and Formulation Core
3. Reagents and Production Core

RAViD Center Project 4: Aerosol delivery of ACE2 decoys for coronaviruses



Aim	Task
1A	Produce AT, MDR 503, 504, 505
1B	Aerosol PK/PD in K18 Mice
1C	Aerosol Efficacy Studies in K18 Mice
2A	Produce bacteria expressing decoys
2B	Bacterial PK/PD in K18 mice
2C	Efficacy Studies in K18 mice
3A	Lead compound PK/PD in NHPs
3B	Lead compound efficacy in NHPs

RAViD Center Project 5: Lipid nanoparticle delivery of Cas13-based antiviral therapies



Aim	Task
1A	Identify antiviral targeting sequences for SARS-CoV-2, Lassa and Nipah viruses
1B	Engineer Cas13 to enhance its activity
1C	Determine Cas13 effects on viral evolution 2
1D	Quantify host immune response to Cas13
2A	Downselect lipid nanoparticle (LNP) formulations for Cas13 transfection of cultured cells
2B	Characterization for Cas13 inhalation delivery 1
3A	Using Cas13 to treat SARS-CoV-2 infection in k18-ACE2 mouse models 1,2
3B	Using Cas13 to treat Nipah infection in hamster models 1,2
3C	Using Cas13 to treat Lassa infection in guinea pig models 1,2
3D	Pilot studies in NHP models of infection 1,2

1. Aerobiology and Formulation Core
2. ABSL3 and ABSL4 Core

RAViD Center Administrative Core: Schedule

Task	*2022*			*2023*				*2024*				*2025*				*2026*				*2027*
	Q2	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*	*Q2*	*Q3*	*Q4*	*Q1*
Kickoff meeting at NIH (In-person)	●																			
Reverse Site visits at NIH (In-person)					●				●				●				●			
Annual in-person meeting – key personnel		●				●				●				●				●		
Annual online retreat – all hands (online)				●				●				●				●				●
IAC, Research Project and Core meetings (online)	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
SAB and IAC joint evaluation meeting (online)			●				●				●				●				●	
Solicitation/Review			■	■							■	■								
Developmental Projects Round 1					■	■	■	■	■	■	■	■								
Developmental Projects Round 2													■	■	■	■	■	■	■	■
Mentored Projects Round 1					■	■	■	■	■	■	■	■	■	■	■	■				
Mentored Projects Round 2													■	■	■	■	■	■	■	■

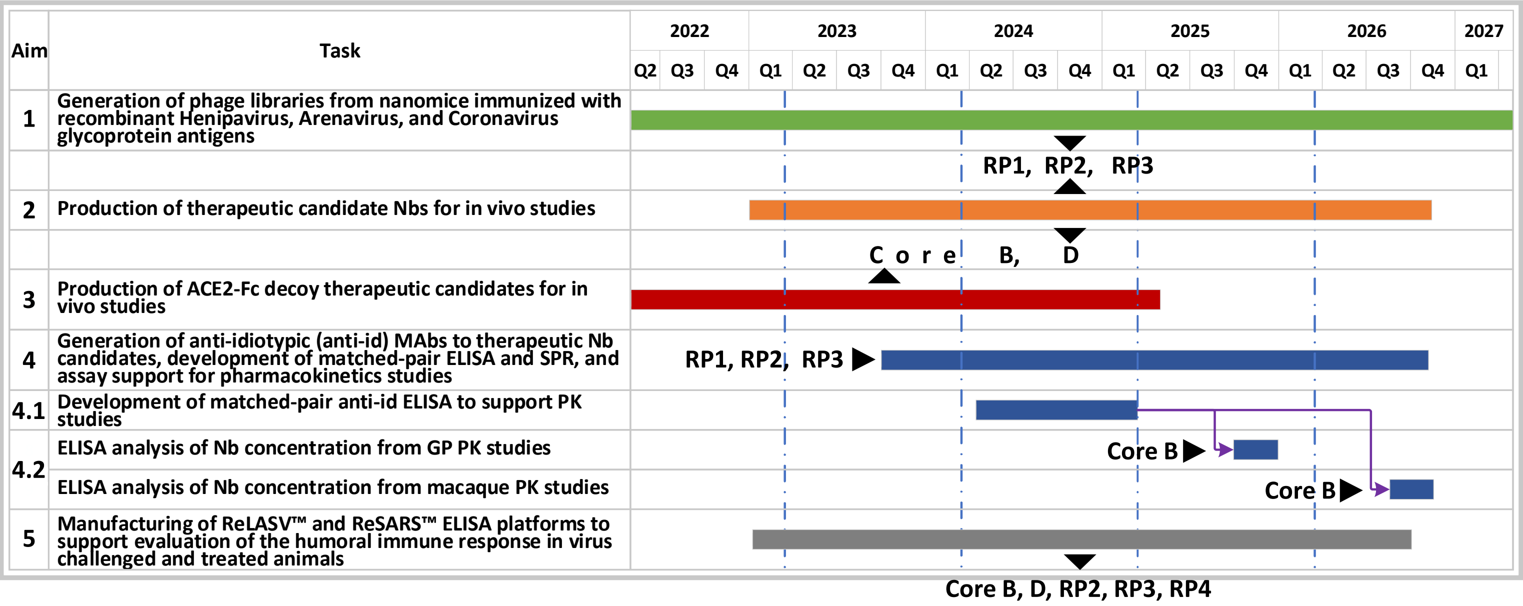
~~RAViD Center Core B: Aerobiology and Formulation Core~~

Aim	Task	2022			2023				2024				2025				2026				2027
		Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
	~~Aerosol characterization of promising lead antiviral candidates~~	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥											
	~~Initial in vivo comparative toxicology and PK studies in multiple species~~					🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥			
	~~Preliminary dose ranging evaluation of leading candidates from individual RPs~~													🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
	~~Therapeutic antiviral dosing in PK/PD and evaluation of leading candidates from individual RPs~~													🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥

RAViD Center Core B: Aerobiology and Formulation Core

Aim	Task	2022			2023				2024				2025				2026				2027
		Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
1	Oversee and harmonize all aerobiology-related studies as related to aerosol infectious challenge	▨	▨	▨	▨	▨	▨	▨	▨	▨											
1A	Support of early animal model efforts by RP individual investigators	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥											
1B	Harmonization of methodologies associated with aerosol/mucosal infectious challenge	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥											
2	Aerosol characterization for inhalation delivery in preclinical antiviral evaluation studies					▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
2A	Lead optimization of aerosol therapies					🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥			
2B	Formulation and aerosol characterization studies					🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥							
2C	Comparative in vivo pharmacokinetics and evaluation studies												🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥

RAViD Center Core C: Reagents and Production



RAViD Center Core D: Animal Biosafety Level (ABSL)-3 and ABSL-4 Core

Aim	Task	2022			2023				2024				2025				2026				2027
		Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1
1	Provide BSL-3, BSL-4 resources, including a secure repository of well characterized seed stocks of SARS-CoV-2, henipaviruses, and Lassa virus	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
2	Provide technical expertise and conduct in vitro assays in BSL-3 and BSL-4 containment	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥
3	Provide technical expertise and conduct animal challenges, treatments, clinical pathology assays, virology assays, and necropsies in ABSL-3, ABSL-4 containment					🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥	🟥

The pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV) cause severe respiratory illness and encephalitis in both animals and humans with human case fatality rates ranging from 40-100%. The NiV-Bangladesh strain (NiV-B) is associated with multiple rounds of human-to-human transmission and is a pathogen of pandemic concern for which there are no vaccines or antivirals approved for human use. We have recently shown that the non-pathogenic Cedar henipavirus (CedV) is useful surrogate platform for high throughput screening (HTS) for antiviral drug discovery, and for virus neutralization assays. This proposal focuses on developing antiviral small molecule and nanobody countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach. We created reporter gene-encoding recombinant CedVs and discovered a series of fused heterocycles, e.g., 4-aminoquinazoline-based, antivirals, and showed that a representative quinazoline CBS1435 was an effective inhibitor of rCedV replication and also both NiV-B and HeV, with EC_{50}s in the low µM range and minimal cytotoxicity. Neutralizing antibodies to the henipavirus F and G glycoproteins have also shown therapeutic promise, but antiviral nanobodies as potential immunotherapeutics represent a novel area of NiV and HeV countermeasure development. This proposal will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Aim 1 will focus on optimizing lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. Lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed by chemical modifications aimed to improve drug-like properties, increase potencies, decrease toxicity, and improve pharmacokinetic properties. Aim 2 will isolate and fully characterize nanobodies broadly neutralizing NiV-B and HeV derived by immunization of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using phage display technology of cloned nanobody gene fragments. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails. Aim 3 will focus on *in vivo* efficacy evaluation of lead compounds and nanobodies in animal models. Both monotherapies and combination therapeutic modalities will be tested, along with testing antiviral nanobody delivery by aerosol. These two unique henipavirus-targeted antiviral development directions will generate novel countermeasures that may display more broad effectiveness.

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] 22 cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antivirals approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus non-pathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC$_{50}$ value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will potentially yield testable antivirals with potential pan-henipavirus effectiveness. Our collaborative team also has an extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and later, best-in-class ~~also aerosol delivery of~~ nanobodies will be also tested by aerosol delivery. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobodies studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

Over the past 40 years the major etiological agents of emerged or identified infectious diseases in humans have been viruses.[11] Among these, the paramyxoviruses, which include many important human and animal pathogens, also include two emerged, zoonotic viral pathogens of importance: the henipaviruses; Hendra virus (HeV) and Nipah virus (NiV).[12,13]~~.~~ In 1994, HeV caused an outbreak of severe respiratory disease in horses in Brisbane, Australia, resulting in the deaths of 14 horses and their trainer.[14,15] NiV was the causative agent of a significant outbreak of severe encephalitis in pig farmers in Malaysia in 1998. Spread of NiV infections typically requires direct contact with an infected source, and transmission can occur between people and from animals to people. Symptoms may culminate in a severe respiratory and/or neurological disease that is often fatal. Complications of the infection can result in relapsed encephalitis following recovery. On September 5, 2021, a twelve-year-old boy in the Indian state of Kerala died after contracting ~~the Nipah virus~~NiV infection. During the process of transporting him to medical centers for a diagnosis, twenty-three health care workers exposed to this boy also died, which underscores both the transmissability and the lethal nature of this virus. *There is presently no vaccine or small molecule therapeutics that have been developed for NiV infections.* Pteropid bats appear to be the major reservoir hosts for henipaviruses.[6,16]~~.~~ HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bat hosts. Among these animals, HeV and NiV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with human fatality rates ranging from 40-100%.[2,17]~~.~~

NiV and HeV are classified as biosafety level-4 (BSL-4) restricted pathogens and both remain transboundary threats to livestock and people throughout South Asia and Australia. NiV and henipaviral diseases have been categorized by the World Health Organization (WHO) as an epidemic threat needing urgent research and countermeasure development and are included in the WHO R&D Blueprint list of priority pathogens with epidemic potential.[18]~~.~~ Presently, there are no prophylactic or therapeutic treatment options for henipavirus infections approved for use in humans. A subunit vaccine, Equivac® HeV, has been available for use in horses in Australia since 2012[19] for the prevention of HeV infection. This equine vaccine received full registration by the Australian Pesticides and Veterinary Medicines Authority in 2015, with all vaccinated horses receiving a microchip with a database being maintained and is the first commercially deployed vaccine against a BSL-4 agent.[20]~~.~~ Although a human monoclonal antibody (mAb) m102.4 has successfully completed a phase I safety trial in Australia,[21]~~,~~ developing vaccines or therapeutics against henipaviruses for use in people remains a high priority. Henipaviruses are single-stranded, negative sense, enveloped RNA viruses with two membrane anchored glycoproteins involved in virus entry; one mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and also the main focus of several vaccine strategies.[2]~~.~~ Although an effective post-exposure human monoclonal antibody (mAb) therapy (anti-NiV/HeV G glycoprotein mAb m102.4) has been extensively examined,[22-26]~~,~~ it must be administered within several days following lethal infection in animal challenge models. In contrast, however, antiviral drug discovery for HeV and NiV has been significantly hampered due to requirements of biosafety level-4 (BSL-4) containment. Recently, a third isolate, namely, Cedar virus (CedV) has been added to the *Henipavirus* genus.[27]~~.~~ However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease and resides in nature in the same bat host species as HeV.[27,28]~~.~~ Nucleic acid based detection studies have identified related henipavirus species, including complete genomic sequences,[29,30]~~,~~ but HeV, NiV, and CedV are the only virus isolates reported.

We have recently optimized a recombinant CedV encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications and have identified several inhibitory compounds that were further validated with the infectious NiV-Bangladesh (NiV-B) strain. The validated compounds provide the foundation for the current application.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of this proposal's investigators. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may will have activity across related members of henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice

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that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification, and finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole ~~inactivated~~ NiV or HeV immune sera ~~will~~ shows cross-reactivity with CedV, however such sera or even specific high-titer anti-CedV F or G ~~glycoprotein~~ antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins.[27] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and ~~it is also~~ the largest polymerase in the order *Mononegavirale*, yet it ~~still~~ shares high aa identity to NiV and HeV L protein. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; ~~the~~ aa identities of ~~the important~~ L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This overall high degree of ~~protein~~ identity, ~~overall,~~ shared between CedV~~,~~ and that of NiV and HeV, suggests that antiviral~~ agent~~s that target ~~for example,~~ the L polymerase, could be identified and perhaps also offer the possibility of pan-henipavirus ~~antiviral~~ countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] ~~Indeed, s~~Several small molecule inhibitors have been validated in vitro using infectious HeV and NiV~~ in BSL-4~~, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support **Aim 1** to optimize our current best-in-class leads and also screen large libraries of antiviral compounds.

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Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[31-33] Nanobodies can be isolated using the same phage library construction methods and phage panning procedures routinely employed for mAb discovery, which allows for in vitro production of various amounts and their rapid characterization. ~~In collaboration~~Nanobody development will be carried out by~~ with~~ **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels~~,~~.[10] ~~we have several l~~Iimmunized mouse cohorts are in progress ~~now underway using native-like oligomeric recombinant~~using recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in **Aim 2**. ~~in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.~~

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By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of **Aim 3**. In addition, we expect to discover, and well-characterize, batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in **Aim 3**. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

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C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet and Irina Gaisina, Chicago BioSolutions** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core Lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company

engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule ~~entry~~ inhibitors of henipaviruses~~Nipah~~.

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C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2 ~~containment~~), cell-based HTS platform to identify small molecule antivirals targeting henipavirus replication without the requirement for BSL-4 containment.[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with that of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera use guidelines and procedures at BSL-2 were reviewed and approved by the USU, Institutional Biosafety Committee (IBC) in accordance with NIH guidelines. These replication competent rCedV chimeras create a very rapid and robust surrogate infection assay for NiV-B and HeV that can be used as a neutralization ~~tool~~ or entry inhibitor assay to measure neutralizing antibodies ~~or sera~~, or as a surrogate for generating virus escape mutants to ~~F or G~~ neutralizing antibodies. Here, the F and G genes of NiV-B (2010 Faridpur isolate) and HeV (2008 Redlands isolate) were synthesized and inserted into our pOLTV5-rCedV anti-genome plasmid. For the HTS neutralization assay, a turbo-GFP gene was inserted between the CedV P and M genes. Each chimera (rCedV-NiV-B-GFP and rCedV-HeV-GFP) were rescued as previously described.[8] Other versions without reporter genes have also been prepared ~~(rCedV-NiV-B and rCedV-HeV)~~. All virus stocks are cloned and genomes confirmed by NGS. Typically, large stocks are prepared and frozen as single use aliquots. Show in **Figure 1** is an example of how these chimeras are used in a virus neutralization test, referred to as a fluorescent reduction neutralization test (FRNT) using two human NiV/HeV cross-reactive anti-G mAbs (HENV-103 and -117) as previously described.[34] Briefly, Vero 76 cells are used in black walled clear bottom 96-well plates, mAbs are serially diluted in medium, and equal volumes of medium containing rCedV-NiV-B or rCedV-HeV are added to each mAb dilution for a final concentration of 2000 PFU, incubated for 2 hrs at 37~~-~~°C. Each virus-mAb mixture (90 µl/well) is added to the pre-seeded Vero 76 cells in triplicate and the assay run for just another 24 h followed by 4% formaldehyde fixation, washing and then scanning using a CTL S6 analyzer. Fluorescent foci ~~were~~ are counted using the CTL Basic Count™ and an IC_{50} is determined as the antibody concentration ~~at which there was a~~yielding 50% reduction in fluorescent foci versus ~~untreated~~ control wells. **Table 1** shows the calculated ~~EC_{50}~~ IC_{50} values using rCedV chimeras vs. those determined by conventional PRNT assay with authentic NiV-B and HeV ~~in BSL-4~~, and they are remarkably within a 2-fold range.

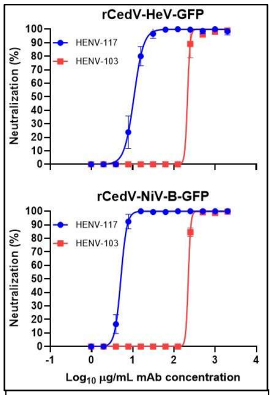


Figure 1. Neutralization curves for HENV-117 and HENV-103 mAbs against rCedV-HeV-GFP (top) and rCedV-NiV-B-GFP (bottom). Data are from a single experiment performed in technical triplicate.

IC50 (ng/mL)	HeV	rCedV-HeV	NiVB	rCedV-NiVB
HENV-103	445	216	507	238
HENV-117	14	11	15	6

Table 1. IC_{50} values of cross-reactive anti-G human mAbs HENV-103 and -117) as determined by rCedV chimera FRNT assay and NiV-B and HeV conventional PRNT assay.

In addition to conventional single agent neutralization tests, the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras can also be used in rapid HTS ~~neutralization~~ assays to measure synergistic activities of inhibitors, such as antibodies or drugs. The synergistic neutralizing activity of the HENV-103 and HENV-117 mAbs were described utilizing the rCedV chimera assay.[34] Shown in **Figure 2** is a neutralization synergy assay conducted with mAbs m102.4 and nAH1.3 (both NiV/HeV cross-reactive anti-G mAbs) where they are individually diluted 2-fold in a 7x7 concentration matrix. The diluted mAbs are added 1:1 to 4000 PFU per well of rCedV-NiV-B-GFP and the assay essentially carried out as above, with percent neutralization calculated by normalizing foci to a virus only control. Matrices are imported into SynergyFinder and analyzed Neutralization synergy was calculated by comparing treatment to virus only control wells. Values were imported into SynergyFinder software[35] using a

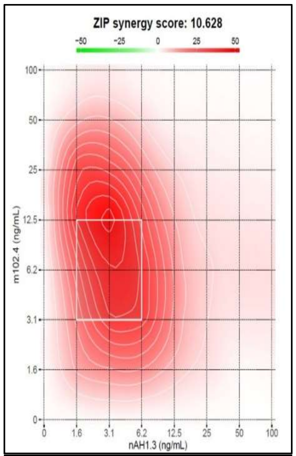


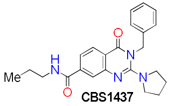
Figure 2. Synergy map with calculated synergy score using the zero interactions potency (ZIP) model generated by SynergyFinder for neutralization of rCedV-NiV-B-GFP by serially diluted m102.4 and nAH1.3 mAbs.

Zero Interactions Potency (ZIP) statistical model; delta scores >10 indicate likely synergy as shown here. ~~m102.4 and nAH1.3 indicate here.~~

Antiviral evaluation and combination testing of small molecules and nanobodies. These rCedV-based tools and rCedV chimera viruses will allow for rapid antiviral activity assessment and an ability to measure synergistic antiviral activities for both small molecule inhibitors derived from **Aim 1** and nanobodies derived from **Aim 2**; and will also allow the novel approach of testing small molecules and nanobody combinations in a rapid and quantitative manner, in vitro, to select best-in-class candidates for both monotherapeutic and combination therapeutic modalities prior to in vivo animal model testing.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called "SMART Set") was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 µM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 µM, and 47 hits showed only minimal cytotoxicity at 33 µM.

Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.

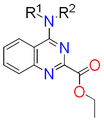


CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC$_{50}$ (µM)	**CC$_{50}$ (µM)**	**EC$_{50}$ (µM)**	**CC$_{50}$ (µM)**	**EC$_{50}$ (µM)**
0.70	100	2.1	100	3.3

In **Table 2** is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437**. Note that both HEK293T and Vero cell lines were employed in these assays, and that CC$_{50}$ values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC$_{50}$ value of 3.3 µM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 µM).

Table 3 shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14,** bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent, **CBS1437**, as well as specific plans for developing the SARs.*

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.

	N	**Compound ID**	**R^1**	**R^2**	**% inhib at 10 µM**
	**1**	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
	2	**G577-0016**	H	2,4-difluorophenyl	18
	3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
	4	**G577-0022**	H	3-methoxyphenyl	46
	5	**G577-0028**	Me	cyclohexyl	93
	6	**G577-0084**	H	4-isopropylphenyl	85
	7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
	8	**G577-0112**	Me	2-methylphenyl	94

C2.2.3. Generation of Nipah and Hendra G and F nanobodies. We recently initiated a collaborative project in nanobody development against NiV and HeV, as well as other henipavirus glycoproteins, with Dr. Jianliang Xu (**RAViD, Nanobody Core Lead**). The Xu lab has engineered and established transgenic mice that produce

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VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, and has published on the power and utility of anti-SARS-CoV-2 nanobodies(REF). We have already initiated the immunizations of several nanomouse cohorts using native-like oligomeric recombinant sF (**Veesler lab**) and sG glycoproteins (**Broder lab**) of NiV-B and HeV. Immune responses are excellent and sera measured by ELISA using the immunogens. The first of these cohorts will be processed in the coming weeks, and the Xu lab will be constructing phage libraries of polyclonal VHHs from each cohort. We except to be able to make rapid progress in **Aim 2**, in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

Commented [CB5]: Xu, J. et al. Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. Nature 595, 278-282, doi:10.1038/s41586-021-03676-z (2021).

D. RESEARCH DESIGN AND METHODS

D1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 μM) and low cytotoxicity (CC_{50} >100 μM).

D1.1. Synthetic Chemistry. In **Figure 3** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**F577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. In addition, we will optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be more chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 1** is shown a brief synopsis of the general method used for the synthesis of 4-aminoquinazolines. The specific procedures for the three steps vary, but all initiate with anthranilamides. This general route will allow us to prepare compounds with varied R_1, R_2 and R_3 groups as well as compounds with substituents that replace the carboethoxy functionality at the 2-position. Several anthranilamides are commercially available, which will allow us to initially access starting materials with a variety of R_3 groups. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[36-37] as well as other methods.[38,39] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible.

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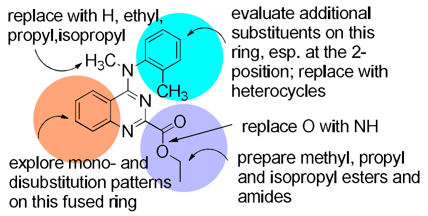
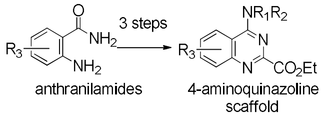
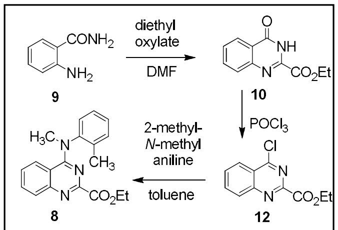


Figure 3. SAR development for 4-aminoquinazolines

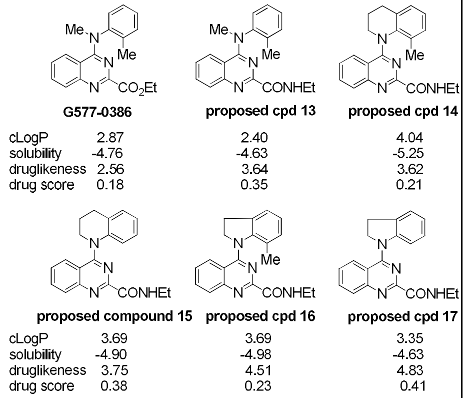


Scheme 1. General synthesis for 4-aminoquinazolines.



Scheme 2. Synthesis of **G577-0112** (cmpd **8**).

In **Scheme 2** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) produces 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline.



	G577-0386	proposed cpd 13	proposed cpd 14
cLogP	2.87	2.40	4.04
solubility	-4.76	-4.63	-5.25
druglikeness	2.56	3.64	3.62
drug score	0.18	0.35	0.21

	proposed compound 15	proposed cpd 16	proposed cpd 17
cLogP	3.69	3.69	3.35
solubility	-4.90	-4.98	-4.63
druglikeness	3.75	4.51	4.83
drug score	0.38	0.23	0.41

Figure 4. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.

In **Figure 4** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[40]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112** (compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester to an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scres have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding.

In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. Additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

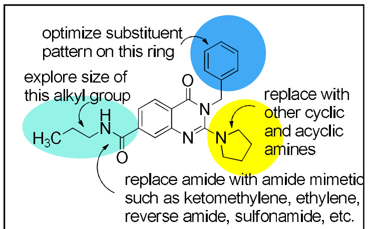


Figure 5. SAR development for **CBS1437**.

D.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5×10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at predetermined nontoxic concentrations for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation ~~(PerkinElmer, Waltham, MA) as per the manufacturer's instructions~~. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T ~~cells~~ and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 μM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

Screening Libraries. These libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of >150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions,**

Inc. *Hit Selection Algorithm.* A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used again as a specificity control. We expect that the majority of the "hit" compounds will not inhibit the VSV-G-mediated viral entry. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (potent inhibitor, EC_{50} less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. *Hit Validation, Cytotoxicity Assessment and Ranking.* We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" ~~(Promega, Madison, WI)~~ as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. *Hit Quality Control and Identity Assurance.* The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds ~~found to be~~ <80% pure will be purified and retested.

D.1.3. Evaluation of ~~protective properties~~antiviral activity of ~~the~~ advanced candidates. ~~in the henipavirus infectious assays~~ Inhibitors will be ~~done~~ tested against authentic NiV and HeV as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2 × 10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to ~~that from~~ parallel control cultures ~~with~~ of virus and DMSO ~~vehicle only~~. This work will be performed in the BSL-4 facility at UTMB (~~by the~~ **Geisbert ~~Lab (Core ?~~BSL-4 Core lead)** .

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Gaisina and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy ~~based on a~~using a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or ~~the additional~~other HTS hits identified ~~with the HTS of the~~using the expanded libraries of compounds ~~for defining lead compounds for advancement~~.

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D.2.1. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with ~~cell growth~~ medium containing either DMSO or selected furopyrimidines at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by ~~standard~~ western blot techniques or viral RNA levels by ~~quantitative real time polymerase chain reaction assays (~~q-RT-PCR~~)~~. CedV specific antibodies against ~~the attachment (~~G~~) and fusion (~~F~~) that were generated in the **Broder Lab** and antibodies to CedV-~~N and ~~CedV-~~P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene, and ~~. The one-step reaction will be~~ performed using the ~~Applied Biosystems (~~ABI~~)~~ 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies,

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HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus.** ***Target identification for small molecules.*** The rCedV tools used in ~~the small molecule inhibitor identification assays (~~**Aim 1**~~)~~ can also be exploited for antiviral target identification. In vitro selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutants~~ viruses~~. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[41,42] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a "drug resistant" virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any "drug resistant" virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L ~~polymerase~~ gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inibitors using authentic NiV-B and HeV in BSL-4 by the UTMB BSL-4 Core.

D.2.2. Assess the binding properties by surface plasmon resonance (SPR). The ~~Nipah~~ NiV polymerase (L protein and a phosphoprotein P) will be prepared using a previously published protocol. Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[43] Prefusion-stabilized RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 μM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab**.

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D.2.3. Structure determination of recombinant proteins with bound inhibitors by X-Ray crystallography and CryoEM. High-resolution structures of the ~~Nipah~~ NiV RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compound to be developed will be obtained using both x-ray crystallography and CryoEM by Dr. Vessler Lab. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands. Initial crystallization screens will be set up robotically using in-house Gryphon crystallization robotics. Biophysical techniques such as native mass spectrometry and thermal shift assays, light scattering, and fluorophore analysis to measure protein stability and folding are routine and will be used to ensure that bound compounds are not destabilizing domain structures to prevent crystallization. X-ray data collection will be done where? Crystal structures will be determined by molecular replacement using existing structures as search models. For inhibitors binding to regions other than above or if co-crystals cannot be obtained, cryo-EM will be used to determine the complex structure. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[44] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including cryoSparc[45], EMAN[46], and RELION[47]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed by molecular dynamics (MD) studies. While cryo-EM can provide access to the near-atomic resolution structures of large biomolecular systems--in their near native environment, MD can be used to reveal its dynamic behavior and refine low-resolution regions of cryo-EM reconstructions[48,49]. Furthermore, as X-ray/cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[50-54], to explore the conformational landscape[55] of ligand binding events, and to capture conformational variability of intermediate states[48,56], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches. David, please look at this section. Make necessary corrections.

Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds. *Milestone: Designate **advanced lead compounds** exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the **advanced lead compounds**.*

D.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.
Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-T properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out using the CYP3A4, and CYP2D6 Human Cytochrome P450 High Throughput Inhibitor Screening Kits (BD Gentest Corp.)~~, Woburn, MA)~~ to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[57] Compounds with IC_{50} values ≥100 μM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds by the method of Kuhnz *et al*.[58] Compounds with >30% recovery (1 h) will be considered for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed in a [^{3}H] dofetilide-binding assay as previously described~~ by Finlayson *et al*~~.[59] using SHSY5Y cells. Compounds that do not prevent [^{3}H]dofetilide binding (IC_{50} >10 μM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method ~~as described by Gres *et al*.~~[60] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P_{app}) greater than 1 × 10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels of the compound up to a solubility-limiting level in the presence or absence of rat liver enzymes (rat S9 liver fraction) acting in the normal process of metabolism and any reverse mutations will be determined. ***In vitro* mammalian chromosomal aberration test**: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes.

D.3.2. PK studies: Naïve male/female mice (equal number, 3 groups of 36 animals total) will be treated with a single dose via i.v., i.p., or oral gavage administration and blood will be collected and processed to obtain plasma and serum from three animals/group/time point at up to 12 time points through 24 h post dose. Two doses will

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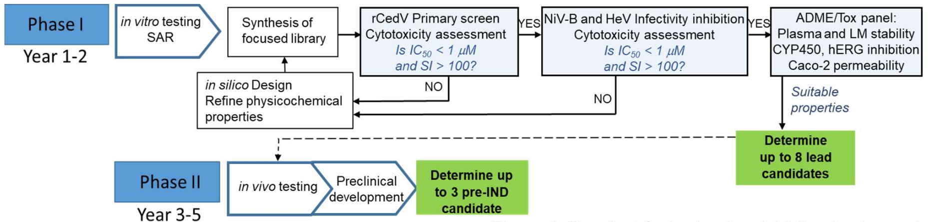

Figure 4. Flowchart for henipavirus inhibitor development

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be used to establish the drug exposure-dose range relationship: the estimated efficacious dose (ED), based upon preliminary data at 25 mg/kg i.p. or p.o. and an observable adverse effect dose (OAED) (to be determined). Compounds of interest will be extracted from biological specimens by protein precipitation, analyzed on a triple-quadruple mass spectrometer (LC-MS/MS) and quantified using an internal standard method with a minimum of a five-point calibration curve. PK parameters including AUC, C_0, C_{max}, $T_{1/2}$, T_{max}, and F(%) will be determined. Depending on the estimation of T_{max}, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc. and Reaction Biology, Corp., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim.

We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 5**).

D.4. AIM 2: Characterization of the potency and breadth of selected nanobodies.

Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. **Aim 1** will also collaborate with the ~~core???~~ UTMB, BSL-4 Core to evaluate lead candidates with authentic NiV and HeV and also in vivo in **Aim 3**. Batteries of nanobodies to the F and G glycoproteins of NiV and HeV will be isolated from phage libraries constructed using cohorts of nanomice immunized with sF and sG recombinant glycoproteins (**C2.2.3. Preliminary Data**). The Core will conduct preliminary characterizations on binding and specificities and then provide purified nanobody panels to the **Veesler** and **Broder labs** for detailed studies.

D.4.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth.

D.4.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including NiV ~~pah virus~~ (Bengladesh and Malaysia strains) and ~~Hendra~~ HeV ~~virus~~ (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[61-63] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with ~~Nipah and Hendra virus~~NiV and HeV glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

D.4.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit ~~Nipah virus and Hendra virus~~NiV and HeV, we will use a high-throughput neutralization assay ~~developed in the~~ (conducted in the **Broder lab**). This assay is based on ~~green fluorescent protein (~~GFP~~)~~-encoding, recombinant replication-competent, Cedar ~~(~~henipa~~)~~virus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bengladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins (see: C.2.1. Preliminary Data).[7,64] This platform is safe, enabling utilization at BSL-2 compared to BSL-4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the **Broder lab** recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described ~~Hendra virus~~HeV variant.[65] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond ~~Nipah and Hendra viruses~~NiV and HeV (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[66,67]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.

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Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against ~~Nipah virus and Hendra virus~~NiV and HeV. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.4.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition.

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The **Veesler lab** is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The **Veesler lab** has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software which runs 24 hours per day and enables automated targeting and image acquisition while keeping a relational database of all micrographs to expedite subsequent processing.[68] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[44,69] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[61,63,70-72] mechanical stage tilting with gold grids[73,74] or detergents to modify surface tension[75]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[73,76-80] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[81-83]

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The ~~Nipah virus~~NiV attachment (G) glycoprotein engages host receptors, is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the ~~Nipah virus~~NiV G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3) which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

D.4.2.1. High-resolution structural studies using X-ray crystallography. Some ~~of the~~ nanobodies might bind to regions that are intrinsically flexible thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[84] and BUSTER-TNT.[85]~~.~~

D.4.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[61-63,71,86,87]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the formulation of cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[88-90] and high-resolution noise substitution prior to Fourier shell correlation calculations[91] to avoid overfitting. Atomic models will be assessed using Molprobity,[92]~~,~~ EMringer[93] and Privateer.[94]~~.~~ The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails.

D.4.3. AIM 2C. Structure-guided formulation of nanobody cocktails.

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[95-97] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected in vivo, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in Aim 2A. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.5. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with Core D to evaluate lead candidates *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in a well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster ~~(refs)~~ and AGM (refs). PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV_B and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV_B and HeV infected AGM models.

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Animal Challenge Models of Henipavirus Infection and Pathogenesis
T. W. Geisbert, H. Feldmann and C. C. Broder
Curr Top Microbiol Immunol 2012 Vol. 359 Pages 153-77
DOI: 10.1007/82_2012_208

D.5.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed ~~by Core D~~by the UTMB Core. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC_{90} in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of heniavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.5.2. *In vivo* testing of lead compounds NiV/HeV infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 pfu of NiV_B or HeV (refs). Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.5.3. Define breakthrough conditions for anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antivirals fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^6$ PFU of NiV or HeV (refs). Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.5.4. Combination therapy of anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising antivirals from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^5$ PFU of NiV or HeV (refs). Treatment with at least two different antivirals will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

D.5.5. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ $5x10^6$ PFU of NiV~~-~~B or HeV as determined in henipavirus dose confirmation studies conducted by Core D (see Core D). Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by Aerosol Delivery Core~~Core X~~ (see Core X) at various times after henipavirus infection.

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D.5.6. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the MAD system as previously described (ref) or by small particle aerosol as previously described (refs) with a uniformly lethal challenge dose (~~~-~~20,000 PFU of NiV or HeV. The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by **Aerosol Delivery Core**~~Core X~~ up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

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Potential Pitfalls and Alternative Strategies. It is possible that **Aerosol Delivery Core**~~ X ~~may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antivirals may not improve protective efficacy or extend the therapeutic window of antivirals. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and ~~monoclonal antibodies~~mAbs to protect animals against lethal henipavirus disease to de-risk this Aim.

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General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues, i.e., antiviral drugs will be coded so that researchers will not know which compounds were given to which animals until experiments and analysis are completed. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the emergence and re-emergence of ~~Nipah virus~~NiV infections throughout the world, coupled with its high fatality rate, provide very significant motivation for the preparation of small molecule inhibitors of this virus to create a readiness for the next ~~Nipah virus~~NiV or other related pathogenic henipavirus outbreaks. We have discovered two differentiated series of compounds that show early promise as agents that have potential for treating ~~Nipah virus~~NiV infections, and we have designed a series of optimized druglike candidates for future preparation and development.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *N Engl J Med* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Desselberger, U. Emerging and re-emerging infectious diseases. *J Infect* **40**, 3-15 (2000).

12 Luby, S. P. & Broder, C. C. in *Viral Infections of Humans, Epidemiology and Control* (eds R. A. Kaslow, L. R. Stanberry, & J. W. Le Duc) Ch. 22, 519-536 (Springer Science+Business Media, 2014).

13 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

14 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

15 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *Med J Aust* **162**, 642-645 (1995).

16 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

17 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

18 Sweileh, W. M. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health* **13**, 9, doi:10.1186/s12992-017-0233-9 (2017).

19 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379, doi:10.3201/eid2003.131159 (2014).

20 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

21 Playford, E. G. *et al.* Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*, doi:10.1016/s1473-3099(19)30634-6 (2020).

22 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).

23 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

24 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

25 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

26 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

27 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

28 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

29 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

30 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

31 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

32 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

33 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

34 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell reports* **36**, 109628, doi:10.1016/j.celrep.2021.109628 (2021).

35 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493, doi:10.1093/nar/gkaa216 (2020).

36 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

37 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

38 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

39 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

40 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

41 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

42 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

43 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Anal Biochem* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

44 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *Journal of structural biology* **166**, 95-102 (2009).

45 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

46 Ludtke, S. J. 3-D structures of macromolecules using single-particle analysis in EMAN. *Methods in molecular biology* **673**, 157-173, doi:10.1007/978-1-60761-842-3_9 (2010).

47 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *Journal of structural biology* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).
48 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).
49 Hughes, T. E. T. *et al.* Structural insights on TRPV5 gating by endogenous modulators. *Nature communications* **9**, 4198, doi:10.1038/s41467-018-06753-6 (2018).
50 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).
51 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).
52 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).
53 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).
54 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).
55 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).
56 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).
57 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).
58 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).
59 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).
60 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).
61 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).
62 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).
63 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).
64 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).
65 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).
66 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).
67 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).
68 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).
69 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

70 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

71 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

72 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

73 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

74 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).

75 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).

76 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).

77 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).

78 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).

79 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).

80 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).

81 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).

82 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).

83 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).

84 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).

85 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).

86 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).

87 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).

88 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

89 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

90 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).

91 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).

92 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).

93 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).

94 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).

95 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).

96 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).

97 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).

From: Gaisina, Irina N on behalf of Gaisina, Irina N <igaysina@uic.edu>
To: Geisbert, Thomas W.; CHRISTOPHER BRODER
Cc: Rong, Lijun; David Veesler; norton peet; Moushimi Amaya
Subject: RE: Project 1 RAViD version for editing
Date: Wednesday, November 3, 2021 3:16:36 PM
Attachments: Tulane_Res_strategy_Nipah-Final-for-revision .docx

---

Yes, thank you.

I changed it the text and fig 6

From: Geisbert, Thomas W. <twgeisbe@UTMB.EDU>
Sent: Wednesday, November 3, 2021 2:06 PM
To: CHRISTOPHER BRODER <christopher.broder@usuhs.edu>; Gaisina, Irina N <igaysina@uic.edu>
Cc: Rong, Lijun <lijun@uic.edu>; David Veesler <dveesler@uw.edu>; norton peet <norton.peet@chicagobiosolutions.com>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>
Subject: Re: Project 1 RAViD version for editing

Please check the reference to the Aerobiology Delivery Core. I think that may be Core B and not Core A.

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Wednesday, November 3, 2021 1:56 PM
To: Gaisina, Irina N <igaysina@uic.edu>
Cc: Rong, Lijun <lijun@uic.edu>; David Veesler <dveesler@uw.edu>; Geisbert, Thomas W. <twgeisbe@UTMB.EDU>; norton peet <norton.peet@chicagobiosolutions.com>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>
Subject: Re: Project 1 RAViD version for editing

if all OK with you ALL
then i am going to make the edits to
cut off 6 lines of text
and return to Irina

On Wed, Nov 3, 2021 at 2:49 PM Broder, Christopher <christopher.broder@usuhs.edu> wrote:

> going over it now
>
> On Wed, Nov 3, 2021 at 2:40 PM Gaisina, Irina N <igaysina@uic.edu> wrote:
>
> > Please use this one for final review.

One more time…

From: Rong, Lijun <lijun@uic.edu>
Sent: Wednesday, November 3, 2021 1:37 PM
To: David Veesler <dveesler@uw.edu>
Cc: Broder, Christopher <christopher.broder@usuhs.edu>; Gaisina, Irina N <igaysina@uic.edu>; twgeisbe@utmb.edu; norton peet <norton.peet@chicagobiosolutions.com>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>
Subject: RE: Project 1 RAViD version for editing

No we are cool
Thank you!

LR

Lijun Rong, PhD
Professor
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From: David Veesler <dveesler@uw.edu>
Sent: Wednesday, November 3, 2021 10:35 AM
To: Rong, Lijun <lijun@uic.edu>
Cc: Broder, Christopher <christopher.broder@usuhs.edu>; Gaisina, Irina N <igaysina@uic.edu>; twgeisbe@utmb.edu; norton peet <norton.peet@chicagobiosolutions.com>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>
Subject: Re: Project 1 RAViD version for editing

The current text looks great. Do we need to cut it further?

On Wed, Nov 3, 2021 at 9:41 AM Rong, Lijun <lijun@uic.edu> wrote:

> Once Irina has a new version, I can cut some in moa studies
>
> Sent from my iPhone
>
> > On Nov 3, 2021, at 8:24 AM, Broder, Christopher <christopher.broder@usuhs.edu> wrote:

> > > Yes this is looking great guys.
> > >
> > > We need to be orderly.
> > > Perhaps Irinia shrink figures a bit and reduce font sizes in AIm 1 figures.
> > > Figs in preliminary data could be a tad smaller. and the sG structure reduce a bit as well
> > > Do anything else to cut down text. Make a final formatted version as soon as you can do it,
> > >
> > > Then send to all of us.   But in the meantime we can look at this version and mark your own changes in your own version
> > > to get a head start,   then move your edits into her version when we get it.
> > >
> > > On Tue, Nov 2, 2021 at 11:57 PM Rong, Lijun <lijun@uic.edu> wrote:
> > >
> > > > Dear all,
> > > >
> > > > This is a well prepared document and I really like it. I did not try to cut it yet, but if we want to cut, we can shorten the MOA studies for small molecules. Maybe David can shorten the MOA studies for nanobodies?
> > > >
> > > > Will work with you all to make a great project tomorrow.
> > > >
> > > > Best,
> > > > Lijun
> > > >
> > > > Lijun Rong, PhD
> > > > Professor
> > > > Dept of Microbiology and Immunology
> > > > College of Medicine
> > > > University of Illinois at Chicago (UIC)
> > > > 8133 COMRB, 909 S Wolcott Ave
> > > > Chicago, IL 60612
> > > > 312-355-0203 (office phone)
> > > > lijun@uic.edu
> > > >
> > > > ---
> > > >
> > > > **From:** Gaisina, Irina N <igaysina@uic.edu>
> > > > **Sent:** Tuesday, November 2, 2021 7:34 PM
> > > > **To:** Rong, Lijun <lijun@uic.edu>; Broder, Christopher <christopher.broder@usuhs.edu>; twgeisbe@utmb.edu
> > > > **Cc:** norton peet <norton.peet@chicagobiosolutions.com>; Moushimi Amaya

<moushimi.amaya.ctr@usuhs.edu>; David Veesler <dveesler@uw.edu>
Subject: RE: Project 1 RAViD version for editing

Please use this one for editing.

We will format the tables and figures afterwards.

Thanks

From: Rong, Lijun
Sent: Tuesday, November 02, 2021 5:27 PM
To: Gaisina, Irina N <igaysina@uic.edu>
Cc: Broder, Christopher <christopher.broder@usuhs.edu>; twgeisbe@utmb.edu; norton peet <norton.peet@chicagobiosolutions.com>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>; David Veesler <dveesler@uw.edu>
Subject: Re: Project 1 RAViD

Hi Irina,

Pls send us the updated version with everything incorporated so we can edit. Thank you!

LR

Sent from my iPhone

> On Nov 2, 2021, at 4:41 PM, Gaisina, Irina N <igaysina@uic.edu> wrote:
>
> I noticed that NiV-B was replaced with NiV_B in aim 3 (same way as "NiV Bangladesh (NiV_B)" in Doyle's publication).
> Should we change it everywhere in the text?
>
> ---
>
> **From:** Gaisina, Irina N
> **Sent:** Tuesday, November 02, 2021 4:03 PM
> **To:** Rong, Lijun <lijun@uic.edu>; David Veesler <dveesler@uw.edu>
> **Cc:** Broder, Christopher <christopher.broder@usuhs.edu>; norton peet <norton.peet@chicagobiosolutions.com>; twgeisbe@utmb.edu; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>; Gaisina, Irina N <igaysina@uic.edu>

Subject: RE: Project 1 RAViD

Here is the intermediate version – for everyone to see what we have – where we keep everything as Lijun suggested.

my next task will be to replace the yellow section.

You can still use this version for any additional corrections and comments.

thanks

From: Rong, Lijun
Sent: Tuesday, November 02, 2021 3:44 PM
To: David Veesler <dveesler@uw.edu>
Cc: Gaisina, Irina N <igaysina@uic.edu>; Broder, Christopher <christopher.broder@usuhs.edu>; norton peet <norton.peet@chicagobiosolutions.com>; twgeisbe@utmb.edu; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>
Subject: Re: Project 1 RAViD

David,

Do not worry about space now. We will do it once we have the entire doc.

LR

Sent from my iPhone

> On Nov 2, 2021, at 3:37 PM, David Veesler <dveesler@uw.edu> wrote:
>
> I was thinking of having it near the description of the cryoEM work.
>
> As space is limited, I added headings to avoid adding more text. I can provide a caption but it will take space. Just let me know
>
> Sent from my iPhone
>
>> On Nov 2, 2021, at 3:58 PM, Rong, Lijun <lijun@uic.edu> wrote:

I think once we have the text, we can decide how to cut it.
My understanding is Chris will edit the first part of the strategy?
The project is looking better and better.

LR

Lijun Rong, PhD
Professor
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From: Gaisina, Irina N <igaysina@uic.edu>
Sent: Tuesday, November 2, 2021 11:54 AM
To: Rong, Lijun <lijun@uic.edu>; David Veesler <dveesler@uw.edu>; Broder, Christopher <christopher.broder@usuhs.edu>
Cc: norton peet <norton.peet@chicagobiosolutions.com>; twgeisbe@UTMB.EDU; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>
Subject: RE: Project 1 RAViD

Where do you want it to be? Pls indicate paragraph ID (ex. C1.2.....)
Do you have a fig legend or a short description to be incorporated in the text?

From: Rong, Lijun <lijun@uic.edu>
Sent: Tuesday, November 2, 2021 2:50 PM

To: David Veesler
<dveesler@uw.edu>; Broder,
Christopher
<christopher.broder@usuhs.edu>
Cc: Gaisina, Irina N
<igaysina@uic.edu>; norton peet
<norton.peet@chicagobiosolutions.com>; twgeisbe@UTMB.EDU;
Moushimi Amaya
<moushimi.amaya.ctr@usuhs.edu>
Subject: RE: Project 1 RAViD

David and Chris,

I love it!
Irina, pls add it and will see how to shorten other parts. As Chris said, Aim 1 is too long.

LR

Lijun Rong, PhD
Professor
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From: David Veesler
<dveesler@uw.edu>
Sent: Tuesday, November 2, 2021
11:29 AM
To: Broder, Christopher
<christopher.broder@usuhs.edu>
Cc: Gaisina, Irina N
<igaysina@uic.edu>; norton peet
<norton.peet@chicagobiosolutions.com>; Rong, Lijun <lijun@uic.edu>;
twgeisbe@UTMB.EDU; Moushimi
Amaya
<moushimi.amaya.ctr@usuhs.edu>

Subject: Re: Project 1 RAViD

How about that?

<image001.jpg>

On Tue, Nov 2, 2021 at 2:53 PM Broder, Christopher <christopher.broder@usuhs.edu> wrote:

> David
> it would be awesome yes, but we have to cut text in AIM 1
> insert were you mention nAH1.3?
>
> On Tue, Nov 2, 2021 at 2:48 PM David Veesler <dveesler@uw.edu> wrote:
>
> > Hi Chris,
> >
> > Is there room for me to include a figure about NiV G?
> >
> > David
> >
> > On Tue, Nov 2, 2021 at 2:08 PM Broder, Christopher <christopher.broder@usuhs.edu> wrote:
> >
> > > Hi all
> > >
> > > Irina,
> > >
> > > As I mentioned this AM, **please use this attached tracked version. There are many edits.** Incorporate Norton's into this one.
> > >
> > > We are down to details and every edit helps with space

and length

As soon as Irina gets another version everyone should please at least check their sections. I have added the missing sections on nanobody development and sources
to preliminary data and to Aim 2 Vessler.

Then we need to remove all hanging sentences to tighten and save space

I am rewriting the background (Yellow highlighted here) as I said earlier. It is not
the version from Sunday and has major errors.

General, Aim #1 needs to be cut down, either by detail or removing some figures,
It is too long and out of balance with Aim 2 and 3 in scope.

Do you need refs for Tom's section Aim 3 ? I can do that also

Did all of you send your consortium letter of agreement to Chris and Bob?

Irina is doing this lifting here now, if someone wants to make
a list of bullets of the major AIMS and SubAIMS we can sent
to Bob and Luis and Luis said

he make our missing Gantt chart for us
We need this or the attached ppt file list

CB

--

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SUMMARY STATEMENT
(Privileged Communication)

PROGRAM CONTACT:
Eun-Chung Park
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Release Date: 11/02/2021
Revised Date:

Application Number: 1 R01 AI168287-01

Principal Investigator

GURLEY, EMILY SUZANNE

Applicant Organization: JOHNS HOPKINS UNIVERSITY

Review Group: CRFS
Clinical Research and Field Studies of Infectious Diseases Study Section

Meeting Date: 10/21/2021
Council: JAN 2022
Requested Start: 04/01/2022
RFA/PA: PA20-185
PCC: M32A

Project Title: Solving Opportunities for Spillover (SOS): Frequency and Mechanisms of Cross-species Transmission of Henipaviruses in Bangladesh
SRG Action: Impact Score:31 Percentile:21
Next Steps: Visit https://grants.nih.gov/grants/next_steps.htm
Human Subjects: 30-Human subjects involved - Certified, no SRG concerns
Animal Subjects: 10-No live vertebrate animals involved for competing appl.
Gender: 1A-Both genders, scientifically acceptable
Minority: 5A-Only foreign subjects, scientifically acceptable
Age: 1A-Children, Adults, Older Adults, scientifically acceptable

Project Year	Direct Costs Requested	Estimated Total Cost
1	456,749	601,580
2	454,825	599,046
3	454,577	598,719
4	454,477	598,587
5	454,632	598,792
TOTAL	2,275,260	2,996,724

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.
EARLY STAGE INVESTIGATOR
NEW INVESTIGATOR

1R01AI168287-01 GURLEY, EMILY

EARLY STAGE INVESTIGATOR

NEW INVESTIGATOR

BIOHAZARDS

SELECT AGENTS

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

RESUME AND SUMMARY OF DISCUSSION: This application proposes to investigate the drivers of spillover events of henipaviruses from bats to domesticated animals to humans in Bangladesh. The characterization of the routes of transmission and molecular and serological analyses to assess exposure to henipaviruses is highly significant and addresses an important knowledge gap. The Principal Investigator (PI) and the investigative team are strong with complimentary expertise and a record of previous successful collaborations and are well-positioned to carry out the proposed studies. Additional strengths of this well put together application are its data collection and analysis and the use of camera trapping observations to determine interactions between domesticated animals with bats. A number of weaknesses were identified. Some reviewers expressed concern about the lack of detail for the secondary antibodies in the multiplex assay or what the expected benchmarks are for success of this assay. There was also considerable concern related to the biosafety level of the henipavirus, which is a BSL-3/BSL-4 pathogen. Questions were raised whether this was adequately addressed in the application. For example, inactivation of the samples, the human transmission risk to study personnel handling the virus in the different work environments, and appropriate biohazard containment at one of the institutions that only has a BSL-2 facility. Overall, the weaknesses dampened the enthusiasm of the review committee.

DESCRIPTION (provided by applicant): Until and unless we better understand and prevent spillovers of bat-borne viruses into intermediate hosts and humans, we will be severely limited in our ability to stop pandemics. Henipaviruses are bat-borne, RNA viruses that spillover through this route. Nearly half of all reported human henipavirus infections have been associated with contact with sick domesticated animals, though our understanding of drivers of henipavirus spillovers and specific transmission pathways remains severely limited. Building on preliminary data about bat, domesticated animal and human infections, this multidisciplinary study integrates epidemiology, ecology, and anthropology, will identify spillover pathways for henipaviruses into domesticated animals in Bangladesh and the risk they pose to human health. Our first aim is to identify drivers of henipavirus spillovers into domesticated animals in Faridpur, Bangladesh. We will use multiplex pan-henipavirus assays to identify infections in Pteropus medius bats and domesticated animals living nearby this roost through cross-sectional and prospective studies. Combined with intensive studies of bat-domesticated animal interactions and weather data, we will build statistical models to identify the relative contribution of each of these factors. Our second aim is to describe which henipaviruses are being transmitted from bats to domesticated animals. Serologic studies of animals in Bangladesh show that they are frequently infected with non-Nipah henipaviruses. Through surveillance for and sampling of sick domesticated animals, we will describe the specific viruses that spillover from bats in Faridpur. Our third aim is to determine the risk of henipavirus transmission from domesticated animals to humans. We hypothesize that undetected henipavirus spillovers in humans are occurring through contact with sick domesticated animals and will conduct cross-sectional and prospective serosurveys of humans who have close contact with sick animals in Faridpur. We remain ignorant about henipavirus spillovers through intermediate hosts – including the specific viruses spilling over, the frequency and distribution of spillovers, and the pathways of transmission – at our own peril. The knowledge gained from this study will be immediately applicable to human and animal health programs in Bangladesh and other countries where henipaviruses circulate in bats. By learning about which henipaviruses infect humans, and how they are infected, we can advise public health surveillance programs on how to optimize detection and epidemiologic investigation of cases across Bangladesh. Our investigations about spillovers in Faridpur can also be scaled- up to other areas of Bangladesh and countries where henipaviruses circulate in bats so that we can truly begin to appreciate the scale of henipavirus spillovers in the global landscape.

PUBLIC HEALTH RELEVANCE: We remain ignorant about bat henipavirus spillovers through intermediate hosts – including the specific viruses spilling over, the frequency and distribution of spillovers, and the pathways of transmission – at our own peril. Until and unless we better understand and prevent spillovers of bat-borne viruses into intermediate hosts and humans, we will be severely limited in our ability to stop pandemics. The knowledge gained from this study will be immediately applicable to human and animal health programs because if we know which henipaviruses infect humans and domesticated animals, and how they are infected, we can advise public health surveillance programs on how to optimize detection and prevention of infections.

CRITIQUE 1:

Significance: 2
Investigator(s): 1
Innovation: 2
Approach: 2
Environment: 1

Overall Impact: The proposed project aims to improve our current understanding of Henipavirus spillovers from animals to humans. Henipaviruses are a highly relevant viral group that have been associated with significant mortality outbreaks in humans. Importantly, in most cases human infections have been associated to contact with an animal reservoir including wild animals (e.g., bats) or domestic livestock species (e.g., pigs). The proposed study will investigate risk factors and drivers of henipavirus spillovers into humans, identify which henipaviruses are being transmitted to humans and determine the risk of transmission of henipavirus from domestic animals to humans. The project will be conducted in a henipavirus endemic area in Bangladesh and will involve field epidemiological investigations as well as prospective sample collection and testing of both human and animals in close contact. This is a highly relevant study, especially when considering the context of the current COVID-19 pandemic. Identification and characterization of pathways of henipavirus spillover is critical to devise strategies that could help to prevent the next epidemic or pandemic.

1. Significance:

Strengths

- This is a field study that will investigate spillover events of henipaviruses from animals to humans in a setting that has seen multiple outbreaks in humans.
- The study will involve field epidemiology-, ecology-, and anthropology and will focus on sampling of both humans and animals that live in henipavirus endemic areas.
- The investigators propose to look at multiple animal species that are raised in the target study area in Bangladesh.
- Outcomes of the study will clearly help and improve our understanding of the factors and risks associated with spillover events of henipaviruses into humans.
- The study will identify the henipaviruses that have the potential to spill over into humans.

Weaknesses

- Most henipavirus outbreaks described to date, have been linked to palm tree sap contact or ingestion. The study will target several domestic animal species that may not serve as intermediate hosts.

2. Investigator(s):

Strengths

- The team of investigators is very strong and have a good track record of collaboration.
- Experience in conducting the proposed field epi studies is well documented and the PI and Co-PI have active and ongoing collaborations.
- The PI has first-hand knowledge of the institution in Bangladesh that will be conducting the field trial in the henipavirus endemic area.

Weaknesses

- None noted.

3. Innovation (2):

Strengths

- The study will undertake an interdisciplinary approach to dissect and improve understanding of spillover events of henipaviruses between animals and humans.
- This will involve field-based surveillance in both humans and animals.

Weaknesses

- None noted.

4. Approach:

Strengths

- The project will include epidemiological investigations, field observation of animal and human behavior and sample collection and testing.
- Molecular and serological tests will be used to assess exposure to known henipaviruses.
- Epidemiological data will be linked to behavioral data and testing data collected in the field.

Weaknesses

- The assays employed will only target known nipaviruses. The study and work would benefit from unbiased pathogen detection approaches (e.g., sequencing) at least in samples collected from sick people and humans or a subset of those.

5. Environment:

Strengths

- The infrastructure available in the U.S. and in Bangladesh are excellent and the team has a good track record of collaboration and international interactions.

Weaknesses

- None noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- Well-articulated human subjects document was provided and all potential issues related to protection of human subjects has been well thought out.

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically
- Race/Ethnicity: Distribution justified scientifically
- For NIH-Defined Phase III trials, Plans for valid design and analysis:
- Inclusion/Exclusion Based on Age: Distribution justified scientifically

Vertebrate Animals:

Yes, all criteria addressed

- Use of animals in the project is well justified.

Biohazards:

Acceptable

- All institutions involved have the infrastructure in place to handle potentially zoonotic henipaviruses. Work in the U.S. will be conducted at a high biocontainment facility.

Applications from Foreign Organizations:

Justified

- Well-articulated and justified plan to collaborate on with an institution located in a henipavirus endemic area.

Select Agents:

Acceptable

Resource Sharing Plans:

Acceptable

- Resource sharing plan well described and follows NIH guidelines.

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2:

Significance: 3
Investigator(s): 3
Innovation: 3
Approach: 6
Environment: 7

Overall Impact: This is an application from a complementary team of investigators led by an experienced early-stage investigator working in an scientific environment that is partially suitable to the project. The project is highly significant and innovative. The team proposes to identify drivers of henipavirus spillovers into domesticated animals, describe which viruses are being transmitted from bats to domesticated animals and lastly, they will serosurvey people to predict possible transmission. Swabs, urine, and blood samples will be collected from bats and from domesticated animals. A major weakness was identified in the Approach as the project entails a human transmission risk to the personnel conducting the study in the field in Bangladesh and appropriate biocontainment conditions are not listed for the sample collection part of the work. Benchmarks for success are not discussed. The likelihood that this project will exert a sustained powerful influence in the field is moderate.

1. Significance:

Strengths

- The significance of this project is very high. It addresses a critical barrier to progress in the field: understanding the nature and frequency of spillover events of RNA viruses which have high genomic mutation rates that favor onward transmission into humans. The goal of the current application is to focus on transmission of henipaviruses (which include Nipah and Hendra viruses) from bats to domesticated animals to humans.
- The work described will address gaps in knowledge in the field and there is strong scientific premise for the project. They propose to use a multiplex pan-henipavirus assay to investigate exposure to these infections across multiple animal species, including humans.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- This early-stage investigator PI is well suited to this project in terms of experience, expertise and leadership.
- The collaborators have complementary and integrated expertise.
- All established investigators have ongoing records of accomplishments that have advanced the field.

Weaknesses

- None noted.

3. Innovation:

Strengths

- The application challenges current research paradigms as it attempts to simultaneously identify and explain transmission pathways of henipaviruses from bats to intermediate hosts and from intermediate hosts to humans.
- Current human henipaviruses surveillance exclude human infections acquired through contact with sick animals.

Weaknesses

- None noted.

4. Approach:

Strengths

- The team proposes to identify drivers of henipavirus spillovers into domesticated animals, describe which viruses are being transmitted from bats to domesticated animals and lastly, they will serosurvey people to predict possible transmission. Swabs, blood, and urine samples will be collected from bats (urine to check for virus shedding) and from domesticated animals.
- The overall strategy and analysis are well reasoned, and potential problems of the study are discussed for each aim.
- A study timeline/milestones is provided.

Weaknesses

- One major weakness was identified in the methodology. Nipah Virus is a select agent BSL-3/BSL-4 pathogen and Nipah virus shedding in urine has been detected repeatedly in longitudinal surveys of bats in Faridpur District in Bangladesh. Although in the select agent section there’s one sentence on sample collection into a solution containing trizol, there’s not enough biocontainment discussed in the approach to reassure that this work will not inadvertently produce a patient zero for a new pandemic virus. This project entails a considerable human transmission risk to the personnel conducting the study in the field due to exposure to bat excretions and large numbers if multiple infected domestic animals and specific measures for personal protection are not mentioned in the Approach for Aims 1 and 2, which are the major risk factors for collection of samples at the interface of bat/human and infected domesticated animal/human.
- Benchmarks for success are not discussed.

5. Environment.

Strengths

- None noted.

Weaknesses

- The associated institution in Bangladesh does not have appropriate biosafety level facilities (minimum BSL3) to handle the samples they propose to collect and test.
- Equipment is not listed.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- Human serosurveys in native environment.

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically
- Race/Ethnicity: Distribution justified scientifically
- For NIH-Defined Phase III trials, Plans for valid design and analysis: Not applicable
- Inclusion/Exclusion Based on Age: Distribution justified scientifically
- Males, females, children, non-Hispanic Asians will be included.

Vertebrate Animals:

Yes, all criteria addressed

- The face page of the application states No Vertebrate Animals but they addressed this issue.

Biohazards:

Unacceptable

- They propose to capture and sample swabs, urine, and blood from bats and domesticated animals in Bangladesh and use multiplex pan-henipavirus assays to identify infections in bats and domesticated animals. Henipaviruses pose a meaningful pandemic threat. Since there will be exposure of humans to potentially new unknown bat viruses, and they will acquire samples in solution containing trizol before being processed in BSL2 at least a plan to safeguard the personal protection of the people doing the field work needs to be explained.

Applications from Foreign Organizations:

Justified

- Work to be conducted in Bangladesh.

Select Agents:

Unacceptable

- Nipah virus is a select agent to be handled in BSL-3/4 conditions. The facility in Bangladesh that will handle samples at interfaces of bat/human can only handle BSL-2 containment.

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Unacceptable

- All the secondary antibodies used for each one of the species proposed for serosurveys, including bats, must be authenticated. This section was not addressed.

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3:

Significance: 2
Investigator(s): 1
Innovation: 2
Approach: 2
Environment: 1

Overall Impact: The investigators propose a study to investigate routes of spillover of henipaviruses from bats to domestic animals to humans. The study is justified by prior work suggesting that a key route of exposure to henipaviruses is via domestic animals and a gap in the scientific understanding of what contributes to these spillover events. The research includes the collection of ecological, environmental, and anthropological data, as well as sero-surveillance in bats, domestic animals and humans, and sequencing virus obtained from bats and sick domestic animals. The study is well-designed to address the research questions both in terms of the study location, the excellent research team, and the design of data collection and analysis. The investigator's greatest challenges are the many unknowns about henipavirus spillovers which will require the team to be agile (the proposal has well-reasoned contingency plans). However, this is also strong justification for the proposed work. The team is well positioned to carry-out the proposed research and improve our understanding of henipavirus transmission.

1. Significance:

Strengths

- Henipaviruses are a model system for studying spillover of viruses into human populations. The proposed research will shed light on pathways by which henipaviruses move from wild to domestic animal populations and cross over to humans.

- The proposed research can help inform how to improve surveillance systems for emerging henipaviruses, which are currently focused, at least in Bangladesh, on human symptomatic surveillance.

Weaknesses

- The generalizability to other emerging pathogens is unclear as the spillover pathways may vary by pathogen. Nonetheless the wild animal to domestic animal to human pathway has historically been a key route of disease emergence.

2. Investigator(s):

Strengths

- The investigator team is well-positioned to conduct the proposed research.

Weaknesses

- None noted.

3. Innovation:

Strengths

- The proposed research focuses on the wild animal/domestic animal interface which is an important but poorly characterized route of pathogen emergence.
- The integration of diverse data streams is novel and can help fill gaps left by studies focused only on a single host species.

Weaknesses

- None noted.

4. Approach:

Strengths

- The bat and domestic animal surveillance is well-designed, providing temporal data.
- The proposed analyses clearly make use of the data collected and are appropriate for the study aims.
- The qualitative studies in SA1 will add a rich data source that is particularly important given the number of unknowns.
- Camera trapping to assess animal contacts is a key strength to allow objective measurement of this feature.
- The sequencing and phylogenic analysis of samples collected from bats and domestic animals is a key strength and will allow the researchers to assess the relatedness of viruses found in animal and bat populations.

Weaknesses

- The investigators may want to consider chipping or tagging bovines, as they are harder to consistently identify over time than they indicate in their proposal.
- It is unclear how/whether mobile animal or human populations such as the mobile pigs they mention will be surveyed as it is plausible they play a role in transmission.

5. Environment:

Strengths

- The study location is excellent as it is likely a region with concentration of henipaviruses and high human – domestic animal – bat exposures.

Weaknesses

- None noted.

Protections for Human Subjects:

Acceptable Risks and/or Adequate Protections

- Acceptable

Inclusion Plans:

- Sex/Gender: Distribution justified scientifically
- Race/Ethnicity: Distribution justified scientifically
- For NIH-Defined Phase III trials, Plans for valid design and analysis: Not applicable
- Inclusion/Exclusion Based on Age: Distribution justified scientifically
- Will restrict to ages 5 and older. This is scientifically and logistically justified.

Vertebrate Animals:

Yes, all criteria addressed

- Adequate protections for all field staff to prevent virus exposure when handling animals will be paramount.

Biohazards:

Acceptable

- This is addressed in the select agent section.

Applications from Foreign Organizations:

Justified

- There is a partnership with ICDDR,B in Bangladesh which is necessary for the proposed research.

Select Agents:

Acceptable

- The project involves works with Nipah and other henipaviruses.

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:
Acceptable

Budget and Period of Support:
Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS: Acceptable

INCLUSION OF WOMEN PLAN: Acceptable

INCLUSION OF MINORITIES PLAN: Acceptable

INCLUSION ACROSS THE LIFESPAN: Acceptable

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

BIOHAZARDS: Unacceptable

SELECT AGENTS: Unacceptable

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES: Unacceptable

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Footnotes for 1 R01 AI168287-01; PI Name: Gurley, Emily Suzanne

NIH has modified its policy regarding the receipt of resubmissions (amended applications).See Guide Notice NOT-OD-18-197 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER

Clinical Research and Field Studies of Infectious Diseases Study Section
Infectious Diseases and Immunology B Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW
CRFS
10/21/2021 - 10/22/2021

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-14-073 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-073.html, NOT-OD-15-106 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-106.html, and NOT-OD-18-115 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-115.html, including removal of the application from immediate review.

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

From: Emily Gurley on behalf of Emily Gurley <egurley1@jhu.edu>
To: clifton.mckee; islam_ausraf@icddrb.org; Dr. Mohammed Ziaur Rahman; Laing, Eric; Broder, Christopher; sluby@stanford.edu; Henrik Salje; Plowright, Raina; Munster, Vincent (NIH/NIAID) [E]
Subject: comments on henipavirus RO1
Date: Wednesday, November 3, 2021 12:13:40 PM
Attachments: 1R01AI168287-01.pdf

Colleagues,

We've received scores and comments on our RO1. Score was 31, 21st percentile. We need to be around 10-14th percentile to be funded. So, not too bad for our first submission.

Two reviewers scored the proposal very high. Reviewer #2 is very cranky about biosafety issues. Hopefully, we can address these in the next submission. I'll meet with the Program Officer soon to see if she has any insights.

There are also some concerns about validating the serology tests, which was predictable. Perhaps we can think of ways to address that as well.

The plan is to resubmit at the next deadline in Feb.

Feeling hopeful.
Emily

The pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV) cause severe respiratory illness and encephalitis in both animals and humans with human case fatality rates ranging from 40-100%. The NiV-Bangladesh strain (NiV-B) is associated with multiple rounds of human-to-human transmission and is a pathogen of pandemic concern for which there are no vaccines or antivirals approved for human use. We have recently shown that the non-pathogenic Cedar henipavirus (CedV) is useful surrogate platform for high throughput screening (HTS) for antiviral drug discovery, and for virus neutralization assays. This proposal focuses on developing antiviral small molecule and nanobody countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach. We created reporter gene-encoding recombinant CedVs and discovered a series of fused heterocycles, e.g., 4-aminoquinazoline-based, antivirals, and showed that a representative quinazoline CBS1435 was an effective inhibitor of rCedV replication and also both NiV-B and HeV, with EC_{50}s in the low μM range and minimal cytotoxicity. Neutralizing antibodies to the henipavirus F and G glycoproteins have also shown therapeutic promise, but antiviral nanobodies as potential immunotherapeutics represent a novel area of NiV and HeV countermeasure development. This proposal will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Aim 1 will focus on optimizing lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. Lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed by chemical modifications aimed to improve drug-like properties, increase potencies, decrease toxicity, and improve pharmacokinetic properties. Aim 2 will isolate and fully characterize nanobodies broadly neutralizing NiV-B and HeV derived by immunization of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using phage display technology of cloned nanobody gene fragments. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails. Aim 3 will focus on *in vivo* efficacy evaluation of lead compounds and nanobodies in animal models. Both monotherapies and combination therapeutic modalities will be tested, along with testing antiviral nanobody delivery by aerosol. These two unique henipavirus-targeted antiviral development directions will generate novel countermeasures that may display more broad effectiveness.

Commented [d1]: This is unlikely to be the case (at least for the nanobodies)

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] 22 cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antivirals approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus non-pathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 µm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will potentially yield testable antivirals with potential pan-henipavirus effectiveness. Our collaborative team also has an extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and also aerosol delivery of nanobodies. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobodies studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

Over the past 40 years the major etiological agents of emerged or identified infectious diseases in humans have been viruses.[11] Among these, the paramyxoviruses, which include many important human and animal pathogens, also include two emerged, zoonotic viral pathogens of importance: the henipaviruses; Hendra virus (HeV) and Nipah virus (NiV)[12,13]. In 1994, HeV caused an outbreak of severe respiratory disease in horses in Brisbane, Australia, resulting in the deaths of 14 horses and their trainer.[14,15] NiV was the causative agent of a significant outbreak of severe encephalitis in pig farmers in Malaysia in 1998. Spread of NiV infections typically requires direct contact with an infected source, and transmission can occur between people and from animals to people. Symptoms may culminate in a severe respiratory and/or neurological disease that is often fatal. Complications of the infection can result in relapsed encephalitis following recovery. On September 5, 2021, a twelve-year-old boy in the Indian state of Kerala died after contracting the Nipah virus. During the process of transporting him to medical centers for a diagnosis, twenty-three health care workers exposed to this boy also died, which underscores both the transmissability and the lethal nature of this virus. *There is presently no vaccine or small molecule therapeutics that have been developed for NiV infections.* Pteropid bats appear to be the major reservoir hosts for henipaviruses[6,16]. HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bat hosts. Among these animals, HeV and NiV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with human fatality rates ranging from 40-100%[2,17].

NiV and HeV are classified as biosafety level-4 (BSL-4) restricted pathogens and both remain transboundary threats to livestock and people throughout South Asia and Australia. NiV and henipaviral diseases have been categorized by the World Health Organization (WHO) as an epidemic threat needing urgent research and countermeasure development and are included in the WHO R&D Blueprint list of priority pathogens with epidemic potential [18]. Presently, there are no prophylactic or therapeutic treatment options for henipavirus infections approved for use in humans. A subunit vaccine, Equivac® HeV, has been available for use in horses in Australia since 2012 [19] for the prevention of HeV infection. This equine vaccine received full registration by the Australian Pesticides and Veterinary Medicines Authority in 2015, with all vaccinated horses receiving a microchip with a database being maintained and is the first commercially deployed vaccine against a BSL-4 agent [20]. Although a human monoclonal antibody (mAb) m102.4 has successfully completed a phase I safety trial in Australia [21], developing vaccines or therapeutics against henipaviruses for use in people remains a high priority. Henipaviruses are single-stranded, negative sense, enveloped RNA viruses with two membrane anchored glycoproteins involved in virus entry; one mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and also the main focus of several vaccine strategies[2]. Although an effective post-exposure human monoclonal antibody (mAb) therapy (anti-NiV/HeV G glycoprotein mAb m102.4) has been extensively examined[22-26], it must be administered within several days following lethal infection in animal challenge models. In contrast, however, antiviral drug discovery for HeV and NiV has been significantly hampered due to requirements of biosafety level-4 (BSL-4) containment. Recently, a third isolate, namely, Cedar virus (CedV) has been added to the *Henipavirus* genus[27]. However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease and resides in nature in the same bat host species as HeV[27,28]. Nucleic acid based detection studies have identified related henipavirus species, including complete genomic sequences[29,30], but HeV, NiV, and CedV are the only virus isolates reported.

We have recently optimized a recombinant CedV encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications and have identified several inhibitory compounds that were further validated with the infectious NiV-Bangladesh (NiV-B) strain. The validated compounds provide the foundation for the current application.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of this proposal's investigators. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may will have activity across related members of henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice

that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification, and finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole inactivated NiV or HeV immune sera will show cross-reactivity with CedV, however such sera or even specific high-titer anti-CedV F or G glycoprotein antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins. [27] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and it is also the largest polymerase in the order *Mononegavirale*, yet it still shares high aa identity to NiV and HeV L protein. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; the aa identities of the important L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This high degree of protein identity, overall, shared between CedV, and that of NiV and HeV, suggests that antiviral agents that target for example, the L polymerase, could be identified and perhaps also offer the possibility of pan-henipavirus antiviral countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] Indeed, several small molecule inhibitors have been validated in vitro using infectious HeV and NiV in BSL-4, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support Aim 1 to optimize our current best-in-class leads and also screen large libraries of antiviral compounds.

Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[31-33] Nanobodies can be isolated using the same phage library construction methods and phage panning procedure routinely employed for mAb discovery, which allows for in vitro production of various amounts and their rapid characterization. In collaboration with **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, [10] we have several immunized mouse cohorts now underway using native-like oligomeric recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in Aim 2 in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of Aim 3. In addition, we expect to discover, and well-characterize, batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in Aim 3. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet and Irina Gaisina, Chicago BioSolutions** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core Lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company

engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule entry inhibitors of Nipah.

C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2 containment), cell-based HTS platform to identify small molecule antivirals targeting henipavirus replication without the requirement for BSL-4 containment.[7] ***rCedV assays and tools.*** We also adapted the rCedV HTS infection platform to create another innovative toolset using novel chimeric viruses, where the viruses are altered only by the substitution of the F and G genes with that of NiV or HeV. As with the luciferase-encoding rCedV HTS assay, rCedV chimera use guidelines and procedures at BSL-2 were reviewed and approved by the USU, Institutional Biosafety Committee (IBC) in accordance with NIH guidelines. These replication competent rCedV chimeras create a very rapid and robust surrogate infection assay for NiV-B and HeV that can be used as a neutralization tool or entry inhibitor assay to measure neutralizing antibodies or sera, or as a surrogate for generating virus escape mutants to F or G neutralizing antibodies. Here, the F and G genes of NiV-B (2010 Faridpur isolate) and HeV (2008 Redlands isolate) were synthesized and inserted into our pOLTV5-rCedV anti-genome plasmid. For the HTS neutralization assay, a turbo-GFP gene was inserted between the CedV P and M genes. Each chimera (rCedV-NiV-B-GFP and rCedV-HeV-GFP) were rescued as previously described.[8] Other versions without reporter genes have also been prepared (rCedV-NiV-B and rCedV-HeV). All virus stocks are cloned and genomes confirmed by NGS. Typically, large stocks are prepared and frozen as single use aliquots. Show in **Figure 1** is an example of how these chimeras are used in a virus neutralization test, referred to as a fluorescent reduction neutralization test (FRNT) using two human NiV/HeV cross-reactive anti-G mAbs (HENV-103 and -117) as previously described.[34] Briefly, Vero 76 cells are used in black walled clear bottom 96-well plates, mAbs are serially diluted in medium, and equal volumes of medium containing rCedV-NiV-B or rCedV-HeV are added to each mAb dilution for a final concentration of 2000 PFU, incubated for 2 hrs at 37 °C. Each virus-mAb mixture (90 µl/well) is added to the pre-seeded Vero 76 cells in triplicate and the assay run for just another 24 h followed by 4% formaldehyde fixation, washing and then scanning using a CTL S6 analyzer. Fluorescent foci were counted using the CTL Basic Count™ and an IC_{50} is determined as the antibody concentration at which there was a 50% reduction in fluorescent foci versus untreated control wells. **Table 1** shows the calculated EC_{50} values using rCedV chimeras vs. those determined by conventional PRNT assay with authentic NiV-B and HeV in BSL-4, and they are remarkably within a 2-fold range.

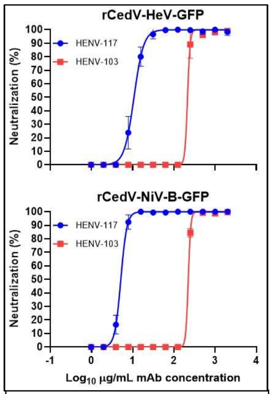


Figure 1. Neutralization curves for HENV-117 and HENV-103 mAbs against rCedV-HeV-GFP (top) and rCedV-NiV-B-GFP (bottom). Data are from a single experiment performed in technical triplicate.

IC50 (ng/mL)	HeV	rCedV-HeV	NiVB	rCedV-NiVB
HENV-103	445	216	507	238
HENV-117	14	11	15	6

Table 1. IC_{50} values of cross-reactive anti-G human mAbs HENV-103 and -117) as determined by rCedV chimera FRNT assay and NiV-B and HeV conventional PRNT assay.

In addition to conventional single agent neutralization tests, the rCedV-NiV-B-GFP and rCedV-HeV-GFP chimeras can also be used in rapid HTS neutralization assays to measure synergistic activities of inhibitors, such as antibodies or drugs. The synergistic neutralizing activity of the HENV-103 and HENV-117 mAbs were described utilizing the rCedV chimera assay.[34] Shown in **Figure 2** is a neutralization synergy assay conducted with mAbs m102.4 and nAH1.3 (both NiV/HeV cross-reactive anti-G mAbs) where they are individually diluted 2-fold in a 7x7 concentration matrix. The diluted mAbs are added 1:1 to 4000 PFU per well of rCedV-NiV-B-GFP and the assay essentially carried out as above, with percent neutralization calculated by normalizing foci to a virus only control. Matrices are imported into SynergyFinder and analyzed Neutralization synergy was calculated by comparing treatment to virus only control wells. Values were imported into SynergyFinder software[35] using a

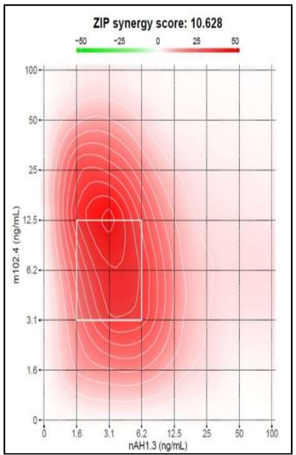


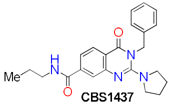
Figure 2. Synergy map with calculated synergy score using the zero interactions potency (ZIP) model generated by SynergyFinder for neutralization of rCedV-NiV-B-GFP by serially diluted m102.4 and nAH1.3 mAbs.

Zero Interactions Potency (ZIP) statistical model; delta scores >10 indicate likely synergy as m102.4 and nAH1.3 indicate here.

Antiviral evaluation and combination testing of small molecules and nanobodies. These rCedV-based tools and rCedV chimera viruses will allow for rapid antiviral activity assessment and an ability to measure synergistic antiviral activities for both small molecule inhibitors derived from **Aim 1** and nanobodies derived from **Aim 2**; and will also allow the novel approach of testing small molecules and nanobody combinations in a rapid and quantitative manner, in vitro, to select best-in-class candidates for both monotherapeutic and combination therapeutic modalities prior to in vivo animal model testing.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called "SMART Set") was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 µM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 µM, and 47 hits showed only minimal cytotoxicity at 33 µM.

Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.



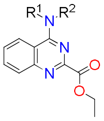
CBS1437

CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)	CC_{50} (µM)	EC_{50} (µM)
0.70	100	2.1	100	3.3

In **Table 2** is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437**. Note that both HEK293T and Vero cell lines were employed in these assays, and that CC_{50} values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC_{50} value of 3.3 µM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 µM).

Table 3 shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14,** bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent,* ***CBS1437****, as well as specific plans for developing the SARs.*

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.



N	Compound ID	R^1	R^2	% inhib at 10 µM
1	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
2	**G577-0016**	H	2,4-difluorophenyl	18
3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
4	**G577-0022**	H	3-methoxyphenyl	46
5	**G577-0028**	Me	cyclohexyl	93
6	**G577-0084**	H	4-isopropylphenyl	85
7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
8	**G577-0112**	Me	2-methylphenyl	94

D. RESEARCH DESIGN AND METHODS

D1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 μM) and low cytotoxicity (CC_{50} >100 μM).

D1.1. Synthetic Chemistry. In **Figure 3** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**F577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. In addition, we will optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be more chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 1** is shown a brief synopsis of the general method used for the synthesis of 4-aminoquinazolines. The specific procedures for the three steps vary, but all initiate with anthranilamides. This general route will allow us to prepare compounds with varied R_1, R_2 and R_3 groups as well as compounds with substituents that replace the carboethoxy functionality at the 2-position. Several anthranilamides are commercially available, which will allow us to initially access starting materials with a variety of R_3 groups. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[36-37] as well as other methods.[38,39] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible.

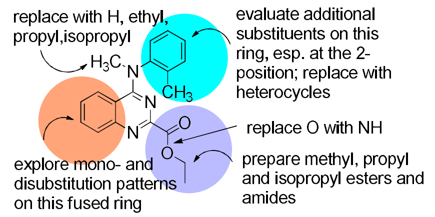
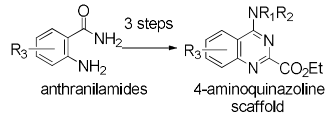
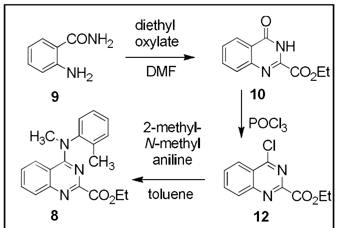


Figure 3. SAR development for 4-aminoquinazolines



Scheme 1. General synthesis for 4-aminoquinazolines.



Scheme 2. Synthesis of **G577-0112** (cmpd **8**).

In **Scheme 2** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) produces 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline.

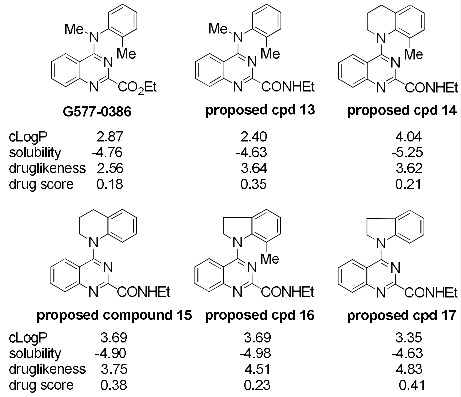


Figure 4. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.

In **Figure 4** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[40]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112** (compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester to an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scres have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding.

In **Figure 5** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 5**, since amides are subject to metabolic cleavage. Additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

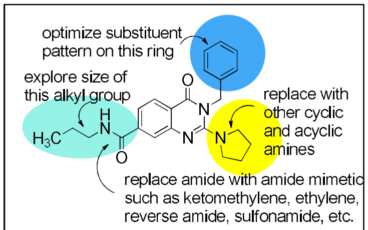


Figure 5. SAR development for **CBS1437**.

D.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5 x 10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at predetermined nontoxic concentrations for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation (PerkinElmer, Waltham, MA) as per the manufacturer's instructions. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T cells and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 μM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

Screening Libraries. These libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of > 150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions, Inc**. *Hit Selection Algorithm.* A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used again as a specificity control. We expect that the majority of the "hit" compounds will not inhibit the VSV-G-mediated viral entry. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (potent inhibitor, EC_{50} less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. *Hit Validation, Cytotoxicity Assessment and Ranking.* We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" (Promega, Madison, WI) as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. *Hit Quality Control and Identity Assurance.* The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds found to be <80% pure will be purified and retested.

D.1.3. Evaluation of protective properties of the advanced candidates in the henipavirus infectious assays will be done as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2 × 10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to that from parallel cultures with virus and DMSO vehicle only. This work will be performed in the BSL-4 facility at UTMB by the **Geisbert Lab (Core ?)** .

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Gaisina and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy based on a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or the additional HTS hits identified with the HTS of the expanded libraries of compounds for defining lead compounds for advancement.

D.2.1. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with cell growth medium containing either DMSO or selected furopyrimidines at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by standard western blot techniques or viral RNA levels by quantitative real time polymerase chain reaction assays (q-RT-PCR). CedV specific antibodies against the attachment (G) and fusion (F) that were generated in the Broder Lab and antibodies to CedV-N and CedV-P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene. The one-step reaction will be performed using the Applied Biosystems (ABI) 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus. *Target identification for small molecules.*** The rCedV tools used in the small molecule inhibitor identification assays (**Aim 1**) can also be exploited for antiviral target identification. In vitro selection of virus escape mutants that acquire resistant to inhibitors is a routine approach for mapping genetic changes associated with escape. We have successfully used authentic NiV and HeV and also rCedV chimeras to generate neutralizing mAb escape mutant viruses. Here, we will test advanced lead compounds for antiviral resistance using techniques similar for generating mAb escape mutants.[41,42] Briefly, a select compound will be used at the sub-optimal inhibition concentrations determined from the studies in **Aim 1**, and virus will be serial passaged in multiple rounds. Amplified virus stocks obtained after several rounds of passage in the presence of inhibitor will be purified by limiting dilution in the presence of inhibitor, and a “drug resistant” virus stock will then be amplified and prepared for genomic sequencing. Full virus genomes of any “drug resistant” virus variant will be derived by deep-sequencing and annotated by a commercial source. All mutations will be mapped to the viral genomes. We expect that many mutations will map to the L polymerase gene of rCedV, however, both P and N proteins also comprise the replicase complex and mutations may appear in those gene products as well, which if identified also suggests the possibility of multiple drug combination testing going forward. We will validate key mutations found in drug resistant rCedV by engineering those mutations directly into the wild-type genome and re-recuse those rCedVs and assay for drug resistance. We will also confirm and correlate our drug targets by performing resistance selection of the best inibitors using authentic NiV-B and HeV in BSL-4 by the UTMB Core.

D.2.2. Assess the binding properties by surface plasmon resonance (SPR). The Nipah polymerase (L protein and a phosphoprotein P) will be prepared using a previously published protocol. Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[43] Prefusion-stabilized RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 μM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab**.

D.2.3. Structure determination of recombinant proteins with bound inhibitors by X-Ray crystallography and CryoEM. High-resolution structures of the Nipah RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compound to be developed will be obtained using both x-ray crystallography and CryoEM by Dr. Vessler Lab. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands. Initial crystallization screens will be set up robotically using in-house Gryphon crystallization robotics. Biophysical techniques such as native mass spectrometry and thermal shift assays, light scattering, and fluorophore analysis to measure protein stability and folding are routine and will be used to ensure that bound compounds are not destabilizing domain structures to prevent crystallization. X-ray data collection will be done where? Crystal structures will be determined by molecular replacement using existing structures as search models. For inhibitors binding to regions other than above or if co-crystals cannot be obtained, cryo-EM will be used to determine the complex structure. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[44] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including cryoSparc[45], EMAN[46], and RELION[47]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed by molecular dynamics (MD) studies. While cryo-EM can provide access to the near-atomic resolution structures of large biomolecular systems--in their near native environment, MD can be used to reveal its dynamic behavior and refine low-resolution regions of cryo-EM reconstructions[48,49]. Furthermore, as X-ray/cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[50-54], to explore the conformational landscape[55] of ligand binding events, and to capture conformational variability of intermediate states[48,56], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches. David, please look at this section. Make necessary corrections.

Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds. *Milestone: Designate **advanced lead compounds** exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the **advanced lead compounds**.*

D.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.
Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-T properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out using the CYP3A4, and CYP2D6 Human Cytochrome P450 High Throughput Inhibitor Screening Kits (BD Gentest Corp., Woburn, MA) to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[57] Compounds with IC_{50} values ≥100 μM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds by the method of Kuhnz *et al*.[58] Compounds with >30% recovery (1 h) will be considered

for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed in a [^{3}H] dofetilide-binding assay as described by Finlayson *et al.*[59] using SHSY5Y cells. Compounds that do not prevent [^{3}H]dofetilide binding (IC$_{50}$ >10 μM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method as described by Gres *et al*.[60] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P$_{app}$) greater than 1 × 10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels of the compound up to a solubility-limiting level in the presence or absence of rat liver enzymes (rat S9 liver fraction) acting in the normal process of metabolism and any reverse mutations will be determined. ***In vitro* mammalian chromosomal aberration test**: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes.

D.3.2. PK studies: Naïve male/female mice (equal number, 3 groups of 36 animals total) will be treated with a single dose via i.v., i.p., or oral gavage administration and blood will be collected and processed to obtain plasma and serum from three animals/group/time point at up to 12 time points through 24 h post dose. Two doses will be used to establish the drug exposure-dose range relationship: the estimated efficacious dose (ED), based upon preliminary data at 25 mg/kg i.p. or p.o. and an observable adverse effect dose (OAED) (to be determined). Compounds of interest will be extracted from biological specimens by protein precipitation, analyzed on a triple-quadruple mass spectrometer (LC-MS/MS) and quantified using an internal standard method with a minimum of a five-point calibration curve. PK parameters including AUC, C$_{0}$, C$_{max}$, T$_{1/2}$, T$_{max}$, and F(%) will be determined. Depending on the estimation of T$_{max}$, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

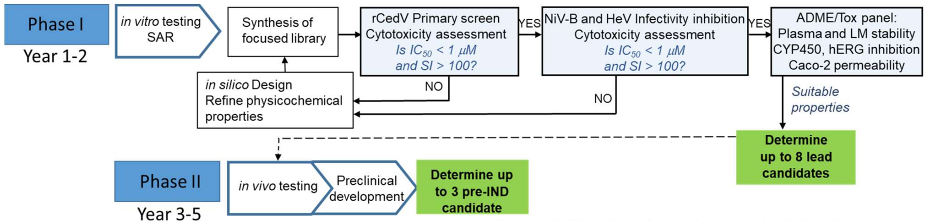


Figure 4. Flowchart for henipavirus inhibitor development

Will be MODIFIED **Figure 5**

Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc. and Reaction Biology, Corp., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim.

We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 5**).

D.4. AIM 2: Characterization of the potency and breadth of selected nanobodies

Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. Aim 1 will also collaborate with the core??? to evaluate lead candidates *in vivo*.

D.4.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth

D.4.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including Nipah virus (Bangladesh and Malaysia strains) and Hendra virus (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[61-63] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with Nipah and Hendra virus glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

D.4.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit Nipah virus and Hendra virus, we will use a high-throughput neutralization assay developed in the Broder lab. This assay is based on green fluorescent protein (GFP)-encoding, replication-competent, Cedar (henipa)virus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bangladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins.[7,64] This platform is safe, enabling utilization at BSL2 compared to BSL4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the Broder lab recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described Hendra virus variant.[65] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond Nipah and Hendra viruses (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[66,67]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.

Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against Nipah virus and Hendra virus. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.4.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The Veesler lab is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The Veesler lab has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software which runs 24 hours per day and enables automated targeting and image acquisition while keeping a relational database of all micrographs to expedite subsequent processing.[68] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[44,69] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[61,63,70-72] mechanical stage tilting with gold grids[73,74] or detergents to modify surface tension[75]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[73,76-80] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[81-83]

The Nipah virus attachment (G) glycoprotein engages host receptors, is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the Nipah virus G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3) which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

D.4.2.1. High-resolution structural studies using X-ray crystallography. Some of the nanobodies might bind to regions that are intrinsically flexible thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[84] and BUSTER-TNT[85].

D.4.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[61-63,71,86,87]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the formulation of cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[88-90] and high-resolution noise substitution prior to Fourier shell correlation calculations[91] to avoid overfitting. Atomic models will be assessed using Molprobity[92], EMringer[93] and Privateer[94]. The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails.

D.4.3. AIM 2C. Structure-guided formulation of nanobody cocktails

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[95-97] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected in vivo, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in Aim 2A. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.5. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with Core D to evaluate lead candidates *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in a well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster (refs) and AGM (refs). PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV_B and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV_B and HeV infected AGM models.

D.5.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed by Core D. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC_{90} in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of heniavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.5.2. *In vivo* testing of lead compounds NiV/HeV infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 pfu of NiV_B or HeV (refs). Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.5.3. Define breakthrough conditions for anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antivirals fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^6$ PFU of NiV or HeV (refs). Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.5.4. Combination therapy of anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising antivirals from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^5$ PFU of NiV or HeV (refs). Treatment with at least two different antivirals will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

D.5.5. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ $5x10^6$ PFU of NiV_B or HeV as determined in henipavirus dose confirmation studies conducted by Core D (see Core D). Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by Core X (see Core X) at various times after henipavirus infection.

D.5.6. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the MAD system as previously described (ref) or by small particle aerosol as previously described (refs) with a uniformly lethal challenge dose (~ 20,000 PFU of NiV or HeV. The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by Core X up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

Potential Pitfalls and Alternative Strategies. It is possible that Core X may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antivirals may not improve protective efficacy or extend the therapeutic window of antivirals. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and monoclonal antibodies to protect animals against lethal henipavirus disease to de-risk this Aim.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues, i.e., antiviral drugs will be coded so that researchers will not know which compounds were given to which animals until experiments and analysis are completed. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the emergence and re-emergence of Nipah virus infections throughout the world, coupled with its high fatality rate, provide very significant motivation for the preparation of small molecule inhibitors of this virus to create a readiness for the next Nipah virus outbreaks. We have discovered two differentiated series of compounds that show early promise as agents that have potential for treating Nipah virus infections, and we have designed a series of optimized druglike candidates for future preparation and development.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *N Engl J Med* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Desselberger, U. Emerging and re-emerging infectious diseases. *J Infect* **40**, 3-15 (2000).

12 Luby, S. P. & Broder, C. C. in *Viral Infections of Humans, Epidemiology and Control* (eds R. A. Kaslow, L. R. Stanberry, & J. W. Le Duc) Ch. 22, 519-536 (Springer Science+Business Media, 2014).

13 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

14 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

15 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *Med J Aust* **162**, 642-645 (1995).

16 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

17 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

18 Sweileh, W. M. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health* **13**, 9, doi:10.1186/s12992-017-0233-9 (2017).

19 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379, doi:10.3201/eid2003.131159 (2014).

20 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

21 Playford, E. G. *et al.* Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*, doi:10.1016/s1473-3099(19)30634-6 (2020).

22 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).

23 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

24 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

25 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

26 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

27 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

28 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

29 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

30 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

31 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

32 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

33 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

34 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell reports* **36**, 109628, doi:10.1016/j.celrep.2021.109628 (2021).

35 Ianevski, A., Giri, A. K. & Aittokallio, T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. *Nucleic Acids Res* **48**, W488-W493, doi:10.1093/nar/gkaa216 (2020).

36 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

37 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

38 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

39 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

40 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

41 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-434, doi:10.1038/s41594-021-00584-8 (2021).

42 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-987, doi:10.1038/s41594-019-0308-9 (2019).

43 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Anal Biochem* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

44 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *Journal of structural biology* **166**, 95-102 (2009).

45 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

46 Ludtke, S. J. 3-D structures of macromolecules using single-particle analysis in EMAN. *Methods in molecular biology* **673**, 157-173, doi:10.1007/978-1-60761-842-3_9 (2010).

47 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *Journal of structural biology* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

48 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).

49 Hughes, T. E. T. *et al.* Structural insights on TRPV5 gating by endogenous modulators. *Nature communications* **9**, 4198, doi:10.1038/s41467-018-06753-6 (2018).

50 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).

51 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).

52 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).

53 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).

54 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).

55 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).

56 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).

57 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).

58 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

59 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

60 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

61 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).

62 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

63 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).

64 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).

65 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).

66 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).

67 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).

68 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

69 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

70 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

71 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

72 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

73 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

74 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).

75 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).

76 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).

77 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).

78 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).

79 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).

80 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).

81 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).

82 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).

83 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).

84 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).

85 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).

86 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).

87 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).

88 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

89 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

90 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).

91 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).

92 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).

93 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).

94 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).

95 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).

96 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).

97 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).

From: Gaisina, Irina N on behalf of Gaisina, Irina N <igaysina@uic.edu>
To: Broder, Christopher; Rong, Lijun; norton peet
Cc: David Veesler; Moushimi Amaya; Thomas Geisbert
Subject: ResStrategy rCedV
Date: Monday, November 1, 2021 11:04:22 PM
Attachments: Tulane_Res_strategy_Nipah-11-1-revised.docx

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please use this latest (for today) version for any corrections and additions. Thank you.
Irina

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Monday, November 1, 2021 8:33 PM
To: Rong, Lijun <lijun@uic.edu>; Gaisina, Irina N <igaysina@uic.edu>
Cc: David Veesler <dveesler@uw.edu>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>; Thomas Geisbert <twgeisbe@utmb.edu>
Subject: rCedV

Irina, Lijun

try putting this rCedV tools into the preliminary data
it covers chimeras to replace the published rCedV-Luc description and existing figure 1 which we should remove. the chimera prelim data supports high throughput nanobody assessment, and synergy assay (which can be mentioned in Aim 1 drugs, and Aim 2 nanobodies.

also there is some text for resistance selection and genome sequencing for target identification if needed somewhere

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV).[1] NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality.[2] Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission.[3] Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors.[4] 22 cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antivirals approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers.[5] As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus non-pathogenic in well-established animal models of NiV and HeV pathogenesis.[6] We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS).[7-9] We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will potentially yield testable antivirals with potential pan-henipavirus effectiveness. Our collaborative team also has an extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity.[10] These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and also aerosol delivery of nanobodies. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobodies studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

A. BACKGROUND AND SIGNIFICANCE

Over the past 40 years the major etiological agents of emerged or identified infectious diseases in humans have been viruses.[11] Among these, the paramyxoviruses, which include many important human and animal pathogens, also include two emerged, zoonotic viral pathogens of importance: the henipaviruses; Hendra virus (HeV) and Nipah virus (NiV)[12,13]. In 1994, HeV caused an outbreak of severe respiratory disease in horses in Brisbane, Australia, resulting in the deaths of 14 horses and their trainer.[14,15] NiV was the causative agent of a significant outbreak of severe encephalitis in pig farmers in Malaysia in 1998. Spread of NiV infections typically requires direct contact with an infected source, and transmission can occur between people and from animals to people. Symptoms may culminate in a severe respiratory and/or neurological disease that is often fatal. Complications of the infection can result in relapsed encephalitis following recovery. On September 5, 2021, a twelve-year-old boy in the Indian state of Kerala died after contracting the Nipah virus. During the process of transporting him to medical centers for a diagnosis, twenty-three health care workers exposed to this boy also died, which underscores both the transmissability and the lethal nature of this virus. *There is presently no vaccine or small molecule therapeutics that have been developed for NiV infections.* Pteropid bats appear to be the major reservoir hosts for henipaviruses[6,16]. HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bat hosts. Among these animals, HeV and NiV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with human fatality rates ranging from 40-100%[2,17].

NiV and HeV are classified as biosafety level-4 (BSL-4) restricted pathogens and both remain transboundary threats to livestock and people throughout South Asia and Australia. NiV and henipaviral diseases have been categorized by the World Health Organization (WHO) as an epidemic threat needing urgent research and countermeasure development and are included in the WHO R&D Blueprint list of priority pathogens with epidemic potential [18]. Presently, there are no prophylactic or therapeutic treatment options for henipavirus infections approved for use in humans. A subunit vaccine, Equivac® HeV, has been available for use in horses in Australia since 2012 [19] for the prevention of HeV infection. This equine vaccine received full registration by the Australian Pesticides and Veterinary Medicines Authority in 2015, with all vaccinated horses receiving a microchip with a database being maintained and is the first commercially deployed vaccine against a BSL-4 agent [20]. Although a human monoclonal antibody (mAb) m102.4 has successfully completed a phase I safety trial in Australia [21], developing vaccines or therapeutics against henipaviruses for use in people remains a high priority. Henipaviruses are single-stranded, negative sense, enveloped RNA viruses with two membrane anchored glycoproteins involved in virus entry; one mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and also the main focus of several vaccine strategies[2]. Although an effective post-exposure human monoclonal antibody (mAb) therapy (anti-NiV/HeV G glycoprotein mAb m102.4) has been extensively examined[22-26], it must be administered within several days following lethal infection in animal challenge models. In contrast, however, antiviral drug discovery for HeV and NiV has been significantly hampered due to requirements of biosafety level-4 (BSL-4) containment. Recently, a third isolate, namely, Cedar virus (CedV) has been added to the *Henipavirus* genus[27]. However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease and resides in nature in the same bat host species as HeV[27,28]. Nucleic acid based detection studies have identified related henipavirus species, including complete genomic sequences[29,30], but HeV, NiV, and CedV are the only virus isolates reported.

We have recently optimized a recombinant CedV encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications and have identified several inhibitory compounds that were further validated with the infectious NiV-Bangladesh (NiV-B) strain. The validated compounds provide the foundation for the current application.

B. INNOVATION

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of this proposal's investigators. *First,* the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may will have activity across related members of henipavirus genus. *Second,* our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice

that produce VHHs (nanobodies). *Third,* together with newly created rCedV chimeras and the establishment of high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. *Fourth,* the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification, and finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Small molecule antiviral drug discovery. Anti-whole inactivated NiV or HeV immune sera will show cross-reactivity with CedV, however such sera or even specific high-titer anti-CedV F or G glycoprotein antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins. [27] Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and it is also the largest polymerase in the order *Mononegavirale*, yet it still shares high aa identity to NiV and HeV L protein. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; the aa identities of the important L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This high degree of protein identity, overall, shared between CedV, and that of NiV and HeV, suggests that antiviral agents that target for example, the L polymerase, could be identified and perhaps also offer the possibility of pan-henipavirus antiviral countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication.[7] Indeed, several small molecule inhibitors have been validated in vitro using infectious HeV and NiV in BSL-4, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support Aim 1 to optimize our current best-in-class leads and also screen large libraries of antiviral compounds.

Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials.[31-33] Nanobodies can be isolated using the same phage library construction methods and phage panning procedure routinely employed for mAb discovery, which allows for in vitro production of various amounts and their rapid characterization. In collaboration with **Dr. Jianliang Xu** (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels, [10] we have several immunized mouse cohorts now underway using native-like oligomeric recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in Aim 2 in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of Aim 3. In addition, we expect to discover, and well-characterize, batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in Aim 3. **Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.**

C. PRELIMINARY RESULTS

C.1. Project Team. All aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet and Irina Gaisina, Chicago BioSolutions** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core Lead). **Chicago BioSolutions, Inc**. is a privately held, product-focused biopharmaceutical company

engaged in the research and development of small molecule antiviral drugs that address commercially significant medical markets and unmet medical needs.

C.2. Preliminary Data: Identification of small molecule entry inhibitors of Nipah.

C.2.1. HTS Assay development. We developed a robust (high signal-to-background ratio), safe (BSL-2 containment), cell-based HTS platform to identify small molecule antivirals targeting henipavirus replication without the requirement for BSL-4 containment. A reverse genetics approach was used to generate rCedV expressing luciferase (rCedV-Luc). A firefly luciferase (Luc) gene was inserted between CedV P and M genes in the rCedV anti-genome clone by standard restriction digest and ligation methods to yield the luciferase reporter rCedV full-length genome plasmid (**Figure 1A**). Successful rescue of rCedV-Luc was demonstrated by observing multiple syncytia on Vero E6 cells inoculated with freeze-thawed cell culture supernatants from BSR-T7/5 cells transfected with rCedV-Luc anti-genome and helper plasmids (yellow box in **Figure 1B**). The growth kinetics of rCedV-Luc to rCedV was compared (**Figure 1C**). Vero E6 cells were infected with either rCedV or rCedV-Luc at an MOI of 0.01. At 24 h intervals, supernatants from infected cells were collected and analyzed by plaque assay for infectious viral titers. At all-time points examined, no statistically significant differences were observed between rCedV and rCedV-Luc, indicating that the recombinant viruses have similar growth kinetics. In parallel, infected cells were lysed and analyzed for intracellular luciferase activity. As seen in **Figure 1C** (blue axis), increasing luciferase activity was detected that reached saturation at ~1 × 108 relative luciferase units (RLU) at 72 hpi (blue dashed line). The increase in luciferase signal correlated with the increase in infectious viral titers. These results indicated that introduction of the luciferase reporter gene did not interfere with the growth of the recombinant virus and that luciferase activity is an indicator of viral genome expression.

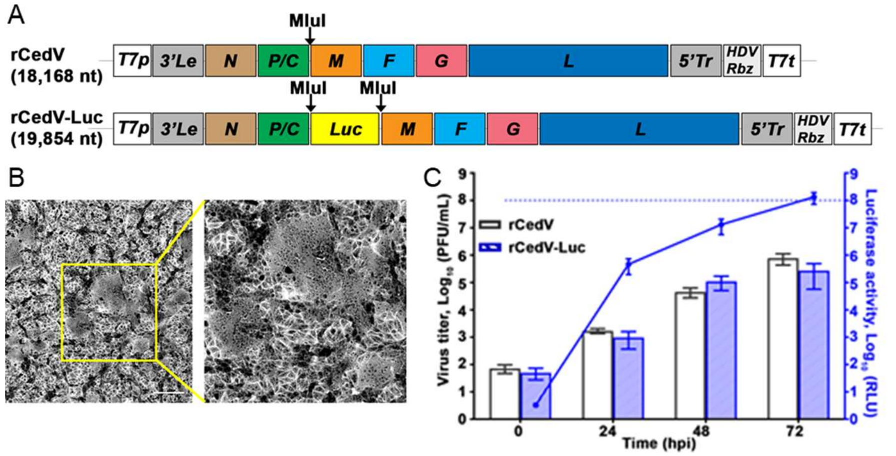


Figure 1. Characterization of recombinant CedV expressing luciferase. A) The firefly luciferase (*Luc*) gene was inserted at the unique MluI restriction site between the *P* and *M* genes to produce rCedV-Luc. B) Vero E6 cells infected with rCedV-Luc C) Vero E6 cells infected with either rCedV or rCedV-Luc at an MOI of 0.01. At 0, 24, 48 and 72 hpi. Viral titers determined by plaque assay and calculated as PFU/ml

Table 1. High throughput screening assay parameters

Assay Parameters	96-well format	384-well format
Z'	0.6 ± 0.02	0.5 ± 0.2
%CV	13.3 ± 1.14	14 ± 0.05
S/B	$> 10^3$	> 40

Table 2. Inhibition of rCedV and NiV-B by the HTS hit **CBS1437**.

CedV (293T)		CedV (Vero)		NiV-B (Vero)
EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)
0.70	100	2.1	100	3.3

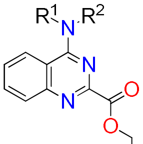
The optimization of cell type, virus MOI, and incubation time was conducted to develop an assay suitable for HTS antiviral screening is described in our recently published manuscript.[7] Shown in **Table 1** are the results of further optimization of the HTS assay and resulting parameters in both 96- and 384-well formats. Z' scores ≥ 0.5, high S/B ratios and CV < 20% were recorded, indicating a robust sensitive high-throughput assay. These data demonstrated that the rCedV-Luc platform is suitable for HTS-based hit identification.

C.2.2. Pilot screening of a small molecule library. A library of 10,000 compounds (preselected from ChemDiv library by the UIC medicinal chemists and called “SMART Set”) was used for a pilot HTS to identify rCedV-Luc inhibitors. This library (1) is pruned for known frequent hitters (PAINS and alike); (2) is balanced in terms of diversity of chemical scaffolds; and (3) has several representative analogs for each of the scaffolds in the library. From the initial single-dose screen, using 80% inhibition as the cutoff, 151 hits (1.51% hit rate) were selected for further evaluation. To eliminate false positive hits due to cytotoxicity, a validation screen alongside a cellular toxicity assay (compound concentration of 33 μM) was carried out. The antiviral activities of 85 compounds were confirmed at 10 μM, and 47 hits showed only minimal cytotoxicity at 33 μM.

In **Table 1** is shown a representative quinazoline compound from our quinazoline series of rCedV inhibitors, designated as **CBS1437** (**Table 2**). Note that both HEK293T and Vero cell lines were employed in these assays, and that CC_{50} values were recorded for these two cell lines. Compound **CBS1437** was also evaluated in the infectious NiV (NiV-B) assay and found to have an EC_{50} value of 3.3 µM. An important reason for producing the data shown in **Table 2** was to validate the primary screening data that was gathered from the rCedV HTS assay. Thus, we concluded that our HTS approach was a valid method for defining NiV inhibitors. Next, target specificity was confirmed in a counterscreen assay using a vesicular stomatitis virus G (VSV-G) protein. This hit showed a lack of detectable inhibition for VSV-G as well as some other unrelated viral proteins, including Lassa glycoprotein, and influenza fusion protein hemagglutinin H1 and H5 (0% inhibition at 12.5 µM).

Table 3 shows eight additional inhibitors in the quinazoline series, namely 4-aminoquinazolines, that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). Note that these 4-aminoquinazolines all bear substituents on the amino group at the 4-position of the quinazoline ring and a carboethoxy substituent at the 2-position. In terms of SAR to be gleaned from the limited data set in **Table 3**, a few features stand out that are worth mentioning. It is clear that a variety of *N*-phenyl substituents give rise to active compounds, and that H, methyl and *n*-propyl groups as the second *N*-substituent all can produce good activity. However, it is also clear that one of the best compounds is compound **14,** bearing an unsubstituted cyclohexyl group, which suggests an area for optimization since compound **14** is the only compound where the phenyl ring has been replaced by a completely saturated (cyclohexyl) substituent. Another point worth making is that the two *n*-propyl compounds (**16** and **18**) are two of the best inhibitors. There may, in fact, be a rough correlation between cLogP and inhibitory activity, which may be worth exploring with a larger set of derivatives. It is clear that additional compounds in our 4-aminoquinazoline series (**Table 3**) would provide excellent opportunities for further optimization of the inhibitory activities. *In the Research Plan we discuss methods for the preparation of new 4-aminoquinazolines in addition to compounds in a backup series defined by our validation agent,* ***CBS1437****, as well as specific plans for developing the SARs.*

Table 3. Antiviral activity of 4-amino-quinazoline-2-carboxylates **1-8** in the rCedV luciferase reporter virus assay.

	N	Compound ID	R^1	R^2	% inhib at 10 µM
	**1**	**G577-0014**	H	3-chloro-4-methoxyphenyl	81
	2	**G577-0016**	H	2,4-difluorophenyl	18
	3	**G577-0021**	H	2-chloro-4-fluorophenyl	58
	4	**G577-0022**	H	3-methoxyphenyl	46
	5	**G577-0028**	Me	cyclohexyl	93
	6	**G577-0084**	H	4-isopropylphenyl	85
	7	**G577-0111**	*n*-propyl	4-fluorophenyl	86
	8	**G577-0112**	Me	2-methylphenyl	94

C3. Any data on monobodies?

D. RESEARCH DESIGN AND METHODS

D1. Aim 1A. Synthesize diverse analogs of the 4-aminoquinazoline hit series to establish structure-activity relationships (SARs) and improve potency and selectivity index. Milestone: Synthesize directed libraries and discrete analogs of hit compounds; using the rCedV-based assay for SAR development define henipavirus replication inhibitors with high potency (EC_{50} <1 µM) and low cytotoxicity (CC_{50} >100 µM).

D1.1. Synthetic Chemistry. In **Figure 2** is shown a summary of how we will develop the SAR plan for the 4-aminoquinazoline scaffold, as exemplified by changes to compound **8** (**F577-0112**). Substituent changes on the core fused scaffold will involve exploration of mono- and disubstitution substituent patterns on the quinazoline core to explore the optimum overall size for the core. Note that we may be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. In addition, we optimize substituents on both aryl rings, as indicated, and optimize the alkyl group on the tertiary amine. In addition, we will optimize the alkyl ester group, and we are very interested in replacing this ester with an amide, which would be more chemically and metabolically more stable. If the amide turns out to be an effective replacement, then we will also explore amide replacement units, such as ketomethylene, reduced ketone, retroamide, ethylene, and sulfonamide units, etc. In **Scheme 1** is shown a brief synopsis of the general method used for the synthesis of 4-aminoquinazolines. The specific procedures for the three steps vary, but all initiate with anthranilamides. This general route will allow us to prepare compounds with varied R_1, R_2 and R_3 groups as well as compounds with substituents that replace the carboethoxy functionality at the 2-position. Several

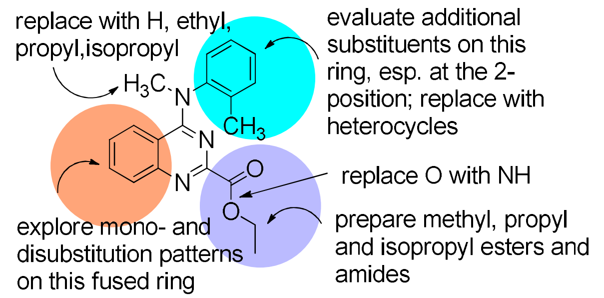
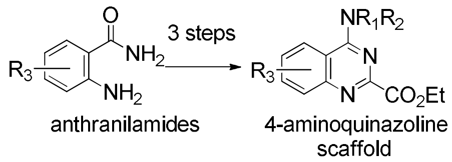


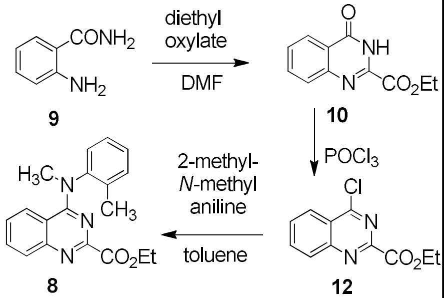
Figure 2. SAR development for 4-aminoquinazolines

anthranilamides are commercially available, which will allow us to initially access starting materials with a variety of R_3 groups. A variety of methods are available for accessing these 4-aminoquinazolines, including initial treatment of the anthranilamides with ethyl ethoxycarbonylformimidate in ether[34-35] as well as other methods.[36,37] Likewise, the additional reagents required for these syntheses are mostly commercially available, as well. Thus, it is very realistic that a large library of these potential inhibitors will be quite accessible.



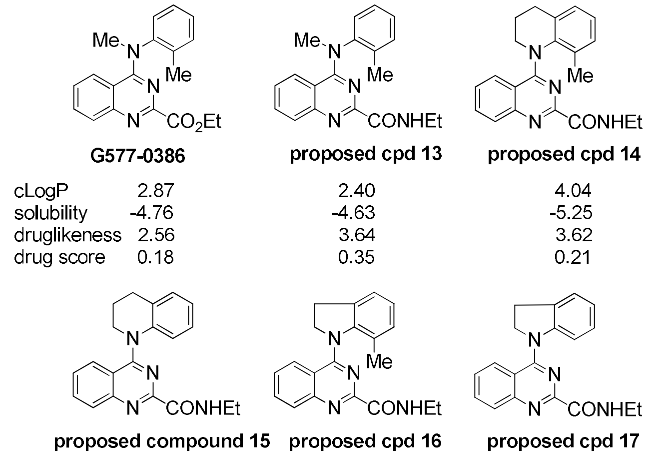
Scheme 1. General synthesis for 4-aminoquinazolines.

In **Scheme 2** is shown a specific synthesis of one of our best inhibitors in this set. Anthranilamide **9** is heated with diethyl oxalate in a solution of dimethylformamide (DMF) produces 2-carboethoxyquinazoline-4-one (**10**) in a condensation/cyclization process. Treatment of **10** with phosphorous oxychloride next provides a reactive intermediate, namely 2-carboethoxy-4-chloroquinazoline-4-one (**12**), which affords the specific inhibitor **G577-0112** (compound **8**) after a displacement reaction with 2-methyl-*N*-methylaniline.



Scheme 2. Synthesis of **G577-0112** (cmpd **8**).

In **Figure 3** is shown a set of proposed compounds related to **G577-0112** (compound **8**) for which we show parameters calculated using Actelion OSIRIS software[38]. The proposed compounds (**13** through **17**) generally show improved druglikeness and drug scores (and other parameters) with respect to **G577-0112** (compound **8**), and therefore represent promising new targets for synthesis and evaluation. Note that a dramatic improvement in all parameters is seen in simply replacing the ester to an amide by substituting NH for O, to provide proposed new compound **13**. In progressing through the constrained compounds **14** through **17**, improvement of parameters culminates with the indoline derivative **17**, whose druglikeness and druglike scres have very dramatically improved. Advantages of imposed conformational restraints, as seen in tetrahydroquinolines **14** and **15** and indolines **16** and **17**, include a reduction in rotatable bonds, and, importantly, a preorganization of an effective conformation that can mitigate the entropic penalty of binding.



	G577-0386	**proposed cpd 13**	**proposed cpd 14**
cLogP	2.87	2.40	4.04
solubility	-4.76	-4.63	-5.25
druglikeness	2.56	3.64	3.62
drug score	0.18	0.35	0.21

	proposed compound 15	**proposed cpd 16**	**proposed cpd 17**
cLogP	3.69	3.69	3.35
solubility	-4.90	-4.98	-4.63
druglikeness	3.75	4.51	4.83
drug score	0.38	0.23	0.41

Figure 3. Proposed inhibitors of rCedV based on Actelion OSIRIS predicted parameters.

In **Figure 4** is shown the SAR development plan for our quinazolin-4-one scaffold. There are three points of optimization that we envision for this trisubstituted fused core. At the 2-position, we will substitute the pyrrolidinyl substituent with additional cyclic and acyclic amines. The unsubstituted benzyl substituent at the 3-position will be optimized in terms of mono- and disubstitution patterns. The position of the carboxamide substituent located at the 7-position will be optimized, as will the *N*-alkyl group on the carboxamide. We will also replace the amide unit with various amide mimetic units, as indicated in **Figure 4**, since amides are subject to metabolic cleavage. Additional synthetic methods are available for the construction of both 4-aminoquinazolines and 2,3,7-trisubstituted quinazolin-4-ones.

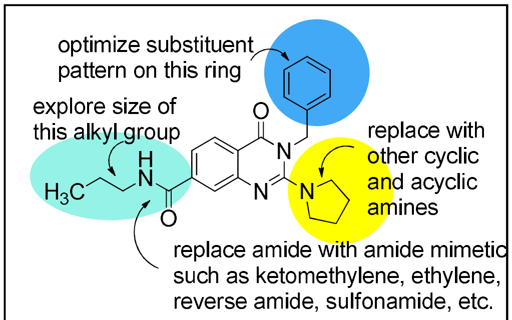


Figure 4. SAR development for **CBS1437**.

D.1.2. Evaluate efficacy of various derivatives in the 4-aminoquinazoline and the quinazolin-4-one series and additional HTS hits to inhibit viral replication in rCedV-Luc infected cells. We will employ our recently published optimized HTS protocol.[7] Cells preseeded in a 96-well plate at a density of 2.5 x 10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at predetermined nontoxic concentrations for 1 h at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation (PerkinElmer, Waltham, MA) as per the manufacturer's instructions. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T cells and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 μM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in the **Broder Lab.**

Screening Libraries. These libraries will be provided by the UIC Research Resource Center (RRC). They are comprised of > 150,000 compounds available in 96- or 384-well plate formats suitable for HTS. Additional libraries from ChemDiv and Enamine, including Antiviral Library composed of 65,937 novel diverse and targeted compounds with privileged scaffolds from ChemDiv's collection, will be purchased by **Chicago BioSolutions, Inc**. _Hit Selection Algorithm._ A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used again as a specificity control. We expect that the majority of the "hit" compounds will not inhibit the VSV-G-mediated viral entry. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (potent inhibitor, EC_{50} less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. _Hit Validation, Cytotoxicity Assessment and Ranking._ We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" (Promega, Madison, WI) as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. _Hit Quality Control and Identity Assurance._ The hits will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds found to be <80% pure will be purified and retested.

D.1.3. Evaluation of protective properties of the advanced candidates in the henipavirus infectious assays will be done as previously described.[7] Vero cells will be seeded in a 96-well plate at a density of 2×10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (final concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 h post infection (hpi), viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to that from parallel cultures with virus and DMSO vehicle only. This work will be performed in the BSL-4 facility at UTMB by the **Geisbert Lab (Core ?)** .

Pitfalls and alternate strategies. The proposed synthetic derivatives will be synthesized under the guidance of **Drs. Gaisina and Moore.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy based on a different reaction sequence. Should we encounter a scaffold-related problem with our 4-aminoquinazoline scaffold, we will switch to our backup quinazolin-4-one scaffold or the additional HTS hits identified with the HTS of the expanded libraries of compounds for defining lead compounds for advancement.

D.2.1. Aim 1B. Investigate the mechanism of action (MOA) of the replication inhibitors. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: **1) Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells**. Confluent HEK293T cells in a 6-well plate will be treated in duplicate with cell growth medium containing either DMSO or selected furopyrimidines at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 hpi cells will be collected to determine viral protein expression by standard western blot techniques or viral RNA levels by quantitative real time polymerase chain reaction assays (q-RT-PCR). CedV specific antibodies against the attachment (G) and fusion (F) that were generated in the Broder Lab and antibodies to CedV-N and CedV-P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the N gene. The one-step reaction will be performed using the Applied Biosystems (ABI) 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; **2) Determine the stage(s) of inhibition of 4-aminoquinazolines and quinazolin-4-ones in the viral lifecycle.** For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 h at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay.[7,8] Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; **3) Analyze escape mutants against the advanced lead compounds with Cedar virus.** Escape mutants provide information on potential targets of small molecule inhibitors as well as the potential for development of drug resistance in the field. We will generate escape mutant rCedV viruses that are resistant to the identified lead compounds and determine the rate at which resistance is developed to our inhibitors. In so doing, we will also identify protein coding as well as regulatory sequences that are altered. HEK293T cells will be infected with rCedV virus at MOI of 1 in the presence of the inhibitors at 10 times the IC_{50} values of each of the compounds. At 72 hpi, viral supernatant will be passaged in Vero cells at MOI of 0.01 in the presence of increasing concentrations of compound for >5 passages to obtain the escape mutants. The escape mutants will be plaque purified and subjected to whole-genome sequencing. The identified mutation(s) will be introduced into the rCedV anti-genome cDNA and upon successful incorporation of the mutation, the mutant rCedV will be rescued and sequenced to ensure retention of the introduced mutation. The rescued mutant rCedV will be fully characterized as described.[8]

D.2.2. Assess the binding properties by surface plasmon resonance (SPR). The Nipah polymerase (L protein and a phosphoprotein P) will be prepared using a previously published protocol. Biacore T200 and Biacore 8KSPR systems will be used to obtain information on binding specificity, binding affinity of the new lead inhibitors (K_D) to the RdRp protein, kinetics [association rate constants (k_a), and dissociation rate constants (k_d)] as previously described.[39] Prefusion-stabilized RdRp protein will be immobilized on a CM5 chip using standard amine coupling protocols. Lead compounds at various concentrations (0.1 - 50 μM) will be injected over the flow channel surfaces, and sensorgrams will be fitted by either steady-state affinity or to a 1:1 Langmuir kinetic binding model. The equilibrium dissociation constants (K_D) will be calculated as $K_D = k_d/k_a$. This kinetic analysis will be performed by UIC RRC, as previously done by the **Rong lab**.

D.2.3. Structure determination of recombinant proteins with bound inhibitors by X-Ray crystallography and CryoEM. High-resolution structures of the Nipah RdRp complex with key inhibitors such as **CBS1437** and the advanced lead compound to be developed will be obtained using both x-ray crystallography and CryoEM by Dr. Vessler Lab. It is expected that these structures will be determined and will reveal the atomic level details of how our inhibitors interact with the polymerase and inhibit its activity, which will be highly informative for developing potent high-affinity ligands. Initial crystallization screens will be set up robotically using in-house Gryphon crystallization robotics. Biophysical techniques such as native mass spectrometry and thermal shift assays, light scattering, and fluorophore analysis to measure protein stability and folding are routine and will be used to ensure that bound compounds are not destabilizing domain structures to prevent crystallization. X-ray data collection will be done where? Crystal structures will be determined by molecular replacement using existing structures as search models. For inhibitors binding to regions other than above or if co-crystals cannot be obtained, cryo-EM will be used to determine the complex structure. For EM studies, we will utilize automated data collection software to facilitate throughput. In brief, data will be processed using the Appion[40] pipeline, which keeps track of all data and processing steps. All data will be processed using multiple single particle EM software packages, including cryoSparc[41], EMAN[42], and RELION[43]. Purified complexes will be deposited onto grids, blotted, and cryo-plunged into liquid ethane to preserve them in a thin layer of vitreous buffer. Images of the complexes will be collected using automated image acquisition software under low dose conditions. Structures generated by cryo-EM will be refined and reconstructed by molecular dynamics (MD) studies. While cryo-EM can provide access to the near-atomic resolution structures of large biomolecular systems--in their near native environment, MD can be used to reveal its dynamic behavior and refine low-resolution regions of cryo-EM reconstructions[44,45]. Furthermore, as X-ray/cryo-EM structures of polymerase in complex with key inhibitors (i.e., **CBS1437** and additional derivatives) become available, MD studies of these systems will be conducted to reveal the dynamic nature of protein/ligand interactions[46-50], to explore the conformational landscape[51] of ligand binding events, and to capture conformational variability of intermediate states[44,52], which can be crucial to gain mechanistic knowledge of biological function, but remain out of reach through experimental approaches. David, please look at this section. Make necessary corrections.

Pitfalls and alternate strategies. We do not anticipate any technical difficulties in this aim as these are commonly utilized techniques employed in the **Broder, Rong, Vessler, and Geisbert labs**. We have previously rescued a GFP reporter rCedV as well as rCedV that lacks a reporter. We do not anticipate that the HEK293T cells will be problematic; however, several other cell lines may be tested in the assay. Successful mechanism studies may allow us to determine a mechanism of action that defines a binding site for inhibitory 4-aminoquinazolines or quinazoline-4-ones.

D.3. Aim 1C. Evaluation of pharmacokinetics/toxicokinetics of the advanced lead compounds. *Milestone: Designate **advanced lead compounds** exhibiting optimal ADME properties in vitro: stability to liver microsomes, low CYP isoform inhibition, Caco-2 permeability, and devoid of hERG channel affinity. Before investigating the efficacy of the compounds in vivo (Aim 3), PK studies will be performed to determine an optimal dosing regimen for the efficacy studies with the **advanced lead compounds**.*

D.3.1. Select NiV-B inhibitors with *in vitro* ADME properties suitable for whole animal testing.
Rationale: Our goal is to transform the validated hit series into **advanced lead compounds** suitable for further therapeutic development. We will assess several critical *in vitro* ADME-T properties and prioritize compounds accordingly. **CYP450 inhibition.** The CYP450 inhibition assays will be carried out using the CYP3A4, and CYP2D6 Human Cytochrome P450 High Throughput Inhibitor Screening Kits (BD Gentest Corp., Woburn, MA) to rapidly screen for potential inhibitors of the principal drug metabolizing cytochrome P450 enzymes.[53]

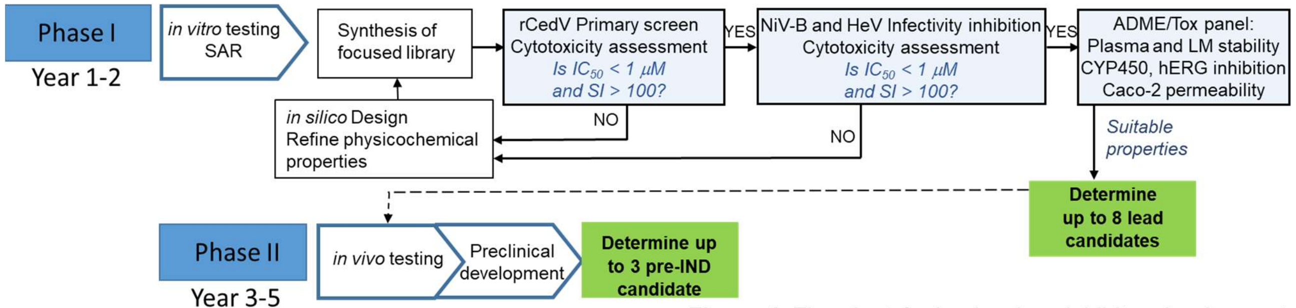


Figure 4. Flowchart for henipavirus inhibitor development

Will be MODIFIED **Figure 5**

Compounds with IC$_{50}$ values ≥100 µM for CYP isoforms will be prioritized for further advancement. **Rat/Human Liver Microsome Stability**. Rat and human liver microsome preparations will be used to evaluate the metabolic stability of compounds by the method of Kuhnz *et al*.[54] Compounds with >30% recovery (1 h) will be considered for further advancement. **hERG channel blockade.** The ability of compounds to block the hERG channel will be assessed in a [^{3}H] dofetilide-binding assay as described by Finlayson *et al.*[55] using SHSY5Y cells. Compounds that do not prevent [^{3}H]dofetilide binding (IC$_{50}$ >10 µM) will advance. **Cellular permeability.** Drug absorption through the intestinal epithelium is critical to good oral bioavailability. We will use the Caco-2 method as described by Gres *et al*.[56] to evaluate the potential for oral bioavailability of the lead inhibitors. Compounds exhibiting a Caco-2 permeability value (P_{app}) greater than 1×10^{-6} cm/sec will be prioritized. **Bacterial reverse mutation assay (Ames):** Four *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and one *Escherichia coli* strain (WP2 uvrA) will be used. The bacteria will be exposed to different dose levels of the compound up to a solubility-limiting level in the presence or absence of rat liver enzymes (rat S9 liver fraction) acting in the normal process of metabolism and any reverse mutations will be determined. ***In vitro* mammalian**

chromosomal aberration test: The purpose of this study is to evaluate the clastogenic potential of the compound based on its ability to induce chromosome aberrations in human peripheral blood lymphocytes.

D.3.2. PK studies: Naïve male/female mice (equal number, 3 groups of 36 animals total) will be treated with a single dose via i.v., i.p., or oral gavage administration and blood will be collected and processed to obtain plasma and serum from three animals/group/time point at up to 12 time points through 24 h post dose. Two doses will be used to establish the drug exposure-dose range relationship: the estimated efficacious dose (ED), based upon preliminary data at 25 mg/kg i.p. or p.o. and an observable adverse effect dose (OAED) (to be determined). Compounds of interest will be extracted from biological specimens by protein precipitation, analyzed on a triple-quadruple mass spectrometer (LC-MS/MS) and quantified using an internal standard method with a minimum of a five-point calibration curve. PK parameters including AUC, C_0, C_{max}, $T_{1/2}$, T_{max}, and F(%) will be determined. Depending on the estimation of T_{max}, PK study time points can be further adjusted to reach more accurate calculations of AUC. Plasma concentration will be established to support dosage selection for efficacy studies. Formulation stability and PK/Toxicity studies will be performed by **CRO**s.

Potential Pitfalls and Alternative Strategies. The proposed ADME/Tox and PK studies will be executed through the testing services provided by Eurofins Panlabs, Inc. and Reaction Biology, Corp., and supervised by **Dr. Peet**. We do not anticipate any problem obtaining high quality data from the assays. If the compounds fail to meet our benchmarks, then we will synthesize additional groups of compounds, or turn to our backup scaffold to meet the objectives in this aim.

We have established rigorous criteria for go-no-go decision-making to ensure the production of suitable **advanced lead compounds**, which will allow for the future selection of a preclinical candidate (**Figure 5**).

D.4. AIM 2: Characterization of the potency and breadth of selected nanobodies

Rationale: Aim 2A will investigate the binding properties, cross-reactivity and inhibition of the nanobodies targeting henipavirus F and G glycoproteins to assess potency and breadth of neutralization. Aim 1 will also collaborate with the core??? to evaluate lead candidates *in vivo*.

D.4.1. AIM 2A. Evaluation of nanobody cross-reactivity, neutralization potency and breadth

D.4.1.1. ELISA and biolayer interferometry assessment of cross-reactivity. The **Veesler lab** has a panel of prefusion-stabilized F ectodomain trimers and G ectodomain tetramers recombinantly expressed in mammalian cells, including Nipah virus (Bangladesh and Malaysia strains) and Hendra virus (both ancestral and the newly described variant) with and without avi-tag for biotinylation.[57-59] These proteins were used to vaccinate transgenic mice for nanobody production and will be used for downstream screening of the nanobody libraries and for exploring cross-reactivity with Nipah and Hendra virus glycoproteins and with more distantly related henipaviruses using high-throughput ELISA assays. Biolayer interferometry analysis of binding kinetics and affinity will be carried out for the most promising nanobodies selected by ELISAs to enable ranking them based on binding affinities and cross-reactivity.

D.4.1.2. Chimeric virus neutralization assays. To evaluate the ability of the nanobodies to inhibit Nipah virus and Hendra virus, we will use a high-throughput neutralization assay developed in the Broder lab. This assay is based on green fluorescent protein (GFP)-encoding, replication-competent, Cedar (henipa)virus (rCedV) chimeras in which the native glycoproteins are substituted with the NiV-B (rCedV-NiV-B-GFP, Bengladesh strain) or the HeV (rCedV-HeV-GFP) F and G glycoproteins.[7,60] This platform is safe, enabling utilization at BSL2 compared to BSL4 for authentic viruses, and has been thoroughly validated and shown to faithfully recapitulate the data obtained with NiV-B and HeV with both monoclonal antibodies and vaccine-elicited polyclonal sera. This method was used to support the preclinical development of G-specific antibodies[5]. Furthermore, the Broder lab recently developed a rCedV-HeVv-GFP using reverse-genetics to study the antigenic properties of a newly described Hendra virus variant.[61] These assays will enable ranking each nanobody based on their neutralization potency and breadth to assist downstream selection. For nanobodies for which cross-reactivity will be detected beyond Nipah and Hendra viruses (e.g. CedV), their inhibitory activity will be tested against authentic CedV. Finally, these chimeric virus neutralization assays can be implemented for viruses for which no isolates are available (as is the case for henipaviruses discovered through metagenomics in bats or other animals).[62,63]

Rigor and reproducibility. All plasmids are purchased from Genescript and delivered with sequencing certificates. Each batch of recombinant glycoprotein and pseudovirus is quality-controlled based on reactivity with or inhibition by well-characterized reagents such as monoclonal antibodies and electron microscopy imaging. All data will be obtained using multiple replicate measurements using independent batches of proteins and viruses and performed by multiple individuals to ensure the reproducibility of our assays.

Anticipated results. This aim will assist the selection of the most promising individual nanobodies with broad and potent neutralizing activity against Nipah virus and Hendra virus. The top candidates will be further studied structurally and functionally and evaluated for *in vivo* efficacy.

D.4.2. AIM 2B: Studies of molecular basis of nanobody-mediated viral inhibition

Rationale: Aim 2B will explore the structural basis of target recognition by the nanobodies to provide an atomic-level understanding of binding and inhibition which will be validated using functional assays. This structural framework will be used to guide the design of nanobody cocktails with enhanced neutralization potency, breadth and resistance to escape mutations. The Veesler lab is a world leader in structural studies of viral glycoproteins and will carry out cryoEM studies of the most potent and broad inhibitors in complex with NiV or HeV F and G glycoproteins to visualize recognition at the atomic level and inform further optimization of target binding affinity. The Veesler lab has developed a streamlined high-resolution cryoEM pipeline enabling high-throughput structural studies of viral glycoproteins. We use the Leginon data collection software which runs 24 hours per day and enables automated targeting and image acquisition while keeping a relational database of all micrographs to expedite subsequent processing.[64] Many image processing tasks are automated as part of a workflow that allows assessing data quality in real time during acquisition, and prevents bookkeeping errors by storing all the details related to image acquisition and processing.[40,65] Complexes of nanobodies with glycoproteins will be vitrified and imaged using cryoEM to visualize directly the epitopes targeted by the different nanobodies. As specimen preparation for cryoEM is often a bottleneck, we will leverage our extensive experience in overcoming specimen preferred orientation upon vitrification using ultrathin continuous carbon films,[57,59,66-68] mechanical stage tilting with gold grids[69,70] or detergents to modify surface tension[71]. Some of the nanobodies might not recognize the glycoproteins rigidly leading to conformational heterogeneity and dampened resolution. We will overcome this using workflows developed in-house specifically for that purpose for previously characterized miniprotein inhibitors and monoclonal antibody Fab fragments based on extensive (focused) 3D classification and local 3D refinement of subregions of the map to achieve 2.5-3.5Å resolutions.[69,72-76] Refinement of atomic models in cryoEM density maps will leverage the Rosetta software developed at the University of Washington.[77-79]

The Nipah virus attachment (G) glycoprotein engages host receptors, is the basis of a commercially available horse vaccine and entered phase I clinical trials for use in humans with funding from the Coalition for Epidemic Preparedness Initiatives. The **Veesler and Broder labs** recently determined a high-resolution cryoEM structure of the Nipah virus G glycoprotein ectodomain tetramer which has resisted structural characterization for 25 years, hindering both our understanding of immunity directed towards G and the rational design of vaccines. The structure was obtained in complex with a potent broadly neutralizing antibody Fab fragment (nAH1.3) which recognizes an epitope distinct from the receptor-binding site and is a candidate for therapeutic development. This study revealed the ultrastructural organization of this key target of the immune system, informs the mechanism of henipavirus entry into host cells and provides a blueprint for guiding vaccine and therapeutic design. The know-how generated to achieve this groundbreaking result will directly support the proposed project.

D.4.2.1. High-resolution structural studies using X-ray crystallography. Some of the nanobodies might bind to regions that are intrinsically flexible thereby limiting resolution using cryoEM. We have already encountered such cases with coronavirus fusion machinery-directed antibodies binding to linear epitopes in the spikes. We will overcome these challenges by determining high-resolution crystal structures of the nanobodies bound to their cognate epitopes, similar to the strategy we used to obtain structural information of the B6 and S2P6 antibodies bound to several β-coronavirus stem helices. Refinement of atomic models will use both Phenix[80] and BUSTER-TNT[81].

D.4.2.2. Functional analysis of nanobodies. The mechanism(s) of viral inhibition of each nanobody inferred from the the structural data will be validated using receptor binding, F cleavage and membrane fusion assays we previously implemented for henipaviruses and coronaviruses.[57-59,67,82,83]

Potential Pitfalls and Alternative Strategies. In the unlikely event we encounter protein complexes that cannot be vitrified using any of the strategies described, we will use the TTP Labtech Chameleon vitrification robot, which has been specifically designed to overcome these issues using self-wicking grids and ultra-fast (80-150ms) spot-to-plunge time (28–30). This device is available at the UW Arnold and Mabel Beckman cryoEM center, which **Dr. Veesler** co-directs.

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the formulation of cocktails. This work will also demonstrate the mechanism(s) of action of nanobodies leading to inhibition of viral entry.

Rigor and reproducibility. Maps and models will be thoroughly validated using standards widely accepted in the field. For cryoEM maps, these will include gold-standard projection matching refinements[84-86] and high-resolution noise substitution prior to Fourier shell correlation calculations[87] to avoid overfitting. Atomic models will be assessed using Molprobity[88], EMringer[89] and Privateer[90]. The coordinates of the structures solved as part of this work, as well as the corresponding experimental data, will be made freely available through deposition in the RCSB Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB).

Anticipated results. The structural studies outlined will provide a blueprint of the atomic interactions between the designed nanobodies and their targets which will enable iterative optimization of binding affinity, cross-reactivity and the design of nanobody cocktails.

D.4.3. AIM 2C. Structure-guided formulation of nanobody cocktails

Rationale: The formulation of mAb cocktails has become a widely used and successful approach to prevent or treat infections with RNA viruses. For instance, although the individual Regeneron 10933 (casirivimab) and 10987 (imdevimab) mAbs are affected by a range of residue substitutions detected in SARS-CoV-2 clinical isolates, the two-mAb cocktail proved more resilient to escape mutations.[91-93] Because experimental passaging of NiV and HeV with low concentrations of neutralizing mAbs can favor the emergence of neutralization escape mutants, although none were ever detected in vivo, we will formulate and test neutralizing nanobody cocktails that targets multiple, yet distinct and conserved, antigenic sites on the NiV and HeV F and G glycoproteins.

The separation of receptor engagement and membrane fusion functions on two distinct glycoproteins represent a unique opportunity for implementing multi-pronged targeting which has not been evaluated previously. We will combine multiple, distinct F-specific, G-specific and F/G-specific nanobodies based on classification of their antigenic sites resulting from our structural studies, biolayer interferometry and competition ELISA data. CedV chimera-based neutralization assays will be used to characterize the cocktails and compare them side-by-side to individual constituting nanobodies with matched composition, as described in Aim 2A. Readouts for down-selection will include maximal neutralization potency and breadth and structural characterization will be used to ensure that multi-pronged binding occurs as designed and to guide future rounds of optimization if necessary.

Anticipated results. The outcome of this aim will be the generation and down-selection of nanobody cocktails with broad and potent neutralizing activity against NiV and HeV ready to be tested for *in vivo* protection.

D.5. AIM 3: Evaluation of lead compounds *in vivo*.

Rationale: Aim 3 will collaborate with Core D to evaluate lead candidates *in vivo*. A critical aspect of antiviral discovery that is often overlooked is the early stage testing of lead compounds in a well-studied and well-validated *in vivo* animal models. Here, our experimental plan has the benefit of such models for pathogenic henipaviruses (NiV and HeV) which includes the Syrian golden hamster (refs) and AGM (refs). PK/PD studies will be carried out in the NiV/HeV hamster model. Protective efficacy studies using select best-in-class drugs and nanobodies will be used in NiV_B and HeV infected hamsters. Final proof-of-concept protective efficacy studies with best-in-class drugs and/or nanobodies will be carried out in the NiV_B and HeV infected AGM models.

D.5.1. Pharmacokinetic studies in hamsters and AGMs. In order to enable *in vivo* efficacy studies, preliminary pharmacokinetic (PK) studies in hamsters and AGMs will be performed by Core D. Using intraperitoneal (i.p.) or intravenous (i.v.) administration for hamsters and i.v. administration for AGMs, these studies will examine the concentration of lead compounds in plasma over time in order to establish parameters such as bioavailability, volume of distribution, clearance, and half-life. This data will be used to select doses and dosing regimens to be investigated in pharmacodynamic (PD) studies using a commonly used PK/PD parameter for efficacy in antiviral drug development, which is the maintenance of the minimum plasma concentration (Cmin) above the EC_{90} in cellular assays. The PK/PD relationship and impact on antiviral activity will be assessed in hamster models of heniavirus disease. Lead compounds which demonstrate good exposure-response antiviral activity in hamsters will then advance into AGM PK studies using similar dose administration and measured parameters as above. The data will be used to select doses and dosing regimens for the subsequent protection studies in AGMs.

D.5.2. *In vivo* testing of lead compounds NiV/HeV infected hamsters. The *in vivo* activity of the most potent compounds from Aims 1 and 2 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and inoculated intranasally (i.n.) with ~ 5x10^6 pfu of NiV_B or HeV (refs). Animals will then be treated by i.p. or i.v. injection ~ 1 hour after virus challenge with different doses of drugs or nanobodies. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for further analysis in the Subaims below.

D.5.3. Define breakthrough conditions for anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising drugs from Aim 1 and nanobodies from Aim 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antivirals fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^6$ PFU of NiV or HeV (refs). Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

D.5.4. Combination therapy of anti-henipavirus antivirals in hamsters. This Subaim will employ the most promising antivirals from Aims 1 and 2 where breakthrough conditions have been defined in D.5.3. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ $5x10^5$ PFU of NiV or HeV (refs). Treatment with at least two different antivirals will be administered beginning at a time point where substantial breakthrough was shown in D.5.3 for each individual antiviral in D.5.3 to determine if combining at least two different antivirals improves survival. Group sizes may be increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

D.5.5. Evaluation of aerosol delivery of antivirals against aerosol exposure in hamsters. This subaim will employ the most promising antivirals from Aims 1 and 2 individually or in combinations to determine 1) if aerosol delivery can improve efficacy or extend the therapeutic window versus parenteral delivery and 2) if antivirals can protect against small particle aerosol exposure. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated by aerosol exposure with up to ~ $5x10^6$ PFU of NiV_B or HeV as determined in henipavirus dose confirmation studies conducted by Core D (see Core D). Treatments with drugs, nanobodies, or combinations of drugs and/or nanobodies will be initiated by i.v., i.p., or aerosol delivery following optimization by Core X (see Core X) at various times after henipavirus infection.

D.5.6. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates (NHP). A final proof of concept study will be performed in NHPs using the lead candidates providing the best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated either by large particle aerosol using the MAD system as previously described (ref) or by small particle aerosol as previously described (refs) with a uniformly lethal challenge dose (~ 20,000 PFU of NiV or HeV. The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion or aerosol following optimization by Core X up to 5 days after viral challenge (initiation time informed by hamster studies) while control animals will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

Potential Pitfalls and Alternative Strategies. It is possible that Core X may experience difficulties in optimizing aerosol delivery of drugs and/or nanobodies. Likewise, it is possible that aerosol delivery of antivirals may not improve protective efficacy or extend the therapeutic window of antivirals. We will also assess conventional parenteral delivery routes (as noted in the subaims above) which were previously shown for remdesivir and monoclonal antibodies to protect animals against lethal henipavirus disease to de-risk this Aim.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues, i.e., antiviral drugs will be coded so that researchers will not know which compounds were given to which animals until experiments and analysis are completed. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

In summary, the emergence and re-emergence of Nipah virus infections throughout the world, coupled with its high fatality rate, provide very significant motivation for the preparation of small molecule inhibitors of this virus to create a readiness for the next Nipah virus outbreaks. We have discovered two differentiated series of compounds that show early promise as agents that have potential for treating Nipah virus infections, and we have designed a series of optimized druglike candidates for future preparation and development.

REFERENCES

1 Eaton, B. T., Broder, C. C., Middleton, D. & Wang, L. F. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* **4**, 23-35, doi:10.1038/nrmicro1323 (2006).

2 Geisbert, T. W., Feldmann, H. & Broder, C. C. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* **359**, 153-177, doi:10.1007/82_2012_208 (2012).

3 Nikolay, B. *et al.* Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. *N Engl J Med* **380**, 1804-1814, doi:10.1056/NEJMoa1805376 (2019).

4 Arunkumar, G. *et al.* Outbreak Investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis* **219**, 1867-1878, doi:10.1093/infdis/jiy612 (2019).

5 Pernet, O. *et al.* Evidence for henipavirus spillover into human populations in Africa. *Nat Commun* **5**, 5342, doi:10.1038/ncomms6342 (2014).

6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses Public Health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).

7 Amaya, M. *et al.* A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery. *Antiviral Res* **193**, 105084, doi:10.1016/j.antiviral.2021.105084 (2021).

8 Laing, E. D. *et al.* Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J* **15**, 56, doi:10.1186/s12985-018-0964-0 (2018).

9 Laing, E. D. *et al.* Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus. *Proc Natl Acad Sci U S A* **116**, 20707-20715, doi:10.1073/pnas.1911773116 (2019).

10 Xu, J. *et al.* Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. *Nature* **595**, 278-282, doi:10.1038/s41586-021-03676-z (2021).

11 Desselberger, U. Emerging and re-emerging infectious diseases. *J Infect* **40**, 3-15 (2000).

12 Luby, S. P. & Broder, C. C. in *Viral Infections of Humans, Epidemiology and Control* (eds R. A. Kaslow, L. R. Stanberry, & J. W. Le Duc) Ch. 22, 519-536 (Springer Science+Business Media, 2014).

13 Wang, L.-F., Mackenzie, J. S. & Broder, C. C. in *Fields Virology* Vol. 1 (eds D. M. Knipe & P. M. Howley) Ch. 37, 1070-1085 (Lippincott Williams & Wilkins, 2013).

14 Murray, K. *et al.* A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* **1**, 31-33, doi:10.3201/eid0101.950107 (1995).

15 Selvey, L. A. *et al.* Infection of humans and horses by a newly described morbillivirus. *Med J Aust* **162**, 642-645 (1995).

16 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).

17 Broder, C. C. *et al.* A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* **100**, 8-13, doi:10.1016/j.antiviral.2013.06.012 (2013).

18 Sweileh, W. M. Global research trends of World Health Organization's top eight emerging pathogens. *Global Health* **13**, 9, doi:10.1186/s12992-017-0233-9 (2017).

19 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379, doi:10.3201/eid2003.131159 (2014).

20 Amaya, M. & Broder, C. C. Vaccines to Emerging Viruses: Nipah and Hendra. *Annu Rev Virol* **7**, 447-473, doi:10.1146/annurev-virology-021920-113833 (2020).

21 Playford, E. G. *et al.* Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of henipaviruses in healthy adults: a first-in-human, randomised, controlled, phase 1 study. *Lancet Infect Dis*, doi:10.1016/s1473-3099(19)30634-6 (2020).

22 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep* **6**, 30916, doi:10.1038/srep30916 (2016).

23 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Science translational medicine* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

24 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS pathogens* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

25 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Science translational medicine* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).

26 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS pathogens* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

27 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS pathogens* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).

28 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

29 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

30 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796, doi:10.1038/ncomms1796 (2012).

31 Muyldermans, S. Nanobodies: natural single-domain antibodies. *Annual review of biochemistry* **82**, 775-797, doi:10.1146/annurev-biochem-063011-092449 (2013).

32 Muyldermans, S. Applications of Nanobodies. *Annual review of animal biosciences* **9**, 401-421, doi:10.1146/annurev-animal-021419-083831 (2021).

33 Scully, M. *et al.* Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. *The New England journal of medicine* **380**, 335-346, doi:10.1056/NEJMoa1806311 (2019).

34 Gomez, E., Avendano, C. & Mckillop, A. Ethyl Carboethoxyformimidate in Heterocyclic Chemistry. *Tetrahedron* **42**, 2625-2634, doi:Doi 10.1016/S0040-4020(01)90547-2 (1986).

35 Mckillop, A., Henderson, A., Ray, P. S., Avendano, C. & Molinero, E. G. Heterocyclic Synthesis Using Ethyl Carboethoxyformimidate. *Tetrahedron Lett* **23**, 3357-3360, doi:Doi 10.1016/S0040-4039(00)87614-5 (1982).

36 Suesse, M. A., F.; Johne, S. Quinazolinecarboxylic acid. Synthesis of alkyl[2-(ethoxycarbonyl)-3,4-dihydro-4-oxoquinazolin-3-yl]-, [2-(ethoxycarbonyl)quinazolin-4-yloxy]- and (5,6,7,8-tetrahydro-2-phenylquinazolin-4-ylthio)alkanoates. *Helvetica Chimica Acta* **69**, 1017-1024 (1986).

37 Sugiyama, Y., Sasaki, T. & Nagato, N. Acid-Catalyzed Reactions of Ethyl Cyanoformate with Aromatic-Amines in Acetic-Acid - Facile Synthesis of N-Substituted Amidinoformic Acids and Ethyl 4-Quinazolone-2-Carboxylate. *J Org Chem* **43**, 4485-4487, doi:DOI 10.1021/jo00417a020 (1978).

38 Ursu, O., Rayan, A., Goldblum, A. & Oprea, T. I. Understanding drug-likeness. *WIRES Comp. Mol. Sci.* **1**, 760-781, doi:10.1002/wcms.52 (2011).

39 Markgren, P. O., Hamalainen, M. & Danielson, U. H. Kinetic analysis of the interaction between HIV-1 protease and inhibitors using optical biosensor technology. *Anal Biochem* **279**, 71-78, doi:DOI 10.1006/abio.1999.4467 (2000).

40 Lander, G. C. *et al.* Appion: an integrated, database-driven pipeline to facilitate EM image processing. *Journal of structural biology* **166**, 95-102 (2009).

41 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

42 Ludtke, S. J. 3-D structures of macromolecules using single-particle analysis in EMAN. *Methods in molecular biology* **673**, 157-173, doi:10.1007/978-1-60761-842-3_9 (2010).

43 Scheres, S. H. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *Journal of structural biology* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

44 Nierzwicki, Ł. & Palermo, G. Molecular Dynamics to Predict Cryo-EM: Capturing Transitions and Short-Lived Conformational States of Biomolecules. *Front Mol Biosci* **8**, 641208, doi:10.3389/fmolb.2021.641208 (2021).

45 Hughes, T. E. T. *et al.* Structural insights on TRPV5 gating by endogenous modulators. *Nature communications* **9**, 4198, doi:10.1038/s41467-018-06753-6 (2018).

46 Wu, Y. *et al.* Flipping in the Pore: Discovery of Dual Inhibitors That Bind in Different Orientations to the Wild-Type versus the Amantadine-Resistant S31N Mutant of the Influenza A Virus M2 Proton Channel. *Journal of the American Chemical Society* **136**, 17987-17995, doi:10.1021/ja508461m (2014).

47 Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-Bonded Water Molecules in the M2 Channel of the Influenza A Virus Guide the Binding Preferences of Ammonium-Based Inhibitors. *J. Phys. Chem. B* **119**, 1173-1183, doi:10.1021/jp506807y (2015).

48 Wang, J. *et al.* Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. *PNAS* **110**, 1315-1320, doi:10.1073/pnas.1216526110 (2013).

49 Wang, J. *et al.* Molecular Dynamics Simulation Directed Rational Design of Inhibitors Targeting Drug-Resistant Mutants of Influenza A Virus M2. *Journal of the American Chemical Society* **133**, 12834-12841, doi:10.1021/ja204969m (2011).

50 Gianti, E., Delemotte, L., Klein, M. L. & Carnevale, V. On the role of water density fluctuations in the inhibition of a proton channel. *PNAS* **113**, E8359-E8368, doi:10.1073/pnas.1609964114 (2016).

51 Casalino, L. *et al.* Beyond Shielding: The Roles of Glycans in the SARS-CoV-2 Spike Protein. *ACS Cent Sci* **6**, 1722-1734, doi:10.1021/acscentsci.0c01056 (2020).

52 Delemotte, L., Tarek, M., Klein, M. L., Amaral, C. & Treptow, W. Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations. *PNAS* **108**, 6109-6114, doi:10.1073/pnas.1102724108 (2011).

53 Perni, R. B. *et al.* Preclinical profile of VX-950, a potent, selective, and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease. *Antimicrob Agents Chemother* **50**, 899-909, doi:50/3/899 [pii]

10.1128/AAC.50.3.899-909.2006 (2006).

54 Kuhnz, W. & Gieschen, H. Predicting the oral bioavailability of 19-nortestosterone progestins in vivo from their metabolic stability in human liver microsomal preparations in vitro. *Drug Metab Dispos* **26**, 1120-1127 (1998).

55 Finlayson, K., Pennington, A. J. & Kelly, J. S. [3H]dofetilide binding in SHSY5Y and HEK293 cells expressing a HERG-like K+ channel? *Eur J Pharmacol* **412**, 203-212, doi:S0014299901007312 [pii] (2001).

56 Gres, M. C. *et al.* Correlation between oral drug absorption in humans, and apparent drug permeability in TC-7 cells, a human epithelial intestinal cell line: comparison with the parental Caco-2 cell line. *Pharm Res* **15**, 726-733 (1998).

57 Dang, H. V. *et al.* Broadly neutralizing antibody cocktails targeting Nipah virus and Hendra virus fusion glycoproteins. *Nat Struct Mol Biol* **28**, 426-+ (2021).

58 Cheliout Da Silva, S. *et al.* Functional Analysis of the Fusion and Attachment Glycoproteins of Mojiang Henipavirus. *Viruses* **13**, doi:10.3390/v13030517 (2021).

59 Dang, H. V. *et al.* An antibody against the F glycoprotein inhibits Nipah and Hendra virus infections. *Nat Struct Mol Biol* **26**, 980-+ (2019).

60 Doyle, M. P. *et al.* Cooperativity mediated by rationally selected combinations of human monoclonal antibodies targeting the henipavirus receptor binding protein. *Cell Rep* **36** (2021).

61 Wang, J. N. *et al.* A new Hendra virus genotype found in Australian flying foxes. *Virol J* **18**, doi:ARTN 197

10.1186/s12985-021-01652-7 (2021).

62 Drexler, J. F. *et al.* Henipavirus RNA in African Bats. *Plos One* **4**, doi:ARTN e6367

10.1371/journal.pone.0006367 (2009).

63 Weiss, S. *et al.* Henipavirus-related Sequences in Fruit Bat Bushmeat, Republic of Congo. *Emerg Infect Dis* **18**, 1536-+, doi:10.3201/eid1809.111607 (2012).

64 Suloway, C. *et al.* Automated molecular microscopy: The new Leginon system. *J Struct Biol* **151**, 41-60, doi:10.1016/j.jsb.2005.03.010 (2005).

65 Tegunov, D. & Cramer, P. Real-time cryo-electron microscopy data preprocessing with Warp. *Nat Methods* **16**, 1146-+, doi:10.1038/s41592-019-0580-y (2019).

66 Park, Y. J. *et al.* Structures of MERS-CoV spike glycoprotein in complex with sialoside attachment receptors. *Nat Struct Mol Biol* **26**, 1151-+, doi:10.1038/s41594-019-0334-7 (2019).

67 Tortorici, M. A. *et al.* Broad sarbecovirus neutralization by a human monoclonal antibody. *Nature* **597**, 103-+, doi:10.1038/s41586-021-03817-4 (2021).

68 Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein (vol 180, 281.e1, 2020). *Cell* **183**, 1735-1735, doi:10.1016/j.cell.2020.11.032 (2020).

69 Piccoli, L. *et al.* Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. *Cell* **183**, 1024-+, doi:10.1016/j.cell.2020.09.037 (2020).

70 Pinto, D. *et al.* Cross-neutralization ofSARS-CoV-2 by a human monoclonal SARS-CoV antibody. *Nature* **583**, 290-+, doi:10.1038/s41586-020-2349-y (2020).

71 Tortorici, M. A. *et al.* Structural basis for human coronavirus attachment to sialic acid receptors. *Nat Struct Mol Biol* **26**, 481-+, doi:10.1038/s41594-019-0233-y (2019).

72 Cao, L. X. *et al.* De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* **370**, 426-+, doi:10.1126/science.abd9909 (2020).

73 Hunt, A. C. *et al.* Multivalent designed proteins protect against SARS-CoV-2 variants of concern. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.07.07.451375 (2021).

74 McCallum, M. *et al.* SARS-CoV-2 immune evasion by the B.1.427/B.1.429 variant of concern. *Science* **373**, 648-654, doi:10.1126/science.abi7994 (2021).

75 McCallum, M. *et al.* N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. *bioRxiv : the preprint server for biology*, doi:10.1101/2021.01.14.426475 (2021).

76 Tortorici, M. A. *et al.* Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. *Science* **370**, 950-957, doi:10.1126/science.abe3354 (2020).

77 Frenz, B., Walls, A. C., Egelman, E. H., Veesler, D. & DiMaio, F. RosettaES: a sampling strategy enabling automated interpretation of difficult cryo-EM maps. *Nat Methods* **14**, 797-800, doi:10.1038/nmeth.4340 (2017).

78 Frenz, B. *et al.* Automatically Fixing Errors in Glycoprotein Structures with Rosetta. *Structure* **27**, 134-139 e133, doi:10.1016/j.str.2018.09.006 (2019).

79 Wang, R. Y. *et al.* Automated structure refinement of macromolecular assemblies from cryo-EM maps using Rosetta. *eLife* **5**, doi:10.7554/eLife.17219 (2016).

80 Liebschner, D. *et al.* Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix. *Acta crystallographica. Section D, Structural biology* **75**, 861-877, doi:10.1107/S2059798319011471 (2019).

81 Blanc, E. *et al.* Refinement of severely incomplete structures with maximum likelihood in BUSTER-TNT. *Acta crystallographica. Section D, Biological crystallography* **60**, 2210-2221, doi:10.1107/S0907444904016427 (2004).

82 Pinto, D. *et al.* Broad betacoronavirus neutralization by a stem helix-specific human antibody. *Science* **373**, 1109-1116, doi:10.1126/science.abj3321 (2021).

83 Sauer, M. M. *et al.* Structural basis for broad coronavirus neutralization. *bioRxiv : the preprint server for biology*, doi:10.1101/2020.12.29.424482 (2021).

84 Scheres, S. H. W. RELION: Implementation of a Bayesian approach to cryo-EM structure determination. *J Struct Biol* **180**, 519-530, doi:10.1016/j.jsb.2012.09.006 (2012).

85 Punjani, A., Zhang, H. W. & Fleet, D. J. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. *Nat Methods* **17**, 1214-+, doi:10.1038/s41592-020-00990-8 (2020).

86 Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* **333**, 721-745, doi:DOI 10.1016/j.jmb.2003.07.013 (2003).

87 Chen, S. X. *et al.* High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. *Ultramicroscopy* **135**, 24-35, doi:10.1016/j.ultramic.2013.06.004 (2013).

88 Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr D* **66**, 12-21, doi:10.1107/S0907444909042073 (2010).

89 Barad, B. A. *et al.* EMRinger: side chain directed model and map validation for 3D cryo-electron microscopy. *Nat Methods* **12**, 943-946, doi:10.1038/Nmeth.3541 (2015).

90 Agirre, J. *et al.* Privateer: software for the conformational validation of carbohydrate structures. *Nat Struct Mol Biol* **22**, 833-834, doi:Doi 10.1038/Nsmb.3115 (2015).

91 Hansen, J. *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* **369**, 1010-+, doi:10.1126/science.abd0827 (2020).

92 Baum, A. *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science* **369**, 1014-+, doi:10.1126/science.abd0831 (2020).

93 Baum, A. *et al.* REGN-COV2 antibodies prevent and treat SARS-CoV-2 infection in rhesus macaques and hamsters. *Science* **370**, 1110-+, doi:10.1126/science.abe2402 (2020).

From: Gaisina, Irina N on behalf of Gaisina, Irina N <igaysina@uic.edu>
To: Broder, Christopher; Rong, Lijun
Cc: David Veesler; Moushimi Amaya; Thomas Geisbert
Subject: Re: rCedV
Date: Monday, November 1, 2021 9:56:06 PM
Attachments: Tulane_Res_strategy_Nipah-11-1-combined.docx

Chris, I'm still working on it, and this is my current version. I had to use an old one for Tom's edits.
Still need to fix refs in your "Background" portion to be able to put it in.
Although, it seems even longer than the one in the submitted Alabama grant.
Would it be possible to reduce it a little bit?

And I'll use the new figures that you just sent us.
Thanks.

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Monday, November 1, 2021 8:33 PM
To: Rong, Lijun <lijun@uic.edu>; Gaisina, Irina N <igaysina@uic.edu>
Cc: David Veesler <dveesler@uw.edu>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>; Thomas Geisbert <twgeisbe@utmb.edu>
Subject: rCedV

Irina, Lijun

try putting this rCedV tools into the preliminary data
it covers chimeras to replace the published rCedV-Luc description and existing
figure 1 which we should remove. the chimera prelim data supports high throughput
nanobody assessment, and synergy assay (which can be mentioned in Aim 1 drugs, and Aim 2
nanobodies.

also there is some text for resistance selection and genome sequencing for
target identification if needed somewhere

SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV)(REF). NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality(REF). Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission(REF). Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors(REF). 22 cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antivirals approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers(REF). As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus non-pathogenic in well-established animal models of NiV and HeV pathogenesis{Marsh, 2012 #2}. We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS)(REFS). We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will potentially yield testable antivirals with potential pan-henipavirus effectiveness. Our collaborative team also has an extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity(REF). These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and also aerosol delivery of nanobodies. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobodies studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

Commented [CB1]: Hendra and Nipah viruses: different and dangerous
B. T. Eaton, C. C. Broder, D. Middleton and L. F. Wang
Nat Rev Microbiol 2006 Vol. 4 Issue 1 Pages 23-35
Accession Number: 16357858

Commented [CB2]: Animal Challenge Models of Henipavirus Infection and Pathogenesis
T. W. Geisbert, H. Feldmann and C. C. Broder
Curr Top Microbiol Immunol 2012 Vol. 359 Pages 153-77
Accession Number: 22476556 DOI: 10.1007/82_2012_208

Commented [CB3]: Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh
B. Nikolay, H. Salje, M. J. Hossain, A. Khan, H. M. S. Sazzad, M. Rahman, et al.
N Engl J Med 2019 Vol. 380 Issue 19 Pages 1804-1814
Accession Number: 31067370

Commented [CB4]: Outbreak investigation of Nipah Virus Disease in Kerala, India, 2018
G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.
J Infect Dis 2018 Vol. 219 Pages 1867-1878
Accession Number: 30364984 DOI: 10.1093/infdis/jiy612

Commented [CB5]: Evidence for henipavirus spillover into human populations in Africa
O. Pernet, B. S. Schneider, S. M. Beaty, M. LeBreton, T. E. Yun, A. Park, et al.
Nat Commun 2014 Vol. 5 Pages 5342
Accession Number: 25405640 PMCID: 4237230 DOI: 10.1038/ncomms6342

Commented [CB6]: A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery
M. Amaya, H. Cheng, V. Borisevich, C. K. Navaratnarajah, R. Cattaneo, L. Cooper, et al.
Antiviral Res 2021 Vol. 193 Pages 105084
Accession Number: 34077807 DOI: 10.1016/j.antiviral.2021.105084

Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species
E. D. Laing, M. Amaya, C. K. Navaratnarajah, Y. R. Feng, R. Cattaneo, L. F. Wang, et al.
Virol J 2018 Vol. 15 Issue 1 Pages 56
Accession Number: 29587789 PMCID: PMC5869790 DOI: 10.1186/s12985-018-0964-0

Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus
E. D. Laing, C. K. Navaratnarajah, S. Cheliout Da Silva, S. R. Petzing, Y. Xu, S. L. Sterling, et al.
Proc Natl Acad Sci U S A 2019 Vol. 116 Issue 41 Pages 20707-20715
Accession Number: 31548390

Commented [CB7]: Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants
J. Xu, K. Xu, S. Jung, A. Conte, J. Lieberman, F. Muecksch, et al.
Nature 2021 Vol. 595 Issue 7866 Pages 278-282
Accession Number: 34098567

RESEARCH STRATEGY

A) Background and Significance

Over the past 40 years the major etiological agents of emerged or newly identified infectious diseases in humans have been viruses{Desselberger, 2000 #1}. Among these, two zoonotic paramyxoviruses of particular importance are Nipah virus (NiV) and Hendra virus (HeV) which are the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae* and are single-stranded, negative sense, enveloped RNA viruses.{Wang, 2013 #1} HeV was discovered first in 1994, in an outbreak of severe respiratory disease in horses in Brisbane, Australia, resulting in the deaths of 14 horses and their trainer{Murray, 1995 #109;Selvey, 1995 #110}. NiV (Malaysia strain NiV-M) was discovered a few years later as the causative agent of a significant outbreak of severe encephalitis in pig farmers in Malaysia in 1998(REF). NiV outbreaks have also been recorded in Bangladesh and India by a closely related strain NiV-Bangladesh (NiV-B)(REF). Pteropid bats are the major reservoir hosts for henipaviruses{Clayton, 2013 #3;Halpin, 2011 #58}. HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bat hosts. Among these animals, NiV and HeV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with human fatality rates ranging from 40-100%{Broder, 2013 #11;Geisbert, 2012 #13}. A third henipavirus isolate, Cedar virus (CedV), was discovered in 2010.{Marsh, 2012 #2} However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease{Lieu, 2015 #642;Marsh, 2012 #2}(REF). Two additional henipaviruses are also recognized, Ghana bat virus and Mojiang virus, but are known only from sequence data{Wu, 2014 #4;Drexler, 2012 #5}(REF). Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and three outbreaks in India (reviewed(REF)). In 2014, an outbreak of NiV-M encephalitis occurred in the Philippines with 9 fatalities of 11 human cases, along with horse-to-human and human-to-human transmission{Ching, 2015 #66}.The combined NiV case fatality rate is ~60% across a large region of South Asia and Southeast Asia in five countries(REF). The recent 2018 NiV outbreak in Kerala, India was significant, having occurred in a new geographic region, a high case fatality rate of 91% and 22 of 23 cases the result of from human-to-human transmission, underscoring both the transmissibility and the lethal nature of this virus{Arunkumar, 2018 #927}.

Human NiV/HeV infection is generally accepted to occur via the oronasal route with an incubation period estimated to be 1 to 2 weeks(REF). Acute NiV/HeV infection in people is systemic and likely via hematogenous spread of the virus from the respiratory system(REF). Disease onset is characterized by fever, myalgia, shortness of breath, and cough(REF). Human HeV infections have resulted in both fatal respiratory or encephalitic disease, and also recovery from infection(REF). The predominant clinical feature in the NiV-M outbreak in Malaysia was encephalitis, but respiratory symptoms were also common, with fever, cough, and headache(REF). The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease and in the 2018 NiV-B outbreak in Kerala, India, 83% of cases presented with acute respiratory distress syndrome (ARDS)(REF). The key findings of human NiV/HeV infection are a wide-spread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected(REF). However, human NiV/HeV infection can also be prolonged following apparent recovery, and some patients can experience a late-onset encephalitis or a relapsed encephalitis in patients who previously recovered(REF). Relapsed encephalitis caused by NiV appears to result from a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later(REF). NiV and HeV are also biosafety level-4 (BSL-4) viruses and classified as Category C priority pathogens and transboundary agents, infecting both humans and economically important livestock by several US Government agencies and both NiV-M and NiV-B can cause lethal infection in nonhuman primates (NHPs), the African green monkey (AGM) when delivered by small particle aerosol(REF). NiV and henipaviral diseases (HeV) are also included on the WHO's Blueprint List of Priority Pathogens(REF). NiV stands out as a potential pandemic threat because of its associated high case-fatality rates, capacity for human-to-human transmission, potential to mutate, and if a human-adapted strain were to infect communities in South Asia, the high regional population densities could lead to rapid infection spread(REF). *There is presently no approved vaccines or therapeutic modalities for preventing or treating NiV/HeV infections in people.*

The viral RNA-dependent-RNA polymerase of paramyxoviruses are attractive targets for antiviral compound discovery, but antiviral drug discovery for NiV and HeV has been significantly hampered due to requirements of BSL-4 containment in research and development process. We recently developed and optimized an authentic henipavirus replication cell-based system using a recombinant CedV (rCedV) encoding luciferase

Commented [CB8]: K. B. Chua, K. J. Goh, K. T. Wong, A. Kamarulzaman, P. S. Tan, T. G. Ksiazek, et al.
Lancet 1999 Vol. 354 Issue 9186 Pages 1257-9
Accession Number: 10520635 DOI: S0140-6736(99)04299-3 [pii]
10.1016/S0140-6736(99)04299-3 [doi]

Nipah virus: a recently emergent deadly paramyxovirus
K. B. Chua, W. J. Bellini, P. A. Rota, B. H. Harcourt, A. Tamin, S. K. Lam, et al. ... [1]

Commented [CB9]: Genetic characterization of Nipah virus, Bangladesh, 2004 ... [2]

Commented [CB10]: Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters ... [3]

Commented [CB11]: Taxonomy of the order Mononegavirales: second update 2018 ... [4]

Commented [CB12]: Vaccines to Emerging Viruses: Nipah and Hendra
M. Amaya and C. C. Broder ... [5]

Commented [CB13]: Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh ... [6]

Commented [CB14]: G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.
J Infect Dis 2018 Vol. 219 Pages 1867-1878 ... [7]

Commented [CB15]: K. T. Wong, W. J. Shieh, S. R. Zaki and C. T. Tan ... [8]

Commented [CB16]: L. A. Selvey, R. M. Wells, J. G. McCormack, A. J. Ansford, K. Murray, R. J. Rogers, et al.
Med J Aust 1995 Vol. 162 Issue 12 Pages 642-5 ... [9]

Commented [CB17]: J. D. O'Sullivan, A. M. Allworth, D. L. Paterson, T. M. Snow, R. Boots, L. J. Gleeson, et al.
Lancet 1997 Vol. 349 Issue 9045 Pages 93-5 ... [10]

Commented [CB18]: N. I. Paton, Y. S. Leo, S. R. Zaki, A. P. Auchus, K. E. Lee, A. E. Ling, et al.
Lancet 1999 Vol. 354 Issue 9186 Pages 1253-6 ... [11]

Commented [CB19]: M. J. Hossain, E. S. Gurley, J. M. Montgomery, M. Bell, D. S. Carroll, V. P. Hsu, et al.
Clin Infect Dis 2008 Vol. 46 Issue 7 Pages 977-84 ... [12]

Commented [CB20]: K. T. Wong, W. J. Shieh, S. Kumar, K. Norain, W. Abdullah, J. Guarner, et al.
Am J Pathol 2002 Vol. 161 Issue 6 Pages 2153-67. ... [13]

Commented [CB21]: C. T. Tan, K. J. Goh, K. T. Wong, S. A. Sarji, K. B. Chua, N. K. Chew, et al.
Ann Neurol 2002 Vol. 51 Issue 6 Pages 703-8. ... [14]

Commented [CB22]: S. Abdullah, L. Y. Chang, K. Rahmat, K. T. Goh and C. T. Tan
Neurology Asia 2012 Vol. 17 Issue 1 Pages 71-74

Commented [CB23]:
D. A. Hammoud, M. R. Lentz, A. Lara, J. K. Bohannon, I. Feuerstein, L. Huzella, et al. ... [15]

Commented [CB24]: ... [16]

Commented [CB25]: The pandemic potential of Nipah virus
S. P. Luby ... [17]

(rCedV-Luc) for high-throughput screening (HTS) applications at BSL-2 containment and identified several inhibitory compounds that were further validated with authentic NiV-B and HeV(REF). Those validated lead compounds provide a foundation and rationale for the current application. This ability to employ rCedV provides an opportunity for anti-henipavirus drug discovery not previously available, allowing for inhibitor discovery across the virus replication cycle, and not restricted to only the viral polymerase. Another well-exploited stage of virus infection that has been successfully targeted is virus attachment and entry(REF). Henipaviruses possess two membrane anchored glycoproteins involved in virus entry; one mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion(REF). The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and also the main focus of all vaccine strategies(REF). Although an effective post-exposure human monoclonal antibody (mAb) therapy targeting the NiV and HeV G glycoprotein (m102.4) has been extensively examined{Mire, 2016 #322;Geisbert, 2014 #154;Xu, 2013 #152;Bossart, 2011 #153;Bossart, 2009 #125}, it must be administered within several days following a lethal virus challenge in NHP models. An unexplored alternative but related antibody-based therapeutic strategy against henipavirus infection are antiviral nanobodies. A nanobody, also termed single-domain antibody (sdAb), is an antibody fragment comprised of just a single monomeric variable antibody domain(REF). Nanobodies were first engineered from variable heavy chain domains of camelid heavy chain antibodies (VHHs), and camelid VHHs retain full antigen specificity and are only a fraction of the size of mouse or human antibody, and they also have extended complementarity determining regions (CDRs) which can allow them to bind epitopes that are not normally accessible to conventional antibodies such as conserved viral epitopes that are often masked by glycan shields(REF). Here, our expertise in recombinant henipavirus F and G glycoprotein structural and functional analysis together with anti-F and G mAb development and their therapeutic application, will facilitate the development and testing of a new and understudied area of nanobody-based countermeasures for combating NiV and HeV.

B) Innovation

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of this proposal's investigators. Indeed, all aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet and Irina Gaisina, Chicago BioSolutions** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core Lead). First, the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may will have activity across related members of henipavirus genus. Second, our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice that produce VHHs (nanobodies). Third, together with newly created rCedV chimeras and the establishment of high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. Fourth, the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification, and finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Cedar henipavirus. The closely related henipavirus, Cedar henipavirus (CedV), was recovered from *Pteropus* flying fox urine samples in Australia and shown to be nonpathogenic (REF). CedV was isolated in BSL-4 containment, but there was potential to use it a surrogate system for the study of pathogenic henipaviruses at BSL-2. We developed a reverse genetics system for designing and rescuing recombinant CedV (rCedV) (REF). Interestingly, unlike HeV and NiV that use only ephrin-B2 and ephrin-B3 as entry receptors, CedV displays a broader ephrin usage profile, employing human ephrin-B2, ephrin-B1, ephrin-A2 and ephrin-A5 and murine-A1 for infection(REF). However, CedV is nonpathogenic in the well-accepted experimental animal models of HeV and NiV infection and disease including the hamster, ferret, and AGM(REF). CedV attenuation has been attributed to the lack of an RNA editing site in the *P* gene for the production of the interferon antagonist proteins, V and W(REF).

Commented [CB26]: Amaya et al.,
Antiviral Res 2021 Vol. 193 Pages 105084
Accession Number: 34077807 DOI: 10.1016/j.antiviral.2021.105084

Commented [CB27]: Henipavirus outbreaks to antivirals: the current status of potential therapeutics
C. C. Broder
Curr Opin Virol 2012 Vol. 2 Issue 2 Pages 176-87
Accsion Number: 22482714 DOI: 10.1016/j.coviro.2012.02.016

Commented [CB28]: Differential Features of Fusion Activation within the Paramyxoviridae
K. D. Azarm and B. Lee
Viruses 2020 Vol. 12 Issue 2
Accession Number: 32019182 DOI: 10.3390/v12020161

Commented [CB29]: Vaccines to Emerging Viruses: Nipah and Hendra
M. Amaya and C. C. Broder
Annu Rev Virol 2020 Vol. 7 Issue 1 Pages 447-473
Accession Number: 32991264 DOI: 10.1146/annurev-virology-021920-113833

Commented [CB30]: Appl Microbiol Biotechnol
2007 Nov;77(1):13-22. doi: 10.1007/s00253-007-1142-2.
Properties, production, and applications of camelid single-domain antibody fragments
M M Harmsen 1, H J De Haard
DOI: 10.1007/s00253-007-1142-2

Commented [CB31]: Nature 2021 Jul;595(7866):278-282. doi: 10.1038/s41586-021-03676-z.
Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants
Jianliang Xu et al.,

Commented [CB32]: https://pubmed.ncbi.nlm.nih.gov/22879820/

Commented [CB33]: https://pubmed.ncbi.nlm.nih.gov/29587789/

Commented [CB34]: E. D. Laing, C. K. Navaratnarajah, S. Cheliout Da Silva, S. R. Petzing, Y. Xu, S. L. Sterling, et al.
Proc Natl Acad Sci U S A 2019 Vol. 116 Issue 41 Pages 20707-20715

Commented [CB35]: Cedar virus: a novel Henipavirus isolated from Australian bats
G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.
PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836
DOI: 10.1371/journal.ppat.1002836

Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters
... [18]

Commented [CB36]: Cedar virus: a novel Henipavirus isolated from Australian bats
G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.
PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836
... [19]

Small molecule antiviral drug discovery. Anti-whole inactivated NiV or HeV immune sera will show cross-reactivity with CedV, however such sera or even specific high-titer anti-CedV F or G glycoprotein antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins (REF). Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and it is also the largest polymerase in the order *Mononegavirale*, yet it still shares high aa identity to NiV and HeV L protein. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; the aa identities of the important L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This high degree of protein identity, overall, shared between CedV, and that of NiV and HeV, suggests that antiviral agents that target for example, the L polymerase, could be identified and perhaps also offer the possibility of pan-henipavirus antiviral countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication (REF). Indeed, several small molecule inhibitors have been validated in vitro using infectious HeV and NiV in BSL-4, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support Aim 1 to optimize our current best-in-class leads and also screen large libraries of antiviral compounds.

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Commented [CB38]: https://pubmed.ncbi.nlm.nih.gov/34077807/

Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials(REFs). Nanobodies can be isolated using the same phage library construction methods and phage panning procedure routinely employed for mAb discovery, which allows for in vitro production of various amounts and their rapid characterization. In collaboration with Dr. Jianliang Xu (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels(REF), we have several immunized mouse cohorts now underway using native-like oligomeric recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in Aim 2 in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

Commented [CB39]: Nanobodies: natural single-domain antibodies
S. Muyldermans
Annu Rev Biochem 2013 Vol. 82 Pages 775-97
DOI: 10.1146/annurev-biochem-063011-092449

Applications of Nanobodies
S. Muyldermans
Annu Rev Anim Biosci 2021 Vol. 9 Pages 401-421
DOI: 10.1146/annurev-animal-021419-083831

Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura
M. Scully, S. R. Cataland, F. Peyvandi, P. Coppo, P. Knöbl, J. A. Kremer Hovinga, et al.
N Engl J Med 2019 Vol. 380 Issue 4 Pages 335-346
DOI: 10.1056/NEJMoa1806311

Commented [CB40]: Nature 2021 Jul;595(7866):278-282. doi: 10.1038/s41586-021-03676-z.
Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants
Jianliang Xu et al.,

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of Aim 3. In addition, we expect to discover, and well-characterize, batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in Aim 3. Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.

C) Preliminary Results

Related to AIM 1

Drugs and structures and activities

Related to AIM 2

ELISA data on nanomice
rCedV SNT assays

NOTES to RES PLAN
Synergy
Target identification

Page 2: [1] Commented [CB8] Christopher Broder 10/29/2021 1:00:00 PM

K. B. Chua, K. J. Goh, K. T. Wong, A. Kamarulzaman, P. S. Tan, T. G. Ksiazek, et al.

Lancet 1999 Vol. 354 Issue 9186 Pages 1257-9

Accession Number: 10520635 DOI: S0140-6736(99)04299-3 [pii]

10.1016/S0140-6736(99)04299-3 [doi]

Nipah virus: a recently emergent deadly paramyxovirus

K. B. Chua, W. J. Bellini, P. A. Rota, B. H. Harcourt, A. Tamin, S. K. Lam, et al.

Science 2000 Vol. 288 Issue 5470 Pages 1432-5

Page 2: [2] Commented [CB9] Christopher Broder 10/29/2021 1:10:00 PM

Genetic characterization of Nipah virus, Bangladesh, 2004

B. H. Harcourt, L. Lowe, A. Tamin, X. Liu, B. Bankamp, N. Bowden, et al.

Emerg Infect Dis 2005 Vol. 11 Issue 10 Pages 1594-7

Accession Number: 16318702

Page 2: [3] Commented [CB10] Christopher Broder 10/29/2021 1:14:00 PM

Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters

T. Schountz, C. Campbell, K. Wagner, J. Rovnak, C. Martellaro, B. L. DeBuysscher, et al.

Viruses 2019 Vol. 11 Issue 3

Acession Number: 30909389 PMCID: PMC6466075 DOI: 10.3390/v11030291

Page 2: [4] Commented [CB11] Christopher Broder 10/29/2021 1:18:00 PM

Taxonomy of the order Mononegavirales: second update 2018

P. Maes, G. K. Amarasinghe, M. A. Ayllon, C. F. Basler, S. Bavari, K. R. Blasdell, et al.

Arch Virol 2019 Vol. 164 Issue 4 Pages 1233-1244

Accession Number: 30663023

Page 2: [5] Commented [CB12] Christopher Broder 10/29/2021 2:02:00 PM

Vaccines to Emerging Viruses: Nipah and Hendra

M. Amaya and C. C. Broder

Annu Rev Virol 2020 Vol. 7 Issue 1 Pages 447-473

Accession Number: 32991264 DOI: 10.1146/annurev-virology-021920-113833

Page 2: [6] Commented [CB13] Christopher Broder 10/29/2021 2:11:00 PM

Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh

B. Nikolay, H. Salje, M. J. Hossain, A. Khan, H. M. S. Sazzad, M. Rahman, et al.

N Engl J Med 2019 Vol. 380 Issue 19 Pages 1804-1814

Accession Number: 31067370

Page 2: [7] Commented [CB14] **Christopher Broder** **10/29/2021 2:27:00 PM**

G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.

J Infect Dis 2018 Vol. 219 Pages 1867-1878

Accession Number: 30364984 DOI: 10.1093/infdis/jiy612

E. G. Playford, B. McCall, G. Smith, V. Slinko, G. Allen, I. Smith, et al.

Emerg Infect Dis 2010 Vol. 16 Issue 2 Pages 219-23

Accession Number: 20113550

Page 2: [8] Commented [CB15] **Christopher Broder** **10/29/2021 2:28:00 PM**

K. T. Wong, W. J. Shieh, S. R. Zaki and C. T. Tan

Springer Semin Immunopathol 2002 Vol. 24 Issue 2 Pages 215-28

Accession Number: 12503066

Page 2: [9] Commented [CB16] **Christopher Broder** **10/29/2021 2:29:00 PM**

L. A. Selvey, R. M. Wells, J. G. McCormack, A. J. Ansford, K. Murray, R. J. Rogers, et al.

Med J Aust 1995 Vol. 162 Issue 12 Pages 642-5

K. J. Goh, C. T. Tan, N. K. Chew, P. S. Tan, A. Kamarulzaman, S. A. Sarji, et al.

N Engl J Med 2000 Vol. 342 Issue 17 Pages 1229-35

Accession Number: 10781618 DOI: MJBA-421701 [pii] 10.1056/NEJM200004273421701 [doi]

Page 2: [10] Commented [CB17] **Christopher Broder** **10/29/2021 2:31:00 PM**

J. D. O'Sullivan, A. M. Allworth, D. L. Paterson, T. M. Snow, R. Boots, L. J. Gleeson, et al.

Lancet 1997 Vol. 349 Issue 9045 Pages 93-5

E. G. Playford, B. McCall, G. Smith, V. Slinko, G. Allen, I. Smith, et al.

Emerg Infect Dis 2010 Vol. 16 Issue 2 Pages 219-23

Accession Number: 20113550

Page 2: [11] Commented [CB18] Christopher Broder 10/29/2021 2:32:00 PM

N. I. Paton, Y. S. Leo, S. R. Zaki, A. P. Auchus, K. E. Lee, A. E. Ling, et al.

Lancet 1999 Vol. 354 Issue 9186 Pages 1253-6

Accession Number: 10520634

K. J. Goh, C. T. Tan, N. K. Chew, P. S. Tan, A. Kamarulzaman, S. A. Sarji, et al.

N Engl J Med 2000 Vol. 342 Issue 17 Pages 1229-35

Accession Number: 10781618 DOI: MJBA-421701 [pii] 10.1056/NEJM200004273421701 [doi]

Page 2: [12] Commented [CB19] Christopher Broder 10/29/2021 2:34:00 PM

M. J. Hossain, E. S. Gurley, J. M. Montgomery, M. Bell, D. S. Carroll, V. P. Hsu, et al.

Clin Infect Dis 2008 Vol. 46 Issue 7 Pages 977-84

Accession Number: 18444812 DOI: 10.1086/529147 [doi]

G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.

J Infect Dis 2018 Vol. 219 Pages 1867-1878

Accession Number: 30364984 DOI: 10.1093/infdis/jiy612

Page 2: [13] Commented [CB20] Christopher Broder 10/29/2021 2:35:00 PM

K. T. Wong, W. J. Shieh, S. Kumar, K. Norain, W. Abdullah, J. Guarner, et al.

Am J Pathol 2002 Vol. 161 Issue 6 Pages 2153-67.

K. T. Wong, T. Robertson, B. B. Ong, J. W. Chong, K. C. Yaiw, L. F. Wang, et al.

Neuropathol Appl Neurobiol 2009 Vol. 35 Issue 3 Pages 296-305

Accession Number: 19473296 DOI: NAN991 [pii]

10.1111/j.1365-2990.2008.00991.x [doi]

Page 2: [14] Commented [CB21] Christopher Broder 10/29/2021 2:37:00 PM

C. T. Tan, K. J. Goh, K. T. Wong, S. A. Sarji, K. B. Chua, N. K. Chew, et al.

Ann Neurol 2002 Vol. 51 Issue 6 Pages 703-8.

J. D. O'Sullivan, A. M. Allworth, D. L. Paterson, T. M. Snow, R. Boots, L. J. Gleeson, et al.

Lancet 1997 Vol. 349 Issue 9045 Pages 93-5

Page 2: [15] Commented [CB23] Christopher Broder 10/29/2021 3:00:00 PM

D. A. Hammoud, M. R. Lentz, A. Lara, J. K. Bohannon, I. Feuerstein, L. Huzella, et al.

PLoS Negl Trop Dis 2018 Vol. 12 Issue 11 Pages e0006978

J. B. Geisbert, V. Borisevich, A. N. Prasad, K. N. Agans, S. L. Foster, D. J. Deer, et al.

J Infect Dis 2020 Vol. 221 Issue Suppl 4 Pages S414-S418

Accession Number: 31665362

A. N. Prasad, K. N. Agans, S. K. Sivasubramani, J. B. Geisbert, V. Borisevich, C. E. Mire, et al.

J Infect Dis 2020 Vol. 221 Issue Suppl 4 Pages S431-S435

Accession Number: 31665351 PMCID: 7213566 DOI: 10.1093/infdis/jiz391

10.1093/infdis/jiz469

Page 2: [16] Commented [CB24] Christopher Broder 10/29/2021 2:52:00 PM

The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts

M. S. Mehand, F. Al-Shorbaji, P. Millett and B. Murgue

Antiviral Res 2018 Vol. 159 Pages 63-67

Accession Number: 30261226

Page 2: [17] Commented [CB25] Christopher Broder 10/29/2021 2:58:00 PM

The pandemic potential of Nipah virus

S. P. Luby

Antiviral Res 2013 Vol. 100 Issue 1 Pages 38-43

Accession Number: 23911335 DOI: 10.1016/j.antiviral.2013.07.011

Page 3: [18] Commented [CB35] Christopher Broder 10/31/2021 3:43:00 PM

Cedar virus: a novel Henipavirus isolated from Australian bats

G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.

PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836

DOI: 10.1371/journal.ppat.1002836

Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters

T. Schountz, C. Campbell, K. Wagner, J. Rovnak, C. Martellaro, B. L. DeBuysscher, et al.

Viruses 2019 Vol. 11 Issue 3

(Geisbert and Broder unpublished)

Page 3: [19] Commented [CB36] Christopher Broder 10/31/2021 3:47:00 PM

Cedar virus: a novel Henipavirus isolated from Australian bats

G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.

PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836

DOI: 10.1371/journal.ppat.1002836

The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2

K. G. Lieu, G. A. Marsh, L. F. Wang and H. J. Netter

Antiviral Res 2015 Vol. 124 Pages 69-76

DOI: 10.1016/j.antiviral.2015.09.017

From: Broder, Christopher on behalf of Broder, Christopher <christopher.broder@usuhs.edu>
To: Rong, Lijun
Cc: David Veesler; Moushimi Amaya; Gaisina, Irina N; Thomas Geisbert
Subject: Re: Project 1 absrt
Date: Monday, November 1, 2021 9:36:40 PM
Attachments: SpecificAims-ResStrategy-HenipaRP1-RAViD-10312021.docx

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i sent you all the Aims page on Sunday night--
it was page 1 of the document so that the references would be
a simple manner (attached here again)

i have not made any more changes.

On Mon, Nov 1, 2021 at 2:50 PM Rong, Lijun <lijun@uic.edu> wrote:

> Thank you Chris. Do you have an updated Aims page to share with us pls?
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> Lijun Rong, PhD
>
> Professor
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> College of Medicine
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> **From:** Broder, Christopher <christopher.broder@usuhs.edu>
> **Sent:** Monday, November 1, 2021 10:02 AM
> **To:** Rong, Lijun <lijun@uic.edu>; David Veesler <dveesler@uw.edu>; Moushimi Amaya <moushimi.amaya.ctr@usuhs.edu>; Gaisina, Irina N <igaysina@uic.edu>; Thomas Geisbert <twgeisbe@utmb.edu>
> **Subject:** Project 1 absrt

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Christopher C. Broder, Ph.D.
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SPECIFIC AIMS

The emergence and reemergence of pathogenic viruses represent continuous infectious disease threats to public health. Among these, the paramyxoviruses, which include many human and animal pathogens, also include two zoonoses of particular importance: the pathogenic henipaviruses; Nipah virus (NiV) and Hendra virus (HeV)(REF). NiV and HeV cause severe respiratory illness and encephalitis in both animals and humans with impressive lethality(REF). Two strains of NiV, Malaysia (NiV-M) and Bangladesh (NiV-B) together have human case fatality rates ranging from 40-100%, and some outbreaks of NiV-B are associated with multiple rounds of human-to-human transmission(REF). Indeed, the 2018 NiV-B outbreak in Kerala, India had a 91% case fatality rate among 23 total cases, leaving only 2 survivors(REF). 22 cases were from virus transmission in 3 hospital settings, with coughing and close proximity to aerosol droplet infection were major risk factors. NiV-B is a pathogen of pandemic concern, and there are no vaccines or antivirals approved for human use. Indeed, the detection of cross-reactive henipavirus antibodies in humans and Pteropus bats in Africa underscored that 2 billion people worldwide live in regions threatened by spillovers(REF). As part of the Respiratory Pathogen Aerosol Antiviral Drug Discovery (RAViD) Center, this proposal focuses on developing countermeasures to combat the pathogenic henipaviruses including therapeutic modalities by an aerosol delivery approach.

Recently, Cedar henipavirus (CedV) was identified in bats and is the only known henipavirus non-pathogenic in well-established animal models of NiV and HeV pathogenesis{Marsh, 2012 #2}. We developed a reverse genetics platform for recombinant CedV (rCedV) construction which is suitable for BSL-2 use, and performed an extensive characterization of its cellular infection tropism and also developed reporter rCedVs suitable for high throughput antiviral screening (HTS)(REFS). We validated and published on the utility of rCedV encoding luciferase in HTS assays and discovered a series of fused heterocycles, e.g., 4-aminoquinazolines, and showed a representative quinoline was an effective inhibitor of rCedV replication that also extended to both NiV-B and HeV, with an IC_{50} value of 3.3 μm against NiV-B replication with minimal cytotoxicity. The further development of small molecules together with more extensive compound library screening using rCedV will potentially yield testable antivirals with potential pan-henipavirus effectiveness. Our collaborative team also has an extensive experience in developing henipavirus neutralizing antibodies to both the fusion (F) and attachment (G) proteins. In this proposal, we have initiated a completely different approach for developing antiviral nanobodies which are acceptable for the goals of this program. Nanobodies are versatile potential immunotherapeutics and have been shown to recognize epitopes that are often inaccessible to conventional antibodies and to achieve ultra-potent antiviral activity(REF). These nanobody accessible epitopes may also have the potential to be more conserved across viral strains, potentially exhibiting greater cross-reactivity. In this proposal we will screen, identify, develop, characterize and test both small molecule inhibitors and antiviral nanobodies to pathogenic henipaviruses. Specifically, we will:

Aim 1. Optimize lead anti-henipavirus small molecules and conduct cell-based antiviral HTS assays. First, the 4-aminoquinazoline scaffold will be used to develop and test improved drug candidates. We will also conduct new compound library HTS assays and identify new lead candidates. We will use resistance selection assays for target identification; advanced lead molecules will be selected using NiV-B and HeV assays, and best-in-class drugs will be developed using predictive software to improve drug-like properties, increase potencies, decrease toxicity, and improve cell permeability and favorable pharmacokinetic properties.

Aim 2. Isolate and fully characterize nanobodies to the F and G glycoproteins of Nipah and Hendra virus. Immunizations of transgenic mice that produce nanobodies from alpacas, dromedaries and Bactrian camels, using native-like oligomeric G and F proteins will be completed. Phage libraries of cloned nanobody gene fragments will be constructed by the Nanobody Core followed by panning and isolation of F and G specific nanobodies. Best-in-class, cross-reactive nanobodies will then be characterized in binding and neutralization assays and structurally analyzed in complex with their viral targets to delineate the recognition sites, mode of action and guide formulation of nanobody cocktails.

Aim 3. Evaluation of lead compounds *in vivo*. Protective efficacy studies using select best-in-class drugs and nanobodies will be conducted early in NiV/HeV-infected hamsters using both conventional intraperitoneal delivery (drug or nanobody) and also aerosol delivery of nanobodies. PK/PD studies of lead drugs (Aim 1) and nanobodies (Aim 2) will be carried out in hamsters. Combination studies of drug with nanobodies studies will also be conducted and final proof-of-concept protective efficacy studies will be carried out in the HeV and NiV-B African green monkey models.

This project brings together a highly-experienced team in henipavirus countermeasure discovery, development and testing. Together with the expertise, resources and track record within the proposed RAViD center, we will deliver preclinical antiviral candidate treatment modalities against henipavirus mediated disease.

Commented [CB1]: Hendra and Nipah viruses: different and dangerous
B. T. Eaton, C. C. Broder, D. Middleton and L. F. Wang
Nat Rev Microbiol 2006 Vol. 4 Issue 1 Pages 23-35
Accession Number: 16357858

Commented [CB2]: Animal Challenge Models of Henipavirus Infection and Pathogenesis
T. W. Geisbert, H. Feldmann and C. C. Broder
Curr Top Microbiol Immunol 2012 Vol. 359 Pages 153-77
Accession Number: 22476556 DOI: 10.1007/82_2012_208

Commented [CB3]: Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh
B. Nikolay, H. Salje, M. J. Hossain, A. Khan, H. M. S. Sazzad, M. Rahman, et al.
N Engl J Med 2019 Vol. 380 Issue 19 Pages 1804-1814
Accession Number: 31067370

Commented [CB4]: Outbreak investigation of Nipah Virus Disease in Kerala, India, 2018
G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.
J Infect Dis 2018 Vol. 219 Pages 1867-1878
Accession Number: 30364984 DOI: 10.1093/infdis/jiy612

Commented [CB5]: Evidence for henipavirus spillover into human populations in Africa
O. Pernet, B. S. Schneider, S. M. Beaty, M. LeBreton, T. E. Yun, A. Park, et al.
Nat Commun 2014 Vol. 5 Pages 5342
Accession Number: 25405640 PMCID: 4237230 DOI: 10.1038/ncomms6342

Commented [CB6]: A recombinant Cedar virus based high-throughput screening assay for henipavirus antiviral discovery
M. Amaya, H. Cheng, V. Borisevich, C. K. Navaratnarajah, R. Cattaneo, L. Cooper, et al.
Antiviral Res 2021 Vol. 193 Pages 105084
Accession Number: 34077807 DOI: 10.1016/j.antiviral.2021.105084

Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species
E. D. Laing, M. Amaya, C. K. Navaratnarajah, Y. R. Feng, R. Cattaneo, L. F. Wang, et al.
Virol J 2018 Vol. 15 Issue 1 Pages 56
Accession Number: 29587789 PMCID: PMC5869790 DOI: 10.1186/s12985-018-0964-0

Structural and functional analyses reveal promiscuous and species specific use of ephrin receptors by Cedar virus
E. D. Laing, C. K. Navaratnarajah, S. Cheliout Da Silva, S. R. Petzing, Y. Xu, S. L. Sterling, et al.
Proc Natl Acad Sci U S A 2019 Vol. 116 Issue 41 Pages 20707-20715
Accession Number: 31548390

Commented [CB7]: Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants
J. Xu, K. Xu, S. Jung, A. Conte, J. Lieberman, F. Muecksch, et al.
Nature 2021 Vol. 595 Issue 7866 Pages 278-282
Accession Number: 34098567

RESEARCH STRATEGY

A) Background and Significance

Over the past 40 years the major etiological agents of emerged or newly identified infectious diseases in humans have been viruses{Desselberger, 2000 #1}. Among these, two zoonotic paramyxoviruses of particular importance are Nipah virus (NiV) and Hendra virus (HeV) which are the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae* and are single-stranded, negative sense, enveloped RNA viruses. {Wang, 2013 #1} HeV was discovered first in 1994, in an outbreak of severe respiratory disease in horses in Brisbane, Australia, resulting in the deaths of 14 horses and their trainer{Murray, 1995 #109;Selvey, 1995 #110}. NiV (Malaysia strain NiV-M) was discovered a few years later as the causative agent of a significant outbreak of severe encephalitis in pig farmers in Malaysia in 1998(REF). NiV outbreaks have also been recorded in Bangladesh and India by a closely related strain NiV-Bangladesh (NiV-B)(REF). Pteropid bats are the major reservoir hosts for henipaviruses{Clayton, 2013 #3;Halpin, 2011 #58}. HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bat hosts. Among these animals, NiV and HeV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with human fatality rates ranging from 40-100%{Broder, 2013 #11;Geisbert, 2012 #13}. A third henipavirus isolate, Cedar virus (CedV), was discovered in 2010.{Marsh, 2012 #2} However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease{Lieu, 2015 #642;Marsh, 2012 #2}(REF). Two additional henipaviruses are also recognized, Ghana bat virus and Mojiang virus, but are known only from sequence data{Wu, 2014 #4;Drexler, 2012 #5}(REF). Since 2001, nearly annual occurrences of human NiV-B infections have occurred in Bangladesh, and three outbreaks in India (reviewed(REF)). In 2014, an outbreak of NiV-M encephalitis occurred in the Philippines with 9 fatalities of 11 human cases, along with horse-to-human and human-to-human transmission{Ching, 2015 #66}.The combined NiV case fatality rate is ~60% across a large region of South Asia and Southeast Asia in five countries(REF). The recent 2018 NiV outbreak in Kerala, India was significant, having occurred in a new geographic region, a high case fatality rate of 91% and 22 of 23 cases the result of from human-to-human transmission, underscoring both the transmissibility and the lethal nature of this virus{Arunkumar, 2018 #927}.

Human NiV/HeV infection is generally accepted to occur via the oronasal route with an incubation period estimated to be 1 to 2 weeks(REF). Acute NiV/HeV infection in people is systemic and likely via hematogenous spread of the virus from the respiratory system(REF). Disease onset is characterized by fever, myalgia, shortness of breath, and cough(REF). Human HeV infections have resulted in both fatal respiratory or encephalitic disease, and also recovery from infection(REF). The predominant clinical feature in the NiV-M outbreak in Malaysia was encephalitis, but respiratory symptoms were also common, with fever, cough, and headache(REF). The clinical presentation of NiV-B infections in Bangladesh also includes severe respiratory disease and in the 2018 NiV-B outbreak in Kerala, India, 83% of cases presented with acute respiratory distress syndrome (ARDS)(REF). The key findings of human NiV/HeV infection are a wide-spread endothelial cell tropism and systemic vasculitis, with prominent parenchymal cell infection in most major organs with the brain and lung significantly affected(REF). However, human NiV/HeV infection can also be prolonged following apparent recovery, and some patients can experience a late-onset encephalitis or a relapsed encephalitis in patients who previously recovered(REF). Relapsed encephalitis caused by NiV appears to result from a recrudescence of virus replication in the central nervous system (CNS), with cases presenting from a few months to as long as 11 years later(REF). NiV and HeV are also biosafety level-4 (BSL-4) viruses and classified as Category C priority pathogens and transboundary agents, infecting both humans and economically important livestock by several US Government agencies and both NiV-M and NiV-B can cause lethal infection in nonhuman primates (NHPs), the African green monkey (AGM) when delivered by small particle aerosol(REF). NiV and henipaviral diseases (HeV) are also included on the WHO's Blueprint List of Priority Pathogens(REF). NiV stands out as a potential pandemic threat because of its associated high case-fatality rates, capacity for human-to-human transmission, potential to mutate, and if a human-adapted strain were to infect communities in South Asia, the high regional population densities could lead to rapid infection spread(REF). *There is presently no approved vaccines or therapeutic modalities for preventing or treating NiV/HeV infections in people.*

The viral RNA-dependent-RNA polymerase of paramyxoviruses are attractive targets for antiviral compound discovery, but antiviral drug discovery for NiV and HeV has been significantly hampered due to requirements of BSL-4 containment in research and development process. We recently developed and optimized an authentic henipavirus replication cell-based system using a recombinant CedV (rCedV) encoding luciferase

Commented [CB8]: K. B. Chua, K. J. Goh, K. T. Wong, A. Kamarulzaman, P. S. Tan, T. G. Ksiazek, et al.
Lancet 1999 Vol. 354 Issue 9186 Pages 1257-9
Accession Number: 10520635 DOI: S0140-6736(99)04299-3 [pii]
10.1016/S0140-6736(99)04299-3 [doi]

Nipah virus: a recently emergent deadly paramyxovirus
K. B. Chua, W. J. Bellini, P. A. Rota, B. H. Harcourt, A. Tamin, S. K. Lam, et al. ... [1]

Commented [CB9]: Genetic characterization of Nipah virus, Bangladesh, 2004 ... [2]

Commented [CB10]: Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters ... [3]

Commented [CB11]: Taxonomy of the order Mononegavirales: second update 2018 ... [4]

Commented [CB12]: Vaccines to Emerging Viruses: Nipah and Hendra
M. Amaya and C. C. Broder ... [5]

Commented [CB13]: Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh ... [6]

Commented [CB14]: G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.
J Infect Dis 2018 Vol. 219 Pages 1867-1878 ... [7]

Commented [CB15]: K. T. Wong, W. J. Shieh, S. R. Zaki and C. T. Tan ... [8]

Commented [CB16]: L. A. Selvey, R. M. Wells, J. G. McCormack, A. J. Ansford, K. Murray, R. J. Rogers, et al.
Med J Aust 1995 Vol. 162 Issue 12 Pages 642-5 ... [9]

Commented [CB17]: J. D. O'Sullivan, A. M. Allworth, D. L. Paterson, T. M. Snow, R. Boots, L. J. Gleeson, et al.
Lancet 1997 Vol. 349 Issue 9045 Pages 93-5 ... [10]

Commented [CB18]: N. I. Paton, Y. S. Leo, S. R. Zaki, A. P. Auchus, K. E. Lee, A. E. Ling, et al.
Lancet 1999 Vol. 354 Issue 9186 Pages 1253-6 ... [11]

Commented [CB19]: M. J. Hossain, E. S. Gurley, J. M. Montgomery, M. Bell, D. S. Carroll, V. P. Hsu, et al.
Clin Infect Dis 2008 Vol. 46 Issue 7 Pages 977-84 ... [12]

Commented [CB20]: K. T. Wong, W. J. Shieh, S. Kumar, K. Norain, W. Abdullah, J. Guarner, et al.
Am J Pathol 2002 Vol. 161 Issue 6 Pages 2153-67. ... [13]

Commented [CB21]: C. T. Tan, K. J. Goh, K. T. Wong, S. A. Sarji, K. B. Chua, N. K. Chew, et al.
Ann Neurol 2002 Vol. 51 Issue 6 Pages 703-8. ... [14]

Commented [CB22]: S. Abdullah, L. Y. Chang, K. Rahmat, K. T. Goh and C. T. Tan
Neurology Asia 2012 Vol. 17 Issue 1 Pages 71-74

Commented [CB23]:
D. A. Hammoud, M. R. Lentz, A. Lara, J. K. Bohannon, I. Feuerstein, L. Huzella, et al. ... [15]

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Commented [CB25]: The pandemic potential of Nipah virus
S. P. Luby ... [17]

(rCedV-Luc) for high-throughput screening (HTS) applications at BSL-2 containment and identified several inhibitory compounds that were further validated with authentic NiV-B and HeV(REF). Those validated lead compounds provide a foundation and rationale for the current application. This ability to employ rCedV provides an opportunity for anti-henipavirus drug discovery not previously available, allowing for inhibitor discovery across the virus replication cycle, and not restricted to only the viral polymerase. Another well-exploited stage of virus infection that has been successfully targeted is virus attachment and entry(REF). Henipaviruses possess two membrane anchored glycoproteins involved in virus entry; one mediates host cell receptor attachment (G) glycoprotein and the other is a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion(REF). The viral G and F glycoproteins are the major antigenic targets of neutralizing antibodies and also the main focus of all vaccine strategies(REF). Although an effective post-exposure human monoclonal antibody (mAb) therapy targeting the NiV and HeV G glycoprotein (m102.4) has been extensively examined{Mire, 2016 #322;Geisbert, 2014 #154;Xu, 2013 #152;Bossart, 2011 #153;Bossart, 2009 #125}, it must be administered within several days following a lethal virus challenge in NHP models. An unexplored alternative but related antibody-based therapeutic strategy against henipavirus infection are antiviral nanobodies. A nanobody, also termed single-domain antibody (sdAb), is an antibody fragment comprised of just a single monomeric variable antibody domain(REF). Nanobodies were first engineered from variable heavy chain domains of camelid heavy chain antibodies (VHHs), and camelid VHHs retain full antigen specificity and are only a fraction of the size of mouse or human antibody, and they also have extended complementarity determining regions (CDRs) which can allow them to bind epitopes that are not normally accessible to conventional antibodies such as conserved viral epitopes that are often masked by glycan shields(REF). Here, our expertise in recombinant henipavirus F and G glycoprotein structural and functional analysis together with anti-F and G mAb development and their therapeutic application, will facilitate the development and testing of a new and understudied area of nanobody-based countermeasures for combating NiV and HeV.

B) Innovation

There are several innovative aspects within the present application which capitalize on the ongoing collaborative research and expertise of this proposal's investigators. Indeed, all aspects of the proposed research are new extensions of productive research endeavors between: **Dr. Christopher Broder, USU** (Henipavirus Project 1 lead); **Dr. Lijun Rong, UIC** (Co-Investigator lead on small molecule discovery) and **Drs. Norton Peet and Irina Gaisina, Chicago BioSolutions** (Co-Investigators and leads on medicinal chemistry); **Dr. David Veesler, UW** (Co-Investigator lead on structural henipaviral biology), **Dr. Moushimi Amaya, USU** (Co-Investigator lead on rCedV tools), **Dr. Jianliang Xu,** (Nanobody Core Lead, Zalgen Labs), and **Dr. Thomas Giesbert, UTMB** (Co-Investigator and BSL-4 and ABSL-4 Core Lead). First, the cell-based high throughput screening (HTS) assay makes use of an authentic henipavirus infection and replication luciferase reporter-gene system, which provides an HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. Of additional significance, this recombinant Cedar virus (rCedV) based platform requires only BSL-2 containment. Also, because of the close phylogenic relatedness of CedV to NiV and HeV, including the use of the same principal entry receptor (ephrin-B2) and replication mechanisms, there is high potential that identified inhibitors may will have activity across related members of henipavirus genus. Second, our native-like, recombinant soluble tetrameric G and trimeric F NiV and HeV glycoproteins are now being used to immunize novel engineered mice that produce VHHs (nanobodies). Third, together with newly created rCedV chimeras and the establishment of high-throughput NiV/HeV GFP reporter-gene, neutralization assays, the screening, identification and characterization of large numbers of nanobodies for their NiV and HeV specific neutralization potency and cross-reactivity features is now possible. Fourth, the rCedV platform allows for authentic henipavirus antiviral escape studies for variant selection and target identification, and finally, the rCedV based reporter-gene system allows rapid and quantitative antiviral combination testing and the ability to measure synergistic activity with small molecules or nanobodies or combinations of both.

Cedar henipavirus. The closely related henipavirus, Cedar henipavirus (CedV), was recovered from *Pteropus* flying fox urine samples in Australia and shown to be nonpathogenic (REF). CedV was isolated in BSL-4 containment, but there was potential to use it a surrogate system for the study of pathogenic henipaviruses at BSL-2. We developed a reverse genetics system for designing and rescuing recombinant CedV (rCedV) (REF). Interestingly, unlike HeV and NiV that use only ephrin-B2 and ephrin-B3 as entry receptors, CedV displays a broader ephrin usage profile, employing human ephrin-B2, ephrin-B1, ephrin-A2 and ephrin-A5 and murine-A1 for infection(REF). However, CedV is nonpathogenic in the well-accepted experimental animal models of HeV and NiV infection and disease including the hamster, ferret, and AGM(REF). CedV attenuation has been attributed to the lack of an RNA editing site in the *P* gene for the production of the interferon antagonist proteins, V and W(REF).

Commented [CB26]: Amaya et al.,
Antiviral Res 2021 Vol. 193 Pages 105084
Accession Number: 34077807 DOI:
10.1016/j.antiviral.2021.105084

Commented [CB27]: Henipavirus outbreaks to antivirals: the current status of potential therapeutics
C. C. Broder
Curr Opin Virol 2012 Vol. 2 Issue 2 Pages 176-87
Accssion Number: 22482714 DOI:
10.1016/j.coviro.2012.02.016

Commented [CB28]: Differential Features of Fusion Activation within the Paramyxoviridae
K. D. Azarm and B. Lee
Viruses 2020 Vol. 12 Issue 2
Accession Number: 32019182 DOI: 10.3390/v12020161

Commented [CB29]: Vaccines to Emerging Viruses: Nipah and Hendra
M. Amaya and C. C. Broder
Annu Rev Virol 2020 Vol. 7 Issue 1 Pages 447-473
Accession Number: 32991264 DOI: 10.1146/annurev-virology-021920-113833

Commented [CB30]: Appl Microbiol Biotechnol
2007 Nov;77(1):13-22. doi: 10.1007/s00253-007-1142-2.
Properties, production, and applications of camelid single-domain antibody fragments
M M Harmsen 1, H J De Haard
DOI: 10.1007/s00253-007-1142-2

Commented [CB31]: Nature 2021 Jul;595(7866):278-282. doi: 10.1038/s41586-021-03676-z.
Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants
Jianliang Xu et al.,

Commented [CB32]: https://pubmed.ncbi.nlm.nih.gov/22879820/

Commented [CB33]: https://pubmed.ncbi.nlm.nih.gov/29587789/

Commented [CB34]: E. D. Laing, C. K. Navaratnarajah, S. Cheliout Da Silva, S. R. Petzing, Y. Xu, S. L. Sterling, et al.
Proc Natl Acad Sci U S A 2019 Vol. 116 Issue 41 Pages 20707-20715

Commented [CB35]: Cedar virus: a novel Henipavirus isolated from Australian bats
G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.
PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836
DOI: 10.1371/journal.ppat.1002836

Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters
... [18]

Commented [CB36]: Cedar virus: a novel Henipavirus isolated from Australian bats
G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.
PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836
... [19]

Small molecule antiviral drug discovery. Anti-whole inactivated NiV or HeV immune sera will show cross-reactivity with CedV, however such sera or even specific high-titer anti-CedV F or G glycoprotein antisera will not cross-neutralize HeV or NiV. Nevertheless, several CedV proteins show high amino acid (aa) identity to those in NiV and HeV, with highly conserved domains particularly in the N, M and L proteins (REF). Interestingly, the CedV L polymerase protein is 257-aa larger in comparison to NiV and HeV L, and it is also the largest polymerase in the order *Mononegavirale*, yet it still shares high aa identity to NiV and HeV L protein. Indeed, CedV L is ~56% aa identical overall to the NiV-B and HeV L proteins; the aa identities of the important L domains are: catalytic domain ~59.5%; the mRNA capping domain ~64.7%; methyltransferase domain ~50.5% (NiV-B) and ~52% (HeV); C-terminal domain ~51% (NiV-B) and ~52% (HeV); and the 2-O-ribose methyltransferase domain ~60.8% (NiV-B) and ~61.7% (HeV). This high degree of protein identity, overall, shared between CedV, and that of NiV and HeV, suggests that antiviral agents that target for example, the L polymerase, could be identified and perhaps also offer the possibility of pan-henipavirus antiviral countermeasures. We recently tested this possibility and optimized a rCedV encoding firefly luciferase (rCedV-Luc) for HTS assays and conducted a small molecule library screening project to identify inhibitors of rCedV replication (REF). Indeed, several small molecule inhibitors have been validated in vitro using infectious HeV and NiV in BSL-4, demonstrating proof-of-concept and the feasibility of using this CedV-based HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for this drug discovery project. This rCedV HTS system will be used to support Aim 1 to optimize our current best-in-class leads and also screen large libraries of antiviral compounds.

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Nanobodies. In contrast to mouse and human antibody binding domains (which are ~50 kDa in size), camelid VHHs retain full antigen binding specificity but are only ~15 kDa in size. Other advantageous features of nanobodies are their extended CDR elements that engage their antigen targets and thus possess abilities to bind epitopes that are often less accessible to conventional antibodies, and they can also be readily humanized and have been shown to be safe with low immunogenicity in clinical trials(REFs). Nanobodies can be isolated using the same phage library construction methods and phage panning procedure routinely employed for mAb discovery, which allows for in vitro production of various amounts and their rapid characterization. In collaboration with Dr. Jianliang Xu (Nanobody Core Lead), who engineered transgenic mice that produce VHHs (nanobodies) from alpacas, dromedaries and Bactrian camels(REF), we have several immunized mouse cohorts now underway using native-like oligomeric recombinant F and G glycoproteins of NiV-B and HeV. We except to be able to make rapid progress in Aim 2 in generating and characterizing diverse panels of nanobodies to the F and G glycoproteins of NiV and HeV.

Commented [CB39]: Nanobodies: natural single-domain antibodies
S. Muyldermans
Annu Rev Biochem 2013 Vol. 82 Pages 775-97
DOI: 10.1146/annurev-biochem-063011-092449

Applications of Nanobodies
S. Muyldermans
Annu Rev Anim Biosci 2021 Vol. 9 Pages 401-421
DOI: 10.1146/annurev-animal-021419-083831

Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura
M. Scully, S. R. Cataland, F. Peyvandi, P. Coppo, P. Knöbl, J. A. Kremer Hovinga, et al.
N Engl J Med 2019 Vol. 380 Issue 4 Pages 335-346
DOI: 10.1056/NEJMoa1806311

Commented [CB40]: Nature 2021 Jul;595(7866):278-282. doi: 10.1038/s41586-021-03676-z.
Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants
Jianliang Xu et al.,

By utilizing an integrated approach in medicinal chemistry that includes synthesis and antiviral testing of new compounds, *in silico* prediction of compound properties, and the utilization of *in vitro* ADME prediction assays, in both building on our preliminary findings and new discovery initiatives, we expect to derive multiple lead anti-henipaviral molecules that will be testable as part of the goals of Aim 3. In addition, we expect to discover, and well-characterize, batteries of new and novel anti-henipavirus nanobodies specific to the F and G glycoproteins of NiV and HeV, and test their therapeutic activities in Aim 3. Taken together, the combinations of new small molecule drugs and nanobodies to be developed in this proposal, with their specificities tailored to the henipaviruses through the applications of our novel proposed approaches, may lead to novel therapeutic modalities capable of treating NiV and HeV disease.

C) Preliminary Results

Related to AIM 1

Drugs and structures and activities

Related to AIM 2

ELISA data on nanomice
rCedV SNT assays

NOTES to RES PLAN
Synergy
Target identification

Page 2: [1] Commented [CB8] **Christopher Broder** **10/29/2021 1:00:00 PM**

K. B. Chua, K. J. Goh, K. T. Wong, A. Kamarulzaman, P. S. Tan, T. G. Ksiazek, et al.

Lancet 1999 Vol. 354 Issue 9186 Pages 1257-9

Accession Number: 10520635 DOI: S0140-6736(99)04299-3 [pii]

10.1016/S0140-6736(99)04299-3 [doi]

Nipah virus: a recently emergent deadly paramyxovirus

K. B. Chua, W. J. Bellini, P. A. Rota, B. H. Harcourt, A. Tamin, S. K. Lam, et al.

Science 2000 Vol. 288 Issue 5470 Pages 1432-5

Page 2: [2] Commented [CB9] **Christopher Broder** **10/29/2021 1:10:00 PM**

Genetic characterization of Nipah virus, Bangladesh, 2004

B. H. Harcourt, L. Lowe, A. Tamin, X. Liu, B. Bankamp, N. Bowden, et al.

Emerg Infect Dis 2005 Vol. 11 Issue 10 Pages 1594-7

Accession Number: 16318702

Page 2: [3] Commented [CB10] **Christopher Broder** **10/29/2021 1:14:00 PM**

Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters

T. Schountz, C. Campbell, K. Wagner, J. Rovnak, C. Martellaro, B. L. DeBuysscher, et al.

Viruses 2019 Vol. 11 Issue 3

Acession Number: 30909389 PMCID: PMC6466075 DOI: 10.3390/v11030291

Page 2: [4] Commented [CB11] **Christopher Broder** **10/29/2021 1:18:00 PM**

Taxonomy of the order Mononegavirales: second update 2018

P. Maes, G. K. Amarasinghe, M. A. Ayllon, C. F. Basler, S. Bavari, K. R. Blasdell, et al.

Arch Virol 2019 Vol. 164 Issue 4 Pages 1233-1244

Accession Number: 30663023

Page 2: [5] Commented [CB12] **Christopher Broder** **10/29/2021 2:02:00 PM**

Vaccines to Emerging Viruses: Nipah and Hendra

M. Amaya and C. C. Broder

Annu Rev Virol 2020 Vol. 7 Issue 1 Pages 447-473

Accession Number: 32991264 DOI: 10.1146/annurev-virology-021920-113833

Page 2: [6] Commented [CB13] **Christopher Broder** **10/29/2021 2:11:00 PM**

Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh

B. Nikolay, H. Salje, M. J. Hossain, A. Khan, H. M. S. Sazzad, M. Rahman, et al.

N Engl J Med 2019 Vol. 380 Issue 19 Pages 1804-1814

Accession Number: 31067370

Page 2: [7] Commented [CB14] Christopher Broder 10/29/2021 2:27:00 PM

G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.

J Infect Dis 2018 Vol. 219 Pages 1867-1878

Accession Number: 30364984 DOI: 10.1093/infdis/jiy612

E. G. Playford, B. McCall, G. Smith, V. Slinko, G. Allen, I. Smith, et al.

Emerg Infect Dis 2010 Vol. 16 Issue 2 Pages 219-23

Accession Number: 20113550

Page 2: [8] Commented [CB15] Christopher Broder 10/29/2021 2:28:00 PM

K. T. Wong, W. J. Shieh, S. R. Zaki and C. T. Tan

Springer Semin Immunopathol 2002 Vol. 24 Issue 2 Pages 215-28

Accession Number: 12503066

Page 2: [9] Commented [CB16] Christopher Broder 10/29/2021 2:29:00 PM

L. A. Selvey, R. M. Wells, J. G. McCormack, A. J. Ansford, K. Murray, R. J. Rogers, et al.

Med J Aust 1995 Vol. 162 Issue 12 Pages 642-5

K. J. Goh, C. T. Tan, N. K. Chew, P. S. Tan, A. Kamarulzaman, S. A. Sarji, et al.

N Engl J Med 2000 Vol. 342 Issue 17 Pages 1229-35

Accession Number: 10781618 DOI: MJBA-421701 [pii] 10.1056/NEJM200004273421701 [doi]

Page 2: [10] Commented [CB17] Christopher Broder 10/29/2021 2:31:00 PM

J. D. O'Sullivan, A. M. Allworth, D. L. Paterson, T. M. Snow, R. Boots, L. J. Gleeson, et al.

Lancet 1997 Vol. 349 Issue 9045 Pages 93-5

E. G. Playford, B. McCall, G. Smith, V. Slinko, G. Allen, I. Smith, et al.

Emerg Infect Dis 2010 Vol. 16 Issue 2 Pages 219-23

Accession Number: 20113550

Page 2: [11] Commented [CB18] Christopher Broder 10/29/2021 2:32:00 PM

N. I. Paton, Y. S. Leo, S. R. Zaki, A. P. Auchus, K. E. Lee, A. E. Ling, et al.

Lancet 1999 Vol. 354 Issue 9186 Pages 1253-6

Accession Number: 10520634

K. J. Goh, C. T. Tan, N. K. Chew, P. S. Tan, A. Kamarulzaman, S. A. Sarji, et al.

N Engl J Med 2000 Vol. 342 Issue 17 Pages 1229-35

Accession Number: 10781618 DOI: MJBA-421701 [pii] 10.1056/NEJM200004273421701 [doi]

Page 2: [12] Commented [CB19] Christopher Broder 10/29/2021 2:34:00 PM

M. J. Hossain, E. S. Gurley, J. M. Montgomery, M. Bell, D. S. Carroll, V. P. Hsu, et al.

Clin Infect Dis 2008 Vol. 46 Issue 7 Pages 977-84

Accession Number: 18444812 DOI: 10.1086/529147 [doi]

G. Arunkumar, R. Chandni, D. T. Mourya, S. K. Singh, R. Sadanandan, P. Sudan, et al.

J Infect Dis 2018 Vol. 219 Pages 1867-1878

Accession Number: 30364984 DOI: 10.1093/infdis/jiy612

Page 2: [13] Commented [CB20] Christopher Broder 10/29/2021 2:35:00 PM

K. T. Wong, W. J. Shieh, S. Kumar, K. Norain, W. Abdullah, J. Guarner, et al.

Am J Pathol 2002 Vol. 161 Issue 6 Pages 2153-67.

K. T. Wong, T. Robertson, B. B. Ong, J. W. Chong, K. C. Yaiw, L. F. Wang, et al.

Neuropathol Appl Neurobiol 2009 Vol. 35 Issue 3 Pages 296-305

Accession Number: 19473296 DOI: NAN991 [pii]

10.1111/j.1365-2990.2008.00991.x [doi]

Page 2: [14] Commented [CB21] Christopher Broder 10/29/2021 2:37:00 PM

C. T. Tan, K. J. Goh, K. T. Wong, S. A. Sarji, K. B. Chua, N. K. Chew, et al.

Ann Neurol 2002 Vol. 51 Issue 6 Pages 703-8.

J. D. O'Sullivan, A. M. Allworth, D. L. Paterson, T. M. Snow, R. Boots, L. J. Gleeson, et al.

Lancet 1997 Vol. 349 Issue 9045 Pages 93-5

Page 2: [15] Commented [CB23] Christopher Broder 10/29/2021 3:00:00 PM

D. A. Hammoud, M. R. Lentz, A. Lara, J. K. Bohannon, I. Feuerstein, L. Huzella, et al.

PLoS Negl Trop Dis 2018 Vol. 12 Issue 11 Pages e0006978

J. B. Geisbert, V. Borisevich, A. N. Prasad, K. N. Agans, S. L. Foster, D. J. Deer, et al.

J Infect Dis 2020 Vol. 221 Issue Suppl 4 Pages S414-S418

Accession Number: 31665362

A. N. Prasad, K. N. Agans, S. K. Sivasubramani, J. B. Geisbert, V. Borisevich, C. E. Mire, et al.

J Infect Dis 2020 Vol. 221 Issue Suppl 4 Pages S431-S435

Accession Number: 31665351 PMCID: 7213566 DOI: 10.1093/infdis/jiz391

10.1093/infdis/jiz469

Page 2: [16] Commented [CB24] Christopher Broder 10/29/2021 2:52:00 PM

The WHO R&D Blueprint: 2018 review of emerging infectious diseases requiring urgent research and development efforts

M. S. Mehand, F. Al-Shorbaji, P. Millett and B. Murgue

Antiviral Res 2018 Vol. 159 Pages 63-67

Accession Number: 30261226

Page 2: [17] Commented [CB25] Christopher Broder 10/29/2021 2:58:00 PM

The pandemic potential of Nipah virus

S. P. Luby

Antiviral Res 2013 Vol. 100 Issue 1 Pages 38-43

Accession Number: 23911335 DOI: 10.1016/j.antiviral.2013.07.011

Page 3: [18] Commented [CB35] Christopher Broder 10/31/2021 3:43:00 PM

Cedar virus: a novel Henipavirus isolated from Australian bats

G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.

PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836

DOI: 10.1371/journal.ppat.1002836

Differential Innate Immune Responses Elicited by Nipah Virus and Cedar Virus Correlate with Disparate In Vivo Pathogenesis in Hamsters

T. Schountz, C. Campbell, K. Wagner, J. Rovnak, C. Martellaro, B. L. DeBuysscher, et al.

Viruses 2019 Vol. 11 Issue 3

(Geisbert and Broder unpublished)

Page 3: [19] Commented [CB36] Christopher Broder 10/31/2021 3:47:00 PM

Cedar virus: a novel Henipavirus isolated from Australian bats

G. A. Marsh, C. de Jong, J. A. Barr, M. Tachedjian, C. Smith, D. Middleton, et al.

PLoS Pathog 2012 Vol. 8 Issue 8 Pages e1002836

DOI: 10.1371/journal.ppat.1002836

The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2

K. G. Lieu, G. A. Marsh, L. F. Wang and H. J. Netter

Antiviral Res 2015 Vol. 124 Pages 69-76

DOI: 10.1016/j.antiviral.2015.09.017

From: Broder, Christopher on behalf of Broder, Christopher <christopher.broder@usuhs.edu>
To: lijun@uic.edu; Gaisina, Irina N
Cc: Moushimi Amaya; David Veesler; twgeisbe@UTMB.EDU
Subject: RAViD
Date: Sunday, October 31, 2021 9:39:52 PM
Attachments: SpecificAims-ResStrategy-HenipaRP1-RAViD-10312021.docx

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Dear Irina / Iijun
and All
I know you have been working on AIm 1 and David on Aim 2

I said i was revising the Aims page, and working on the bkg and innovation.

Please integrate the attached, which is also a V3 revision to Aims page, into this Doc you just sent, before folks start editing.

Again. as we discussed. UIC will be the holder of the final format and reff generation. So the tedious bit here is the ref insertions into the BKG and Innovation.

I am not sure how all think to move forward from here, regards to editing and working on one document

I will work on Prelim data on nanos and SNT chimera assay tomorrow. with some figs to add
cb

SPECIFIC AIMS.

The COVID-19 pandemic caused by SARS-CoV-2 continues to profoundly impact individuals, communities, and nations. SARS-CoV-2 is rapidly evolving, demonstrating a capacity to persist and cause disease despite effective vaccines. There is a profound need for new antivirals for SARS-CoV-2 and particularly orally bioavailable antivirals for prophylaxis and early treatment. For over 20 years, our NIH-supported studies have advanced understanding of the functions and inhibitors of the CoV replicase, specifically the nsp12 RNA-dependent RNA polymerase (nsp12-RdRp) and the nsp14 proofreading exoribonuclease (nsp14-ExoN), both highly conserved and critical functions for CoV replication and RNA synthesis. Since 2014, our program has led IND-enabling preclinical studies for remdesivir and molnupiravir, including *in vitro* and *in vivo* efficacy, potency, toxicity, mechanism of action (MOA), resistance and combination studies. Thus, our capabilities are well established for coronavirus inhibitor hit identification and lead development using relevant *in vitro* and *in vivo* models, in conjunction with iterative chemical design and modification with industry and academic labs.

In this program, we propose to use novel design and chemistry to: *1)* develop inhibitor hit compounds and validate leads targeting nsp12-RdRp (and cofactors nsp7,8) and nsp14-ExoN (and cofactor nsp10); and *2)* advance available and newly identified priority leads through *in vitro*, *in vivo*, and chemical studies toward oral formulation and IND-ready potent, oral drugs active against SARS-CoV-2 and other present and future zoonotic CoVs.

Aim 1. dentification and Selection of Hit and Lead Compounds Targeting the SARS-CoV-2 nsp12-RdRp and nsp14-ExoN Complexes. *The objective of this aim is to define hit compounds suitable for chemical modification, validation, and advancement to lead optimization*. **Part 1** will test novel nucleoside analogs (NAs) targeting the nsp12-RdRp. In **Part 2**, chemical libraries will be screened for selective activity using *in vitro* biochemical assays against defined components of the replicase core complex, nsp12-7-8, and nsp14-10. **Part 3** will test inhibitors of two-metal-ion-dependent (TMID) enzymes that have demonstrated inhibition of nsp14-ExoN and CoV replication. In **Part 4,** high-impact hits identified using continuous human respiratory cell cultures (A549-hACE2, Calu-3) will be validated in primary human airway epithelial (HAE) cells and against multiple human and model animal CoVs. Hits will be prioritized for lead advancement based on potency, activity in primary cells types, selectivity index, and amenability to SAR and oral administration.

Aim 2. Lead Development for Mechanism of Action (MOA), Viral Determinants of Activity and Resistance, and Combination Testing. *The objective of this aim is to rapidly move validated hits through rigorous* in vitro *testing and further lead advancement to* in vivo *studies*. In **Part 1**, SARS-CoV-2 will be passaged for resistance to lead inhibitors, followed by testing of isolates showing phenotypic resistance in quantitative assays of potency and efficacy, replication kinetics, and cytopathic effect. Viral genomes will be sequenced to define candidate determinants of resistance and known and novel drug targets in nsp12, nsp14, and across the genome. Prioritized candidate mutations will be introduced into isogenic backgrounds of SARS-CoV2 to test and confirm putative drug targets and resistance determinants. This information will be fed to the Structure and Analysis cores to further explore mechanism of action. In **Part 2**, passaged virus populations and engineered mutants will be tested for cross-resistance or increased sensitivity to compounds of similar or different chemical classes and for relative fitness vs. wild-type SARS-CoV-2 in direct co-infection competition experiments. In **Part 3**, lead compounds will be tested in combinations with remdesivir, molnupiravir, and other novel leads from this program to determine whether their interactions are synergistic, additive, or antagonistic.

Aim 3. Lead Evaluation in Rodent Models of Zoonotic CoV Replication, Pathogenesis, Disease, Transmission, and Persistence. *The objective of this aim is to use established, robust, murine and hamster models for SARS-CoV-2, SARS-CoV, and MERS-CoV infection to determine lead efficacy* in vivo. In **Part 1**, validated leads (prioritizing established oral formulations) will be tested in wild-type, young, adult mice at different times of infection for impact on viral replication, disease, and pathology. In **Part 2**, a hamster model will be used to test lead efficacy in a different host species and prevention of transmission to uninfected contacts. In **Part 3**, compounds demonstrating activity in young, immunocompetent mice will be tested in aged and immunocompromised (RAG -/- and MuMT-/- strains) mice for capacity to ameliorate replication and disease in animals predisposed to severe infection. In **Part 4**, the potential and genetic basis for *in vivo* development of SARS-CoV-2 and SARS-CoV resistance to advanced leads will be explored using virus isolated from immunocompromised mice that progress to persistent infection.

RESEARCH STRATEGY

SIGNIFICANCE AND IMPACT.

SARS-CoV-2 and other zoonotic coronaviruses (CoVs) cause devastating disease and remain uncontrolled. During the past 20 years, five novel CoVs have emerged to cause severe illness in new hosts, three of which are human respiratory pathogens: severe acute respiratory syndrome (SARS)-CoV (2002-2004), Middle East respiratory syndrome (MERS)-CoV (2012-present), and SARS-CoV-2 (2019-present)[7-9]. Viruses similar to SARS-CoV, MERS-CoV, and SARS-CoV-2 have been found in reservoir species such as bats and pangolins[10-14]. Thus, in addition to the current pandemic that has killed 1 in 500 Americans, all evidence indicates that new CoV emergence will continuously threaten global health at the animal-to-animal, animal-to-human, and human-to-human interfaces. Potent, broad-spectrum, resistance-averting, and orally-administered antivirals are thus urgently needed to prevent and treat SARS-CoV-2 and allow rapid response to future high-consequence zoonotic CoVs as vaccines and other countermeasures are developed.

The CoV replicase as a target of antiviral drug development. CoVs contain the largest positive sense single-strand RNA of any known human virus, ranging from 28-32 kb in length (Fig. 1). CoV RNA genome replication and subgenomic mRNA transcription are mediated by the replicase nonstructural proteins 1-16 (nsps 1-16) which are translated from input genome RNA as large replicase polyproteins[15] and processed by viral proteases in nsp3 and nsp5 into intermediate and mature nsps 1-16 containing known or predicted functions in RNA synthesis and modification[15-18]. Biochemical, genetic, protein-interaction, cryo-EM, and crystallographic structure studies of replicase components all support a model of a multi-protein replicase holoenzyme with polymerase, RNA-modification, and proofreading functions containing at a minimum nsps 7-8-9-10-12-13-14 (Fig. 1). The **nsp12-RNA-dependent-RNA-polymerase** (**nsp12-RdRp**) is the central component in the RNA synthesis and modification machinery. The nsp12-RdRp folds into canonical "fingers-palm-thumb" domains forming the active-site cleft, which contains SDD metal-coordinating catalytic residues, as well as a novel "NiRAN" domain harboring nucleotidyl and guanylyl transferase activities. The nsp12-RdRp has multiple demonstrated interacting proteins and cofactors, including nsp7, nsp8, nsp9, nsp13, and the bifunctional nsp14-exoribonuclease/ N7-methyltransferase[19-21]. The Denison lab has been a world leader in studies of the **nsp14-exoribonuclease** (**nsp14-ExoN**) and has shown nsp14-ExoN to have critical roles in virus replication, genomic and subgenomic RNA synthesis, high-fidelity replication (RNA-dependent RNA proofreading), RNA recombination, innate immune antagonism, and native resistance to nucleoside analogs (NAs)[19,20,22-28]. The CoV replicase thus constitutes a holoenzyme with arrayed critical enzymatic functions and cofactors that are vulnerable to perturbation by precisely acting small-molecule antivirals.

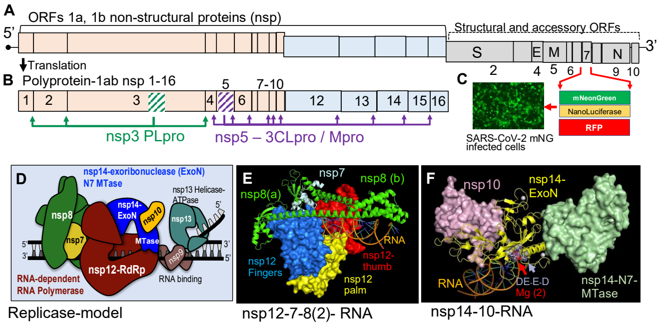


Figure 1. Coronavirus replicase protein expression, reporters, organization and structures of nsp12-7-8 and nsp14-10 subcomplexes. (**A**) Genome organization of SARS-CoV-2. (~30kb ss (+)RNA genome). (**B**) Translation of ORF1ab replicase polyprotein 1ab and processing of nonstructural proteins 1-16 (nsp1-16) by viral encoded proteases nsp3-PLpro and nsp5-3Clpro (Mpro). (**C**) Engineered reporters in SARS-CoV-2 ORF7 for analysis of replication and inhibition: mNeonGreen, mNG; nanoluciferase; red fluorescent protein (RFP)[3,4]. Similarly, reporters for SARS-CoV[5] and MERS-CoV [6] are available. (**D**) Model of known interactions and proposed holoenzyme of the CoV replicase including: nsp12-RdRp; nsp7-8, required nsp12-RdRP cofactors; nsp14-exoribonuclease and N7 methyltransferase (ExoN, N7MTase); nsp10, required nsp14 cofactor. (**E**) Structure of nsp12-RdRp and nsp7/8 cofactors and RNA (PDB 6YYT). Domains of core nsp12-RdRp are shown: blue, fingers; yellow, palm; red, thumb. NiRAN domain not shown. The molecules of nsp8 (green) and nsp7 (light blue) required for nsp12 activity are shown, as well as location of RNA. (**F**) Structure of nsp14, required nsp10 cofactor, and RNA molecule (PDB 7N0B). Locations of active Mg^{2+} (2) (red) and coordinating DE-E-D residues (blue) are indicated.

Research team focus on next-generation inhibitors, oral formulations, and combinations. Prior to the COVID-19 pandemic, very few labs were focused on fundamental CoV biology and fewer still on CoV antiviral drug development. Our CoV antivirals program in the NIAID-U19-supported "Antiviral Drug Discovery and Development Center" (AD3C) between 2014 and 2020 advanced two nucleoside analog (NA) antivirals through preclinical development into IND: remdesivir (Veklury, Gilead Sciences) and molnupiravir (NHC, MK-4482 / EIDD-2801, Emory Institute for

Drug Development). Both showed remarkable potency and efficacy against CoVs, including SARS-CoV-2, and both target the nsp12-RdRp[29-32]. Due in large part to our preclinical development contributions, remdesivir was rapidly moved into human trials and remains the only FDA-approved direct-acting antiviral (DAA) for SARS-CoV-2. Yet, the remdesivir requirement for IV administration limited the scope of trials and its subsequent indications to hospitalized and severely ill patients[33], which excludes treatment of COVID-19 cases comprising the vast majority of infections. Similarly, our studies supported use of the orally bioavailable NA, molnupiravir, now in advanced-stage clinical trials[34]. The recent announcement by MERCK that the phase 3 trial was terminated due to clear benefit of molnupiravir over placebo for prevention of hospitalization and severe disease (awaiting publication and FDA review) is highly encouraging and supportive of our studies in vitro and in animals and demonstrates our ability to identify oral inhibitors of CoV replication and disease. We have shown that both remdesivir and molnupiravir present high genetic barriers to resistance, but just as clearly we have shown that resistance can emerge against either drug[31,32]. Thus, it is imperative to establish additional antiviral strategies for all stages of infection—early, late, and post-exposure prophylaxis—and to include combinations of DAAs to improve outcomes and prevent emergence of resistance.

INNOVATION

Discovery of chemical matter for testing. Multiple parallel and highly innovative approaches will be used to develop hits and identify and optimize leads (see below for team integration and expertise), including: *1)* novel NA chemistry (Arbutus [AR]) and modifications (University of Southern California [USC]); *2)* structure-based design and modification of two-metal-ion-dependent (TMID) enzyme inhibitors active against nsp14-ExoN (University of Connecticut [UConn]); *3)* biochemical screening of active nsp12-7-8 and nsp14-10 replicase subcomplexes against massive compound libraries (Southern Research [SR]); and *4)* integrated molecular modeling, cryo-EM, NMR, crystallography, and fragment-based screening of hits or chemically modified leads (SR Cores).

Established pathways for hit identification and lead development. We have well-established and effective strategies for testing inhibitor efficacy, potency, and toxicity across multiple CoVs and cell lines, which will allow for rapid and accurate assessment of candidate hits meriting modification and/or advancement. Further, we will merge rational drug design and discovery approaches to identify candidate resistance mutations and relevant mutation linkages[25,31] in linear but extensively cross-linked workflows to achieve high-throughput hit evaluation and refinement, rapid down-selection, and seamless progression of our best leads though comprehensive functional analyses *in vitro* and *in vivo.* These approaches include: *1)* numerous relevant *in vitro* systems of HCoV infection utilizing continuous and primary human airway cells[30,35,36]; *2)* physiologic *in vivo* models of HCoV disease[35,37-41]; *3)* precision biochemical assays of priority targets in the viral replicase[1,2]; *4)* a panel of well characterized epidemic and endemic HCoVs [6,12,13,30,40-45]; *5)* reverse-genetics systems for human endemic, zoonotic, and pre-epidemic CoVs[4,6,12,13,42,43,46]; and *6)* deep- and long-read next-generation sequencing (NGS) methods [25].

RESEARCH TEAM

CoV research team and integration of expertise. This project proposes a highly synergistic collaboration incorporating novel target and drug discovery and chemistry, biochemistry, structural biology, and *in vitro* and *in vivo* preclinical models of infection (Fig. 2). The **Denison lab** at **Vanderbilt University Medical Center** (**VUMC**) has been a leader in CoV research for over 30 years and in preclinical development of remdesivir and molnupiravir[29-32,47,48]. The Denison lab houses longstanding fundamental research programs in the virology and genetics of the CoV replicase, particularly nsp12-RdRp and nsp14-ExoN, across SARS-CoV-2, MERS-CoV, and SARS-CoV and the highly tractable animal beta-CoV, murine hepatitis virus (MHV). The Denison lab was the first to confirm and characterize the CoV unique RNA-proofreading function of nsp14-ExoN and its critical role in virus fidelity, replication, and intrinsic resistance to classes of NAs[22,23,32,49-51]. The **Menachery lab** at the **University of Texas Medical Branch** (**UTMB**) will perform hit validation and lead development *in vitro* and *in vivo*. They have established robust small animal models for SARS-CoV-2, SARS-CoV, MERS-CoV, and bat-CoV infection[6,12,13,40-45], including animal-adapted strains of SARS-CoV-2 for use in native mice--young, aged, and immunocompromised animals[40]. Further, they have established models in hamsters for studies of SARS-CoV-2 replication, pathology, and transmission[35,38,39]. In addition to animal models, UTMB has long-standing expertise in highly relevant *in vitro* primary cell-culture systems, including air-liquid-interface (ALI) human airway epithelial (HAE) cultures[35]. Their well-established reverse genetics for multiple CoVs, including SARS-CoV-2, allows for testing of resistance mutations in mouse-adapted viral genetic backgrounds. Finally, Andrew Routh, PhD (UTMB) is an expert in deep sequencing analysis and has developed pipelines for analysis of RNA-seq and long-read (nanopore MinION) data in collaboration with the Denison lab, showing the role of CoV nsp14-ExoN in CoV recombination[25].

Expertise in fundamental CoV biology, replication, and pathogenesis at VUMC and UTMB is complemented by decades of experience and demonstrated success in novel antiviral discovery, chemistry, and development. The **Weller and Wright labs at UConn** have long-standing expertise in viral exonucleases and have established novel approaches for developing inhibitors of TMID enzymes, specifically against viral exonucleases. They have collaborated with the Denison lab to identify exonuclease-targeted compounds that demonstrate inhibition of SARS-CoV-2. **SR will direct the Medicinal Chemistry** (**MedChem**) **Core, Analysis Core, and Structure Core. SR** will perform screening of large libraries in defined biochemical assays of nsp12-7-8 and nsp14-10 to identify hits with potential for active-site and allosteric inhibition of those complexes. In addition, they have established lead compounds of unknown mechanism that will serve as initial development candidates at VUMC and UTMB. The **McKenna lab at USC** has established an approach for tunable chemical modifications to increase the potency and metabolism of NAs. They also will contribute modified NAs with significant *in vitro* efficacy for hit-to-lead validation and development. **AR** is an established company whose scientific director is Michael Sofia, a discoverer of sofosbuvir. Dr. Sofia and his research team have ongoing programs on antivirals against both hepatitis B virus and CoVs. AR has generated a panel of anti-CoV NAs with activity against HCoV-OC43 and/or SARS-CoV-2; these will be tested for hit identification and lead development in collaboration with AR. The drug-development programs are highly integrated and will work with the MedChem Core in the conduct of structure-activity refinement (SAR), biochemical testing, structure determination, modeling, and modifications.

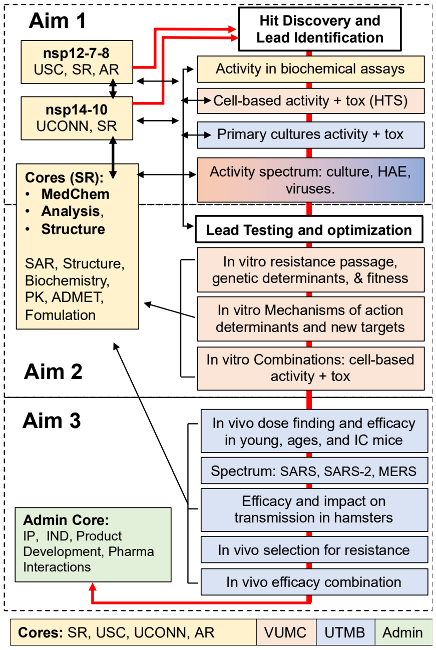


Figure 2. Experimental design and integration of programs for hit discovery and lead development. Schematic of experimental design, hit discovery and validation, and lead development through *in vitro* and *in vivo* testing. Cores: MedChem, Analysis and Structure; SR, Southern Research; USC, McKenna lab (Univ. Southern California); UCONN, Weller/Wright labs, (Univ. Connecticut); AR, Arbutus Biopharma; VUMC, Denison Lab (Vanderbilt University Medical Center); UTMB, Menachery lab (University of Texas Medical Branch); Admin, Admin Core.

Project 1 interactions and with other projects. Project 1 has been designed as an integrated, iterative workflow across the partnering scientific teams and cores. We will promptly communicate about leads that target proteins and functions conserved across virus families. Combination treatments built from leads not only carry the potential to improve breadth, potency, and resistance profiles against CoVs, but also might create bridge regimens effective against disparate virus families. Thus, the preclinical development program for CoV antivirals will promote progress across all AD3C-2 AVIDD projects and potentially define novel, broad-spectrum antivirals.

SCIENTIFIC PREMISE, EXPERIMENTAL RIGOR, AND CONSIDERATION OF RELEVANT BIOLOGICAL VARIABLES

The scientific premise of this program is that small-molecule antivirals, singly and in combination and designed for oral administration, are essential for the control of current (SARS-CoV-2 and MERS-CoV) and future zoonotic CoV epidemics. Additionally, we propose that broad-spectrum therapies can be developed that target more than one virus and more than one virus family to maximize their benefit to global public health. We are committed to conducting rigorous, unbiased, and transparent research that generates repeatable and reproducible data amenable to statistically verifiable outcomes as part of comprehensive laboratory quality assurance. Cell lines, including primary cells, will be routinely monitored for purity by histological analysis of cell morphology and testing for contaminants (e.g., *Mycoplasma*). To reduce biological variability of *in vitro* antiviral analyses, we will perform assays using multiple complementary cell types (continuous cell lines and primary cells) and methods (reporter gene expression, infectious virus production, and viral RNA quantification by RT-qPCR). Experimental animals will be purchased through standard vendors that maintain genotypic control of their colonies. If non-commercially available animals are required, genetic backgrounds will be determined using state-of-the-art genotyping arrays. Any mutant animal strain used in this project will be confirmed within each cohort using validated diagnostic assays. To safeguard reproducibility across the scientific community, we will make novel virus strains, vectored viral cDNAs, and engineered cell lines available through BEI and Addgene.

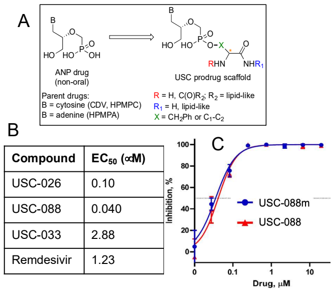
APPROACH

SPECIFIC AIM 1. Identification and Selection of Hit and Lead Compounds Targeting the SARS-CoV-2 nsp12-RdRp and nsp14-ExoN.

Rationale. The objective of this aim is to define hit compounds for chemical modification, in vitro validation, and advancement to lead optimization. These studies focus on the critical conserved replicase complex, specifically nsp12-RdRp, nsp14-ExoN, and cofactors. However, all proteins that assemble in the functional replicase complex and required for RNA synthesis and modification are potential targets that may emerge during the course of the study.

Part 1. NA chemistry and targeting the nsp12-RdRp.

NA modifications for nsp12-RdRp inhibition. Modified NAs have the potential for both enhanced and more universal cell penetration as well as increased efficacy and potential oral bioavailability. **USC** has designed and synthesized a series of NAs[52] (Fig. 3), one of which, USC-088, shows 30x higher potency than remdesivir against a clinical isolate of SARS-CoV-2 in Vero E6 cells (IC_{50} = 0.04 μM; CC_{50} >100 μM). USC-088 incorporates a unique promoiety platform created at USC that provides versatile chemical 'tunability' to enhance the potency and PK properties of antiviral nucleoside phosphonates and phosphates (Fig. 3). In support of Project 1, they will develop this potential lead and its back-up, USC-026. USC-088 and USC-026 will be tested for activity against SARS-CoV-2 in continuous and primary cells (Part 4) and further developed and characterized in Aim 2. Concurrently, USC will collaborate with the **MedChem Core** to perform SAR, ADME, and PK in animal models, with the goal of obtaining an orally effective final lead compound. USC-088 and USC-026 or a lead derived therefrom by SAR will be made available for evaluation to other projects as a broad-spectrum antiviral RdRp inhibitor. The pro-moiety platform will also be applied to enhance potency[53] and oral bioavailability[54-56] of emerging nucleoside/nucleotide leads from Project 1 and MedChem Core.



Compound	EC_{50} (∝M)
USC-026	0.10
USC-088	0.040
USC-033	2.88
Remdesivir	1.23

Figure 3. A) Tunable prodrug scaffold to enhance potency, tissue selectivity, and oral bioavailability. CDV and HPMPA are shown as antiviral nucleoside examples. **B)** Compound activity against SARS CoV-2 in Vero E6 cells (McKenna, USC). **C**) *In vitro* activity profile of USC-88 (two synthetic batches to demonstrate reproducibility) against SARS-CoV-2.

Novel NA inhibitors from structure-based design and pharmacophore screening. Through structure-based modeling and medicinal chemistry approaches, the chemistry team at **Arbutus (AR)** has synthesized or identified novel NAs as candidate broad-spectrum inhibitors of nsp12-RdRp. AR has complemented In-house *de novo* design with *in silico* pharmacophore-based pre-screening of compound libraries to triage a diverse set of known NA inhibitors to be tested against CoVs. In the AR development pipeline, systematic modifications are introduced to trigger antiviral activity and favorable selectivity toward virus vs. host cells. Any identified hits are elaborated further based on emerging SAR and through structure-based computational modeling. Hits validated using SARS-CoV-2-infected Vero cells and/or other CoV-infected cell systems with low micromolar EC_{50} values and no cytotoxicity in the efficacy range will be evaluated in continuous and primary cells (VUMC and UTMB). Results will be communicated with AR for their analysis of desirable drug-like properties, including stability and solubility (See Letter). Advancement of potential leads through Aims 2 and 3 (VUMC) will occur in parallel and iteratively with SAR to improve stability, solubility, and cell permeability for oral bioavailability (AR). Subsequent ADMET and PK analyses will be conducted at AR. Mechanism-of-action (MOA) studies of lead molecules will be performed using synthesized triphosphate metabolites and nsp12-RdRp enzymatic assays (AR).

Part 2. Biochemical high-throughput screening of nsp12-7-8 and nsp14-10 complex inhibitors.

Current Lead Compounds. The existing and productive collaboration between **VUMC and SR** has yielded 4 compounds based on viral cytopathic effect (CPE) screening against SARS-CoV in Vero-E6 cells (Fig 4) The compounds demonstrate >3 log virus titer reduction. sub-μM potency against SARS-CoV-2 in A549 cells at VUMC. These are currently progressing through SAR in the MedChem Core and will immediately be studied *in vitro* and *in vivo* as leads. Because mechanism is unknown, we will pursue passage / resistance / sequencing studies to determine if these leads are virus-targeted and, if so, the specific protein(s) with which they interact.

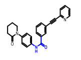
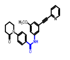
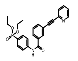
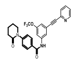
Assay	Target Values				
		SRI-45975	**SRI-46488**	**SRI-46944**	**SRI-46897**
SARS-CoV EC_{50} (μM) (Vero E6-CPE)	<1	1.0	0.209	0.179	0.13
CC_{50} (μM) [SI]	>30	>30 [>30]	>30 [>140]	>30 [>165]	>30 [>230]
SARS-CoV-2 EC_{50} (μM) (A549-hACE2)	<1	0.65	0.102	0.003	0.088
Virus Titer Reduction (VTR) ($\log_{10}$)	>2	2.98	3.08	2.938	2.589

Figure 4. Existing Lead Inhibitors from Southern Research. Compounds identified by previous CPE-based library screening demonstrate activity against both SARS-CoV (VeroE6) and SARS-CoV-2 (A549), meeting criteria for progression as leads according to EC50, CC_{50}, SI, and virus titer reduction.

Biochemical Screening for Novel Inhibitors. Going forward in this proposal, **SR** will not further pursue viral CPE-based screening; in this project SR will deploy biochemical assays using SARS-CoV-2 replicase subunits: nsp12-7-8 as an active polymerase complex and nsp14-10 as an active exoribonuclease/N7-methyltransferase assay. **A) The nsp12-7-8 complex.** A functionally active SARS-CoV-2 RdRp comprised of recombinant nsp12-7-8 has been reported[1] and will be used (Fig. 5, panel A). This assay will enable high-throughput screening for compounds that directly target polymerase activity. The system has the advantage of targeting the core synthetic complex and thus will capture active compounds specific for the nsp12-RdRp active site as well as allosteric or other inhibitors of nsp12, 7 or 8. Hits that advance with priority in Aim 1 will be subjected to MOA studies in Aim 2 and iterative SAR and structural analyses at SR. **B) The nsp14-10 complex**. Nsp14-ExoN function may require or be facilitated or modified by the small non-enzymatic nsp10[57]. Complexes of nsp14-10 have been confirmed to show ExoN function when expressed and purified from bacteria [2,24,26,27,58]. A SARS-CoV-2 nsp14-10 ExoN functional assay sensitive to dsRNA digestion by ExoN will be employed[2] (Fig. 5, panel B). The complex will be screened against the same library as the nsp12-7-8 complex, with similar potential to detect both direct and indirect inhibitors (allosteric and disruption of protein interactions). Further, this biochemical testing approach using nsp12-7-8 and nsp14-10 complexes will allow protein-focused evaluation of confirmed and new hits identified from AR, USC, and UConn libraries.

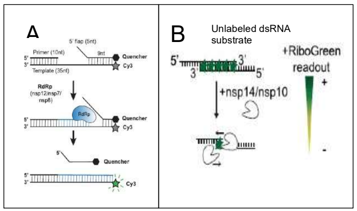
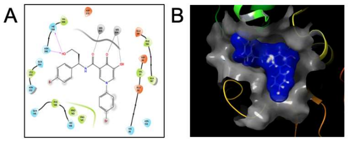


Figure 5. A) FRET-based SARS-CoV-2 recombinant nsp12/7/8 RdRp strand- displacement assay. Figure from (1). **B)** RiboGreen recombinant SARS-CoV-2 nsp14-10 exoribonuclease assay. Figure from (2).

PART 3. TMID enzyme inhibitors of nsp14-ExoN (UConn).

The large size of CoV genomes and inherent error-prone nature of the viral RdRp necessitate a proofreading step (mediated by the ExoN domain of nsp14) to avoid buildup of lethal mutations in progeny genomes[59,60]. The nsp14-ExoN activity also renders CoVs resistant to many classic NAs via ExoN-mediated excision of the mispaired base[24]. Inhibitors of ExoN function are therefore expected to be directly antiviral and are also anticipated to strongly synergize with NAs such as ribavirin and remdesivir [32,49-51]. ExoN belongs to a large superfamily of DE-D-D exonucleases (DE-E-D in CoVs), whose catalytic functions are orchestrated by two Mg^{2+} atoms[16,60]. Both DNA and RNA viruses encode such TMID enzymes, many of which have been shown to perform essential roles in nucleic acid metabolism (e.g., HIV integrase[61-63] and influenza endoribonuclease[64,65]). Despite a common two-metal dependent mechanism, TMID enzymes differ considerably in the overall active site architecture, offering opportunities to engineer both potency and selectivity. The **Weller/Wright labs (UConn)** have developed a small-molecule platform to target viral TMID enzymes and generate novel antivirals, now extended to nsp14-ExoN in collaboration with the Denison lab at VUMC. The high-resolution structure of the nsp10-nsp14 complex enabled rapid virtual screening of a TMID-focused library developed by the Weller/Wright lab. Compounds were flexibly docked to the active site using Schrodinger Maestro Suite, and the resulting poses were ranked by docking score and fit. Visual inspection of top-scoring complexes revealed the promise of a subset of inhibitors, which made contacts in the deeper regions of the active site while retaining key interactions with the metal centers. Figure 6 shows a representative *in silico* complex of one such inhibitor, DDI-117 docked to ExoN. Eight of the compounds were tested for inhibition of ExoN activity using a gel-based assay to monitor cleavage of a ssRNA substrate. While six of the derivatives showed no effect on ExoN activity, two compounds reduced activity significantly. Next, we evaluated direct binding to ExoN protein by measuring the thermal shift



C

Compound	EC50 (µM) SARS-CoV-2	CC50 (µM) (SI)	VTR max $\log_{10}$
DDI 116	~ 0.94	4.11 (4.3)	>2.5
DDI 117	0.18	2.93 (16.3)	~ 2

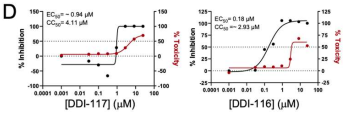


Figure 6. Structure-based compounds against nsp14-ExoN inhibit SARS-CoV-2 (Weller-Wright, UConn). **A)** Ligand plot showing key interactions between DDI-117 and ExoN. **B)** Surface plot of DDI-117 in the ExoN active site (based on PDB:7DIY). **C)** Activity of DDI-116 and DDI-117 against SARS-CoV-2 in human A549-hACE2 cells. CC_{50}: cytotoxicity. SI: selectivity index (CC_{50}/EC_{50}). VTR max: maximum virus titer reduction. **D)** Inhibition and cytotoxicity data used to generate EC_{50} and CC_{50}.

of unlabeled protein in the presence of 20-fold excess of inhibitor (Tycho, Nanotemper). Both DDI-117 and DDI-116 produced a pronounced shift in the inflection temperature (ΔTi = 1.1°C), indicative of significant stabilization of the protein:ligand complex, while two structurally related but inactive compounds had no effect. These four compounds were tested at VUMC to determine anti-SARS-CoV-2 activity in culture. DDI-117 and DDI-116 demonstrated low micromolar level antiviral activity with an SI of >5, while the others exhibited no antiviral activity.

These validated hists demonstrate direct target engagement, functional inhibition, and antiviral activity in cell culture, providing a strong foundation for the ongoing Weller/Wright ExoN inhibitor program. This program will use a combination of structure-based lead optimization, *in vitro* analysis, biophysical evaluations, functional analysis, and antiviral activity to generate an evolving SAR model. Refinements will be made to induce improvements in potency and selectivity at **UConn** and in collaboration with the **MedChem, Structure, and Analysis Cores**. For example, additional contacts with proximate, conserved residues including Pro141, Pro142, His95, Gln145, Gln104, and Phe146 will be pursued. Candidates demonstrating good levels of antiviral activity in cell culture (EC_{50} < 2.5 µM) and an acceptable SI (> 30) will be recommended for synergy testing with nucleoside inhibitors, formulation studies, and *in vivo* evaluation in collaboration.

Part 4. *In vitro* hit determination and validation.

Hit testing in continuous cell lines. Compounds from parts 1-3 above will be tested in SARS-CoV-2-infected cells using recombinant virus expressing mNeonGreen (mNG), nanoluciferase, and red fluorescent protein (RFP) (Fig. 1). We will employ Calu-3 (human lung adenocarcinoma), A549-ACE2 (human lung adenocarcinoma), and Huh-7 (human hepatocellular carcinoma) cells using approaches we have previously reported for remdesivir and molnupiravir[30-32] and as described for preliminary studies with SR compounds and UConn TMID enzyme inhibitors. The use of multiple cell types is important to address potential differences in cell penetration or metabolism, as we observed with remdesivir and its parent compound GS-441524 in Vero and A549 cells[30]. Cultures will be infected in multiple replicates and then left untreated, treated with carrier (such as DMSO), or treated with compound in carrier at concentration ranges designed to either establish or directly test the needed range for a 10-point analysis to calculate potency ($\log_{10}$ virus titer reduction [VTR]) and efficacy (EC_{50}). Virus replication will be initially quantified using luminescence or fluorescence, followed by confirmation with qRT-PCR and/or plaque assay. Parallel uninfected cells will be similarly treated across a broad range of drug concentrations to determine cytotoxicity (CC_{50}) using CellTiter-Glo luminescence assay. Data will be used to calculate compound selectivity index (SI = EC_{50}/CC_{50}) and compared between cell types. Compounds with EC_{50} <10 µm and SI >10 will be considered for further analysis, with priority on compounds demonstrating EC_{50} <2 µM and SI >50. While not a chief criterion, we will further prioritize compounds that show VTR >2 $\log_{10}$. However, compounds with higher EC_{50}s and/or promising feasibility based on modeling, docking, or other assays may be considered for further testing if they are especially relevant and good candidates for SAR.

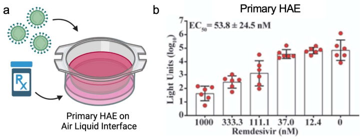


Figure 7. Human airway cultures for anti-SARS-CoV-2 testing. **a)** Primary human airway epithelia (HAE) cultures will be infected with reporter-expressing CoVs and treated with candidate drugs. **b)** Remdesivir was tested in nanoluciferase-SARS-CoV-2-infected HAE cultures. EC_{50} value is shown. Each dot represents one replicate data point.

Hit validation in physiologically relevant primary cells against SARS-CoV-2 and determination of breadth of activity. Compounds of significant interest from testing in continuous cell lines will be assessed for efficacy, potency, and cytotoxicity in SARS-CoV-2-infected primary HAE cultures using methods established in the M**enachery lab at UTMB**[35] (Fig. 7). In this system, Cultures are established from explanted tracheal/bronchial cells on air-liquid-interface (ALI) transwells, which allows differentiation into ciliated cells as natural targets of infection. Cells are infected at the apical surface and may be treated with drug either from the apical or basal compartments depending on formulation, cell penetration, and metabolism. Efficacy, potency, cytotoxicity, and SI will be determined. Compounds also will be examined for breadth of activity in HAE cultures infected with SARS-CoV and MERS-CoV.

Prioritization, capacity, lead identification, optimization, and advancement. We will prioritize initial hits based on multiple factors including potency, activity in primary cell types, SI, potential for SAR, and development potential for oral administration. Prioritized compounds will be further developed within this Aim and down-selected to a top-performing pool of leads for testing in Aim 2. We have significant capacity between VUMC and UTMB for evaluation and validation of up to 20 hits per month, and we are positioned to advance 10 compounds annually into detailed *in vitro* and *in vivo* studies. We have already identified multiple compounds (Figs. 3, 4, 6) satisfying basic criteria for advancement Those will enter studies in Aim 2, and possibly Aim 3, as the pipeline of new hit compounds and validated hits from parts 1-3 above progress. Thus, we anticipate a fully established pipeline of drug candidates, from novel discovery to hit validation to lead optimization at project inception, which will continue toward IND-enabling studies over the course of the project.

SPECIFIC AIM 2: Lead Development for Mechanism of Action (MOA), Viral Determinants of Activity and Resistance, and Combination Testing.

Rationale. The objective of this aim is to rapidly move advanced hits and validated leads through rigorous in vitro virologic assays to define MOA, phenotypic and genotypic resistance, and functional interactions with other priority compounds; inform additional SAR; and guide other modifications based on structural and biophysical analyses, leading to Go/No-Go decisions on progression of leads to in vivo testing.

Published and preliminary results supporting feasibility and capacity. Studies at VUMC over the past eight years have established approaches for lead development that define MOA, resistance, and novel protein determinants and combination studies of NAs, including remdesivir and molnupiravir[29,31,32]. These studies have been IND-enabling and supported advancement of remdesivir (RDV) and molnupiravir (MPV) into clinical trials. We have shown: **1)** RDV and MPV demonstrate high barriers to resistance and follow different genetic pathways impacted by MOA. **2)** Introduction of two nsp12-RdRp mutations associated with MHV resistance to RDV into WT MHV reconstitutes the resistance phenotype, and homologous mutations introduced into SARS-CoV confer RDV resistance[32]. **3)** Resistance of MHV to RDV results in ***increased sensitivity*** to MPV[29] (Fig. 8). **4)** Combination treatment with MPV and RDV show no antagonism and are at least additive in their anti-CoV activity (unpublished-score 1.72). These findings support our proposal that multiple NAs targeting nsp12-RdRp alone or with other replicase inhibitors may be useful combinations to enhance efficacy and limit emergence of resistance.

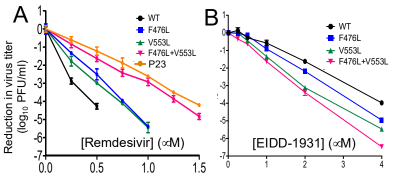


Figure 8. Remdesivir resistance mutations from MHV confer *increased* sensitivity to molnupiravir parent compound EIDD-1931. **A)** Remdesivir-mediated reduction in murine hepatitis virus (MHV) titer. Wildtype MHV (WT, black). Virus passaged 23 times in the presence of remdesivir (P23, orange). nsp12-RdRP resistance mutations F476L (blue), V553I (green) or both (magenta) in the WT MHV genetic background. **B)** EIDD-1931-mediated reduction in titers of viruses from panel A.

Part 1. Identification of resistance determinants. We will serially passage SARS-CoV-2 and MHV in A549-hACE2 and murine delayed brain tumor (DBT) cells, respectively, in the presence of leads to select for resistance. This parallel approach allows for analysis of the trajectory of resistance development along different genetic pathways taken by distantly related beta-CoVs in different cell types. We will serially passage virus in the presence of increasing concentrations of lead compounds in three parallel lineages for each virus and cell type to allow for selection of alternative pathways to resistance. Vehicle-passaged lineages will serve as controls for drug-independent cell-culture adaptations. Passaged virus populations displaying phenotypic resistance, including increased viral titers and CPE at higher drug concentrations, will be tested in EC_{50} and VTR assays to quantify the level of resistance.

Part 2. Sequence analysis, confirmation of targets, determinants of resistance, and biochemical and structural implications. Viruses demonstrating resistance phenotypes (e.g., increased CPE and infectious titers) will be analyzed by combined RNA-seq and long-read RNA sequencing (nanopore MinION) at **VUMC and UTMB (See Routh letter)** to confirm or define targets, mechanism of action (MOA), candidate resistance mutations, and novel physical or functional linkages in known or unpredicted interacting viral proteins or genetic elements. We will initially examine RNA from cells infected with population virus, which represents total diversity of potential mutations selected over passage. If multiple mutations are identified in a known or putative target, additional studies will determine whether mutations occur singly or in specific combinations (i.e., genetic linkage), including selection and sequencing of plaque isolates and MinION sequencing to define mutation haplotypes in sub-lineages or individual genomes. Single and combination mutations that have emerged to dominate WT sequences will be engineered in isogenic clones of SARS-CoV-2 to test/confirm drug targets and candidate resistance mutations. Confirmed mutations impacting resistance or MOA will be engineered at conserved homologous positions of MERS-CoV, MHV, and other beta- and alpha-CoVs to test for shared resistance determinants across divergent CoVs. Finally, confirmed mutations impacting resistance or MOA will be submitted to collaborators and to the Structure Core for potential structural determination and to the Analysis Core for testing in defined biochemical nsp12/7/8 or nsp14-10 systems.

Part 3. Impact of resistance mutations on viral fitness and susceptibility to other compounds. Our previous studies have shown that CoV passage in the presence of an inhibitory drug can select specific resistance mutations and may concurrently select for epistatic (compensatory) or adaptive mutations, which can impact virus replication, fitness, resistance, and general sensitivity to multiple compounds[31,32,50,51]. **1) Replication.** Viruses will be compared in side-by-side replicate assays during single-cycle (high multiplicity of infection [MOI]) and multiple-cycle (low MOI) replication in the presence and absence of compounds. Replication curves will reveal changes in eclipse period, exponential growth kinetics, time to maximum replication, and peak titer. **2) Competitive fitness.** SARS-CoV-2 passaged populations and isogenic clones containing engineered confirmed mutations will be co-infected with WT virus containing a silent (synonymous) barcode of up to 8 nucleotides, which allows quantitative comparison of WT and mutant virus abundances by qRT-PCR using specific primers and probes[51]. WT and mutant viruses will be inoculated onto cells at ratios of 1:1, 1:9, and 9:1, followed by five serial passages of culture supernatants and qRT-PCR for WT and mutant virus in supernatant and total infected-cell RNA at each passage level. Based on our long experience, we predict that selection of resistance mutations in the replicase will confer a fitness cost (less competitive) compared to vehicle-passaged virus in the absence of compound and display distinct replication advantages against all viruses in presence of drug. **3) Resistance/sensitivity to other compounds with similar or different targets.** We will assess the sensitivity of drug-resistant mutants to other leads targeting the same and different proteins. These experiments will define the potential for cross-resistance and sensitivity to leads with distinct target specificities—e.g., nsp14-ExoN inhibitors vs. nsp12-RdRp resistance mutations—and guide selection of combinations.

Part 4. Combination testing. An important goal of this program is to define interactions between lead compounds and establish a basis in support of - or rationale against - combination therapy for CoVs as a strategy to increase potency and prevent emergence of resistance. Most basically, it is critical to establish that a compound does not broadly antagonize other compounds and/or exacerbate cellular toxicity. On the other hand, additivity or synergy with other compounds would favor advancement of a lead. Leads advancing from preceding sections will be tested in two-way dose matrices, initially with clinical-stage and approved anti-CoV DAAs (e.g., remdesivir, molnupiravir, protease inhibitors GC376 and PF-07321332, and then with combinations of compounds targeting nsp12/7/8 and nsp14-10 complexes or individual proteins. Synergy testing will be performed in technical and biological replicates, beginning with EC_{50} concentrations centered in the six-point dose-response curve. Combinations that display neutrality in toxicity assays and either additive or synergistic efficacy as defined by the BLISS synergy score[66] will be prioritized for testing *in vivo*.

SPECIFIC AIM 3. Lead evaluation in rodent models of zoonotic CoV replication, pathogenesis, disease, transmission, and persistence.

Rationale. In vivo models of CoV infection offer a critical, complex system to evaluate drug efficacy[67]. Robust mouse and hamster models have been established at **UTMB** for SARS-CoV, SARS-CoV-2, and MERS-CoV infection. In addition, these systems can be tailored to evaluate drug efficacy against transmission, in aged models, and in immune compromised states. This approach will provide crucial insights into how a drug performs in limiting disease and spread in healthy and vulnerable populations. The models also will allow iterative testing for route of drug administration, formulation, and testing of combinations.

Part 1. Utilize mouse models of SARS-CoV-2, SARS-CoV, and MERS-CoV infection to explore how drug treatment via different routes alters viral replication and pathogenesis. In this sub-aim, we will use established mouse infection models for SARS-CoV-2[40], SARS-CoV[41], and MERS-CoV[37] to explore drug efficacy *in vivo*. Each of these murine models of CoV infection recapitulate key elements of human disease and allow examination of how a drug impacts virus replication, disease progression, and recovery. **Mouse models.** Both SARS-CoV and MERS-CoV models of infection have been previously established and use mouse-adapted

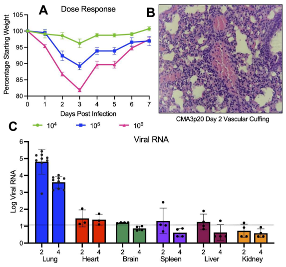


Figure 9. Mouse-adapted SARS-CoV-2 model. A) Dose response of 10 week old BALB/C mice infected with 10^4 (green), 10^5 (blue), or 10^6 (pink) PFU of SARS-CoV-2 CMA3. **B)** Immune infiltration in the lung 2 days post-infection with 10^5 PFU SARS-CoV-2. **C)** Viral RNA in various tissues following 10^5 PFU SARS-CoV-2 infection. Dashed line indicates lower limit of quantification.

strains to demonstrate viral replication in respiratory tissues and significant disease in terms of weight loss, respiratory function, and damage to the lung[37,41]. Our group at UTMB has generated a mouse-adapted SARS-CoV-2 strain (CMA3p20) that induces dose-dependent weight loss, immune infiltration/lung damage, and virus replication limited to the respiratory tract (Fig. 9)[40]. For SARS-CoV and SARS-CoV-2, these studies utilize standard laboratory strains (BALB/C, C57BL/6), while the MERS-CoV model employs human DPP4 transgenic mice[37]. Importantly, these CoV mouse models do not produce disease in off-target tissues like the CNS as seen with human ACE2 transgenic mice[68]. As such, these models provide a more physiologically relevant system to evaluate drug efficacy in the respiratory tract following infection. **Route of treatment.** The mouse models offer several avenues for drug administration. We will utilize two routes for each target drug. The first route will be decided based on expected efficacy (intranasal [i.n.], intraperitoneal [i.p.], intravenous [i.v.], inhalation). In addition, we will also use on oral delivery (gavage) to facilitate transfer to studies in humans. **Evaluation of viral load.** Initial studies will examine the impact a drug has on virus replication in the respiratory tract. In mice, we focus on viral titer found in the upper (trachea) and lower (lung) airways. We anticipate effective drugs will yield a reduction >1 $\log_{10}$ in viral titer relative to control. In addition to plaque assays, lobes of the lung will be examined for changes in viral RNA and antigen staining. From each of these parameters, the impact of the drug on viral load will be comprehensively evaluated. **Disease parameters.** In addition to viral load, we will evaluate the impact of drug treatment on CoV-induced disease. The most common metric, weight loss, will be examined over a 7-day time course with peak disease occurring between days 2-5 post-infection. We also will survey changes in disease score (ruffled fur, hunching, diminished movement) and use whole body plethysmography (WBP) to evaluate changes to respiratory function[69]. Finally, we will utilize histopathology (H&E) to examine immune-cell infiltration and lung damage in the presence of drug treatment. **Experimental design.** Drug efficacy will be evaluated in CoV models in the following order: SARS-CoV-2, SARS-CoV, and MERS-CoV. Briefly, 10 week-old C57BL/6 (SARS-Cov-2, SARS-CoV) or transgenic DPP4 (MERS-CoV) mice will be treated with the drug of interest or vehicle control starting 1 day prior to infection and continued throughout the time course of infection (Fig. 10). Animals subsequently will be infected with a dose of virus inducing up 20% weight loss and evaluated over a 7-day time course. Subsets of mice will be euthanized at days 2, 4, and 7 for determination of viral replication (lung and trachea), viral RNA (lung), and histopathology (lung). We anticipate an effective drug will have a significant impact on viral replication (reduction >1 $\log_{10}$), reduction in weight loss (>10% [absolute magnitude]), improved respiratory function, and/or diminished damage/immune infiltration in the lung. Targets validated with the most sensitive route (i.e., i.v., i.p., inhalation) or oral administration will be evaluated for therapeutic treatment commencing after infection. Because a priority is for development of orally available drugs, any lead advancing through *in vitro* studies that has known oral formulation will be prioritized and immediately tested by oral gavage administration.

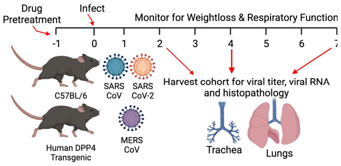


Figure 10. Drug efficacy testing in murine CoV models of infection. Mice will be treated with target drug or vehicle control beginning at day -1 and infected, monitored, euthanized for tissue harvest at days 2, 4, and 7.

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Part 2. Examine drug efficacy in the SARS-CoV-2 hamster model: impact on airway replication, lung damage, disease induction, and transmission. We will utilize the golden Syrian hamster, a second model of SARS-CoV-2 infection, to evaluate efficacy of a drug at limiting CoV replication, disease, and spread. **Hamster model.** Similar to mice, the golden Syrian hamster provides an *in vivo* model that recapitulates key elements of human disease, including replication limited to the airway, disease with non-adapted SARS-CoV-2 strains, and immune infiltration and damage to the lung. Importantly, the model also evaluates how drug treatment impacts virus transmission. Overall, our group has had significant experience utilizing the hamster model to evaluate fundamental aspects of SARS-CoV-2 biology and impact of variants[35,38,39]. **Route of treatment.** Paralleling the mouse studies, we will pursue two routes for drug treatment. The first approach will use best-efficacy route (i.n., i.p., i.v., or inhalation). Subsequent studies will focus on oral delivery (gavage). Selected routes will be informed by efficacy data from mouse studies. **Evaluation of viral load.** We will evaluate viral replication in both lower (lung) and upper (trachea) airways. In addition, we will determine viral titers in nasal washes as a proxy for transmission capacity[38,39]. We also will examine changes in viral RNA and antigen staining in the lung in the presence of drug treatment. **Disease parameters.** Drug-treated and control hamsters will be evaluated for changes in disease by weight loss, disease score, and histopathology. We will perform WBP in the hamster model by leveraging current equipment available in the animal BSL3. **Transmission.** One advantage of the hamster model of SARS-CoV-2 over mice is the capacity to evaluate changes in transmission between animals. While mice fail to transmit virus even via contact, our group has demonstrated efficient transmission of SARS-CoV-2 in the hamster model[38,39]. In this study, we will use drug- and mock-treated hamsters infected with barcoded SARS-CoV-2 as donor animals (Fig. 11). We will subsequently expose infected hamsters to naïve hamsters and evaluate transmission efficiency under drug treatment using NGS (see Letter Andrew Routh). **Experimental design.** 4-6 week old male golden Syrian hamsters will be treated with drug of interest or vehicle 1 day prior to infection with SARS-CoV-2 and continued throughout the experimental time course. Hamsters will be evaluated over a 7-day time course, with subsets euthanized at days 2, 4, and 7 for assessment of viral replication, viral RNA, and histopathology. We anticipate an effective drug will reduce viral replication (>1 $\log_{10}$) and weight loss (>10% [absolute magnitude]), improve respiratory function, and/or diminish lung damage and immune infiltration. Treatments that result in reduced viral replication or improved disease outcomes will be further evaluated for changes in transmission. Finally, targets validated by sensitive routes (i.n., i.v., i.p., inhalation) will tested for oral dosing using same design.

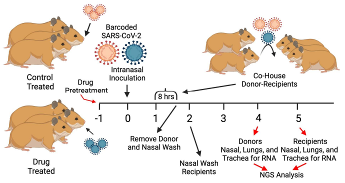

Figure 11. Drug impact on SARS-CoV-2 transmission. Drug- and mock-treated animals infected with barcoded SARS-CoV-2 will be co-housed with naïve animals and transmission evaluated by NGS.

Part 3. Evaluate drug efficacy in reducing disease in the susceptible aged mouse model of CoV infection. Based on demographic data from SARS-CoV-2, SARS-CoV, and MERS-CoV outbreaks, CoV infection causes increased mortality in aged human populations, an age-dependent susceptibility is recapitulated in our mouse models of SARS-CoV and SARS-CoV-2 pathogenesis[70,71]. Therefore, these aging models allow evaluation of drug efficacy in a critical, susceptible population. **Aged mouse model.** While young C57BL/6 mice (10 weeks old) are relatively resistant to SARS-CoV infection, aged mice (20 months) have significantly more disease in terms of weight loss, immune infiltration, and respiratory dysfunction (Fig. 12). Similar age-dependent disease has been observed following SARS-CoV-2 infection of mice[70]. Importantly, drug treatments that reduce or disrupt virus replication may have augmented efficacy in aging mouse models and show utility in treating CoV-mediated disease in this susceptible population. **Viral load and disease parameters.** We anticipate changes in viral replication will be similar between young and aged mice. However, we predict drug treatment will have an increased impact in aged mice due to exacerbated disease. We will focus on several disease readouts to mark improved outcomes, including weight loss, disease course, and survival over a 7-day time period. We will also utilize WBP to evaluate changes in respiratory function with and without drug treatment. Finally, histopathology of the lung will provide insight into damage and immune infiltration in the presence of drug treatment. **Experimental Design.** Aged C57BL/6 mice (14-20 months) will be treated with the drug of interest or vehicle control 1 day prior to infection. Animals will be subsequently infected with mouse-adapted SARS-CoV (MA15) or SARS-CoV-2 (CMA3p20) and evaluated over a 7-day time course. Subsets of mice will be euthanized at days 2, 4, and 7 for analyses of viral replication, viral RNA, and histopathology. We expect treated aged mice will experience a reduction in weight loss (>10% [absolute magnitude), improved respiratory function, and/or diminished damage and immune infiltration in the lung.

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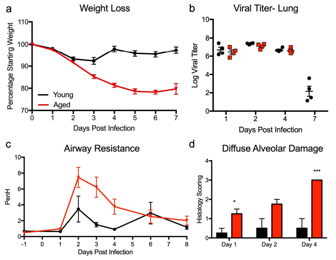


Figure 12. Age-dependent disease in mouse models of SARS-CoV. Young (10 week-old, black) and aged (20 month old, red) C57BL/6 mice infected with 10^4 PFU of SARS-CoV MA15 were monitored for **a)** weight loss, **b)** viral titer in the lung, **c)** airway resistance (penH) by WBP and **d)** diffuse alveolar damage by histopathology.

Part 4. Determine drug efficacy and viral resistance generation in immune compromised mouse models. In addition to aged populations, immunocompromised people represent a major vulnerable population to CoV disease, and specific genetic knockout mouse models recapitulate similar immune deficiencies as found in humans. These models will be used to evaluate drug efficacy as well as define potential routes for drug

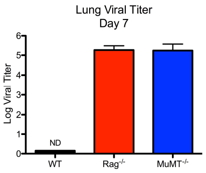


Figure 13. CoV clearance requires adaptive immunity. WT mice clear SARS-CoV by day 7. Mice lacking lymphocytes (Rag-/-) or only B-cells (MuMT-/-) fail to clear SARS-CoV, permitting continued replication.

resistance produced in persistently infected *in vivo* models. **Immune compromised mouse model.** Previous work with mice lacking both B- and T-cell immunity (RAG-/-) or B cells immunity alone (MuMT-/-) has demonstrated persistent infection with SARS-CoV lasting >30 days post-initial challenge[72] (Fig. 13). As such, these mouse models correspond to persistent infection with SARS-CoV-2 observed in immunocompromised humans[73]. Importantly, immune compromised mouse models provide an avenue to explore drug efficacy in a vulnerable population, which often respond poorly to vaccines[74,75]. In addition, the persistent infection model provides an *in vivo* system to explore drug resistance that can expand on *in vitro* studies. **Evaluation of viral load.** We will evaluate virus replication in drug-treated immunocompromised mice over an extended timeline—early (day 2), intermediate (day 7), and extended (day 30) intervals post-infection. We will focus on the lungs in these animals and evaluate viral replication, RNA, and antigen staining in parallel. **Disease parameters.** Previous studies with SARS-CoV demonstrated that, although RAG-/- and MuMT-/- fail to clear virus over time, the disease phenotypes are consistent with WT mice in terms of disease and damage. Therefore, while we will evaluate weight loss, disease score, and histopathology, we do not anticipate significant changes in disease relative to young mice in Part 1. For SARS-CoV-2, the immune compromised model development is ongoing and may reveal distinction in disease that will be pursued as indicated by specific phenotypes. **Resistance mutations.** Consistent with *in vitro* resistance testing, immunocompromised mice used in these studies will allow exploration of the generation of resistance *in vivo*. Using the day 30 samples from persistently infected mice, we will work with Dr. Andrew Routh (see letter) to examine coding changes in the SARS-CoV and SARS-CoV-2 genomes that may contribute to drug resistance. **Experimental Design.** RAG-/- and MuMT-/- mice will be treated with the drug of interest or vehicle control 1 day prior to infection and continued throughout the 30-day time course. Animals will be subsequently infected with either SARS-CoV (MA15) or SARS-CoV-2 (CMA3) and evaluated over a 30-day time course. Subsets of mice will be euthanized at days 2, 7, and 30 for viral replication (lung and trachea), viral RNA (lung), and histopathology (lung). We anticipate an effective drug will have a significant impact on viral replication (reduction >1 log$_{10}$), even in the absence of adaptive immune responses. We do not anticipate significant changes to disease parameters between WT and mutant animals in the SARS-CoV model. For SARS-CoV-2, model development will inform measurable changes to pathogenesis. For resistance, we will focus on virus-coding mutations found at late time points (days 15 and 30), and these mutations will be cross-referenced to Aim 2 results to identify unique and shared features of *in vitro*- and *in vivo*-selected resistance. Critical mutations will be engineered in isogenic backgrounds for in vitro testing and for direct testing of impact of mutations on virus fitness (**VUMC**), attenuation in vivo **(UTMB)**, and in biochemical systems with the **Structure and Analysis Cores** for impact on protein function and sensitivity to the compound in question.

Expected outcomes, potential challenges, and alternative strategies. Our group has extensive experience with *in vivo* models of coronavirus infection[12,13,38-40,76-82]. We anticipate each activity in Aim 3 will be successful based on robust animal model systems, well-established experimental design, and prior experience with the proposed studies. We predict that targets advancing to this aim will show efficacy in both the hamster and mouse models of infection. In addition, the transmission and aged models will provide important insights into the use of these drugs in a metric of spread and a key, susceptible population, respectively. While the models are well characterized, the oral dosing and candidate formulations may require optimization to improve efficacy and may vary across CoV strains. Similarly, while we anticipate generation of resistance, failure to induce resistance *in vivo* would be a positive result, suggesting limited escape from the candidate tested. Absence of resistant mutants, while unlikely, would obviate the need for studies to characterize attenuation of resistant viruses in immune competent animals.

PROJECT DEVELOPMENT PLAN. The table below shows the stages of development. It is broadly useful to look at overall process but less so in regards to specific compounds and our high priority of identifying and rapidly advancing compounds with broad efficacy and potential for oral administration. With the ongoing pandemic, our demonstrated expertise, and the highly integrated labs (VUMC, UTMB, UCONN, SR), collaborators (Arbutus, USC) and Cores (MedChem, Structure, Analysis) we are positioned for rapid-cycle iteration on validated hits and advanced leads to improve potency, physical properties, and toxicity profiles via combination of structural analyses, biochemical assays, and medicinal chemistry. All requisite capabilities exist within Project 1 consortium. Importantly, we start with multiple validated hits from USC, Arbutus, UConn that will enter Aims 1 and 2 and even Aim 3. We will constantly review and prioritize / reprioritize compounds to continue to develop novel approaches while rapidly advancing those most promising.

Based on the monthly progress meetings and annual project reviewers, the AD3C-2 Center will prioritize promising projects and drug candidate for fast-track development. Compounds with *in vivo* efficacy, desired PK, and good safety profile will be prioritized for preclinical development and IND profiling. Please refer the details for the Product Development Plan in the OVERALL write-up of the application.

	Year 1		**Year 2**		**Year 3**		**Year 4**		**Year 5**	
Quarter	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**	**1/2**	**3/4**
SPECIFIC AIM 1: Identification and selection of hit and lead compounds targeting the SARS-CoV-2 nsp12-RdRp and nsp14-ExoN										
Part 1. NA chemistry and targeting the nsp12-RdRp	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Part 2. Biochemical high-throughput screening of nsp12-7-8 and nsp14-10 complex inhibitors	▨	▨	▨	▨						
Part 3. TMID enzyme inhibitors of nsp14-ExoN	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
Part 4. *In vitro* hit identification and validation	▨	▨	▨	▨	▨	▨	▨	▨	▨	▨
SPECIFIC AIM 2: Lead development for mechanism of action, viral determinants of activity and resistance, and combination testing										
Part 1. Identification of resistance determinants			▨	▨	▨	▨	▨	▨	▨	▨
Part 2. Sequence analysis, confirmation of targets, determinants of resistance, and biochemical and structural implications			▨	▨	▨	▨	▨	▨	▨	▨
Part 3. Impact of resistance mutations on viral fitness and susceptibility to other compounds			▨	▨	▨	▨	▨	▨	▨	▨
Part 4. Combination testing			▨	▨	▨	▨	▨	▨	▨	▨
SPECIFIC AIM 3: Lead evaluation in rodent models of zoonotic CoV replication, pathogenesis, disease, transmission, and persistence										
Part 1. Utilize mouse models of SARS-CoV-2, SARS-CoV, and MERS-CoV infection to explore how drug treatment via different routes alters viral replication and pathogenesis			▨	▨	▨	▨	▨	▨	▨	▨
Part 2. Examine drug efficacy in the SARS-CoV-2 hamster model: impact on airway replication, lung damage, disease induction, and transmission			▨	▨	▨	▨	▨	▨	▨	▨
Part 3. Evaluate drug efficacy in reducing disease in the susceptible aged mouse model of CoV infection			▨	▨	▨	▨	▨	▨	▨	▨

PROJECT 1 BIBLIOGRAPHY

1. Bertolin AP, Weissmann F, Zeng JK, et al. Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of nsp12/7/8 RNA-dependent RNA polymerase. Biochem J 2021;478(1):2425-2443. (In English). DOI: 10.1042/Bcj20210200.
2. Canal B, McClure AW, Curran JF, et al. Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of nsp14/ nsp10 exoribonuclease. Biochem J 2021;478(1):2445-2464. (In English). DOI: 10.1042/Bcj20210198.
3. Xie X, Muruato AE, Zhang X, et al. A nanoluciferase SARS-CoV-2 for rapid neutralization testing and screening of anti-infective drugs for COVID-19. Nat Commun 2020;11(1):5214. DOI: 10.1038/s41467-020-19055-7.
4. Xie X, Muruato A, Lokugamage KG, et al. An Infectious cDNA Clone of SARS-CoV-2. Cell Host Microbe 2020;27(5):841-848 e3. DOI: 10.1016/j.chom.2020.04.004.
5. Sims AC, Baric RS, Yount B, Burkett SE, Collins PL, Pickles RJ. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J Virol 2005;79(24):15511-24. DOI: 10.1128/JVI.79.24.15511-15524.2005.
6. Scobey T, Yount BL, Sims AC, et al. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. Proc Natl Acad Sci U S A 2013;110(40):16157-62. DOI: 10.1073/pnas.1311542110.
7. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science 2003;302(5643):276-8. DOI: 10.1126/science.1087139.
8. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 2012;367(19):1814-20. DOI: 10.1056/NEJMoa1211721.
9. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579(7798):270-273. DOI: 10.1038/s41586-020-2012-7.
10. Lam TT, Jia N, Zhang YW, et al. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature 2020;583(7815):282-285. DOI: 10.1038/s41586-020-2169-0.
11. Menachery VD, Dinnon KH, 3rd, Yount BL, Jr., et al. Trypsin Treatment Unlocks Barrier for Zoonotic Bat Coronavirus Infection. J Virol 2020;94(5). DOI: 10.1128/JVI.01774-19.
12. Menachery VD, Yount BL, Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. Nat Med 2015;21(12):1508-+. (In English). DOI: 10.1038/nm.3985.
13. Menachery VD, Yount BL, Jr., Sims AC, et al. SARS-like WIV1-CoV poised for human emergence. Proc Natl Acad Sci U S A 2016;113(11):3048-53. DOI: 10.1073/pnas.1517719113.
14. Woo PC, Lau SK, Li KS, et al. Molecular diversity of coronaviruses in bats. Virology 2006;351(1):180-7. DOI: 10.1016/j.virol.2006.02.041.
15. Perlman S, Netland J. Coronaviruses post-SARS: update on replication and pathogenesis. Nat Rev Microbiol 2009;7(6):439-50. DOI: 10.1038/nrmicro2147.
16. Snijder EJ, Bredenbeek PJ, Dobbe JC, et al. Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. J Mol Biol 2003;331(5):991-1004. DOI: 10.1016/s0022-2836(03)00865-9.
17. Snijder EJ, Decroly E, Ziebuhr J. The Nonstructural Proteins Directing Coronavirus RNA Synthesis and Processing. Adv Virus Res 2016;96:59-126. DOI: 10.1016/bs.aivir.2016.08.008.
18. Ziebuhr J, Snijder EJ, Gorbalenya AE. Virus-encoded proteinases and proteolytic processing in the Nidovirales. J Gen Virol 2000;81(Pt 4):853-79. DOI: 10.1099/0022-1317-81-4-853.
19. Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nature Communications 2019;10 (In English). DOI: ARTN 2342 10.1038/s41467-019-10280-3.
20. Hillen HS, Kokic G, Farnung L, Dienemann C, Tegunov D, Cramer P. Structure of replicating SARS-CoV-2 polymerase. Nature 2020;584(7819):154-+. (In English). DOI: 10.1038/s41586-020-2368-8.
21. Yan L, Yang Y, Li M, et al. Coupling of N7-methyltransferase and 3'-5' exoribonuclease with SARS-CoV-2 polymerase reveals mechanisms for capping and proofreading. Cell 2021;184(13):3474-3485 e11. DOI: 10.1016/j.cell.2021.05.033.
22. Eckerle LD, Lu X, Sperry SM, Choi L, Denison MR. High Fidelity of Murine Hepatitis Virus Replication Is Decreased in nsp14 Exoribonuclease Mutants. J Virol 2007;81:12135-12144. (In English). DOI: 10.1128/JVI.01296-07.

23. Eckerle LD, Becker MM, Halpin RA, et al. Infidelity of SARS-CoV Nsp14-Exonuclease Mutant Virus Replication Is Revealed by Complete Genome Sequencing. Plos Pathog 2010;6(5) (In English). DOI: ARTN e1000896 10.1371/journal.ppat.1000896.
24. Ferron F, Subissi L, Silveira De Morais AT, et al. Structural and molecular basis of mismatch correction and ribavirin excision from coronavirus RNA. Proc Natl Acad Sci U S A 2018;115(2):E162-E171. DOI: 10.1073/pnas.1718806115.
25. Gribble J, Stevens LJ, Agostini ML, et al. The coronavirus proofreading exoribonuclease mediates extensive viral recombination. Plos Pathog 2021;17(1):e1009226. DOI: 10.1371/journal.ppat.1009226.
26. Liu C, Shi W, Becker ST, Schatz DG, Liu B, Yang Y. Structural basis of mismatch recognition by a SARS-CoV-2 proofreading enzyme. Science 2021. DOI: 10.1126/science.abi9310.
27. Ma YY, Wu LJ, Shaw N, et al. Structural basis and functional analysis of the SARS coronavirus nsp14-nsp10 complex. P Natl Acad Sci USA 2015;112(30):9436-9441. (In English). DOI: 10.1073/pnas.1508686112.
28. Smith EC, Sexton NR, Denison MR. Thinking Outside the Triangle: Replication Fidelity of the Largest RNA Viruses. Annu Rev Virol 2014;1:111-132. (In English). DOI: 10.1146/annurev-virology-031413-085507.
29. Sheahan TP, Sims AC, Zhou S, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med 2020;12(541). DOI: 10.1126/scitranslmed.abb5883.
30. Pruijssers AJ, George AS, Schafer A, et al. Remdesivir Inhibits SARS-CoV-2 in Human Lung Cells and Chimeric SARS-CoV Expressing the SARS-CoV-2 RNA Polymerase in Mice. Cell Rep 2020;32(3) (In English). DOI: ARTN 107940 10.1016/j.celrep.2020.107940.
31. Agostini ML, Pruijssers AJ, Chappell JD, et al. Small-molecule antiviral Beta-D-N (4)-hydroxycytidine inhibits a proofreading-intact coronavirus with a high genetic barrier to resistance. J Virol 2019;93(24). DOI: 10.1128/JVI.01348-19.
32. Agostini ML, Andres EL, Sims AC, et al. Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. Mbio 2018;9(2) (In English). DOI: ARTN e00221-18 10.1128/mBio.00221-18.
33. Gilead Sciences I. VEKLURY® (remdesivir) for injection, for intravenous use. 2021.
34. ClinicalTrials.gov. Molnupiravir Studies. (https://clinicaltrials.gov/ct2/results?cond=&term=molnupiravir&cntry=&state=&city=&dist=).
35. Plante JA, Liu Y, Liu J, et al. Spike mutation D614G alters SARS-CoV-2 fitness. Nature 2021;592(7852):116-121. DOI: 10.1038/s41586-020-2895-3.
36. Sims AC, Burkett SE, Yount B, Pickles RJ. SARS-CoV replication and pathogenesis in an in vitro model of the human conducting airway epithelium. Virus Res 2008;133(1):33-44. DOI: 10.1016/j.virusres.2007.03.013.
37. Algaissi A, Agrawal AS, Han S, et al. Elevated Human Dipeptidyl Peptidase 4 Expression Reduces the Susceptibility of hDPP4 Transgenic Mice to Middle East Respiratory Syndrome Coronavirus Infection and Disease. J Infect Dis 2019;219(5):829-835. DOI: 10.1093/infdis/jiy574.
38. Johnson BA, Xie X, Bailey AL, et al. Loss of furin cleavage site attenuates SARS-CoV-2 pathogenesis. Nature 2021;591(7849):293-299. DOI: 10.1038/s41586-021-03237-4.
39. Liu Y, Liu, Jianying, Plante, Kenneth S, Plante, Jessica A, Xie, Xuping, Zhang, Xianwen, Ku, Zhiqiang, An, Zhiqiang, Scharton, Dionna, Schindewolf, Craig, Menachery, Vineet D, Shi, Pei-Yong, Weaver, Scott C. The N501Y spike substitution enhances SARS-CoV-2 transmission. bioRxiv. DOI: https://doi.org/10.1101/2021.03.08.434499.
40. Muruato A, Vu, Michelle N., Johnson, Bryan A., Davis-Gardner, Meredith E., Vanderheiden, Abigail, Lokugmage, Kumari, Schindewolf, Craig, Crocquet-Valdes, Patricia A., Langsjoen, Rose M., Plante, Jessica A., Plante, Kenneth S., Weaver, Scott C., Debbink, Kari, Routh, Andrew L., Suthar, Mehul S., Walker, David, Xie, Xuping, Shi, Pei-Yong, Menachery, Vineet D. Mouse Adapted SARS-CoV-2 protects animals from lethal SARS-CoV challenge. DOI: https://doi.org/10.1101/2021.05.03.442357.
41. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. Plos Pathog 2007;3(1):e5. DOI: 10.1371/journal.ppat.0030005.
42. Becker MM, Graham RL, Donaldson EF, et al. Synthetic recombinant bat SARS-like coronavirus is infectious in cultured cells and in mice. P Natl Acad Sci USA 2008;105(50):19944-19949. (In English). DOI: 10.1073/pnas.0808116105.
43. Yount B, Curtis KM, Fritz EA, et al. Reverse genetics with a full-length infectious cDNA of severe acute respiratory syndrome coronavirus. Proc Natl Acad Sci U S A 2003;100(22):12995-3000. DOI: 10.1073/pnas.1735582100.
44. Douglas MG, Kocher JF, Scobey T, Baric RS, Cockrell AS. Adaptive evolution influences the infectious dose of MERS-CoV necessary to achieve severe respiratory disease. Virology 2017 (In eng). DOI: 10.1016/j.virol.2017.12.006.

45. Cockrell AS, Yount BL, Scobey T, et al. A mouse model for MERS coronavirus-induced acute respiratory distress syndrome. Nat Microbiol 2016;2:16226. DOI: 10.1038/nmicrobiol.2016.226.
46. Hou YJ, Okuda K, Edwards CE, et al. SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell 2020;182(2):429-446 e14. DOI: 10.1016/j.cell.2020.05.042.
47. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 2017;9(396) (In English). DOI: ARTN eaal3653 10.1126/scitranslmed.aal3653.
48. Sheahan TP, Sims AC, Leist SR, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun 2020;11(1):222. DOI: 10.1038/s41467-019-13940-6.
49. Smith EC, Blanc H, Surdel MC, Vignuzzi M, Denison MR. Coronaviruses lacking exoribonuclease activity are susceptible to lethal mutagenesis: evidence for proofreading and potential therapeutics. Plos Pathog 2013;9(8):e1003565. DOI: 10.1371/journal.ppat.1003565.
50. Sexton NR, Smith EC, Blanc H, Vignuzzi M, Peersen OB, Denison MR. Homology-Based Identification of a Mutation in the Coronavirus RNA-Dependent RNA Polymerase That Confers Resistance to Multiple Mutagens. J Virol 2016;90(16):7415-7428. (In English). DOI: 10.1128/Jvi.00080-16.
51. Graepel KW, Lu XT, Case JB, Sexton NR, Smith EC, Denison MR. Proofreading-Deficient Coronaviruses Adapt for Increased Fitness over Long-Term Passage without Reversion of Exoribonuclease-Inactivating Mutations. Mbio 2017;8(6) (In English). DOI: ARTN e01503-17 10.1128/mBio.01503-17.
52. McKenna C, Overhulse J, Lentini N, et al. Novel prodrugs as inhibitors of SARS-coronavirus-2. 34th Int. Conf. Antiviral Res.2021.
53. Lyu J, Hartline C, Keith K, et al. Cidofovir and (S)-HPMPA serinamide and homoserinamide prodrugs potently inhibit DNA viruses in cell culture. 34th Int. Conf. Antiviral Res.2021.
54. Liu D, Lyu J, Lloyd M, et al. Oral USC-373, an HPMPC prodrug, prevents varicella zoster virus replication in a mouse model. 2021.
55. Toth K, Lyu J, Spencer J, et al. Oral USC-093, a novel homoserinamide prodrug of (S)-HPMPA shows equivalent efficacy to USC-087 in preventing mortality against lethal hAdV-C6 challenge in a permissive immunosuppressed Syrian hamster model. 2021.
56. Toth K, Spencer JF, Ying B, et al. USC-087 protects Syrian hamsters against lethal challenge with human species C adenoviruses. Antiviral Res 2018;153:1-9. DOI: 10.1016/j.antiviral.2018.03.001.
57. Smith EC, Case JB, Blanc H, et al. Mutations in Coronavirus Nonstructural Protein 10 Decrease Virus Replication Fidelity. J Virol 2015;89:6418-6426. (In English). DOI: 10.1128/JVI.00110-15.
58. Ogando NS, Zevenhoven-Dobbe JC, van der Meer Y, Bredenbeek PJ, Posthuma CC, Snijder EJ. The Enzymatic Activity of the nsp14 Exoribonuclease Is Critical for Replication of MERS-CoV and SARS-CoV-2. J Virol 2020;94(23) (In English). DOI: ARTN e01246-20 10.1128/JVI.01246-20.
59. Denison MR, Graham RL, Donaldson EF, Eckerle LD, Baric RS. Coronaviruses: an RNA proofreading machine regulates replication fidelity and diversity. RNA Biol 2011;8:270-279. (In English). DOI: 10.4161/rna.8.2.15013.
60. Gorbalenya AE, Enjuanes L, Ziebuhr J, Snijder EJ. Nidovirales: Evolving the largest RNA virus genome. Virus Research 2006;117:17-37. (In English). DOI: 10.1016/j.virusres.2006.01.017.
61. Esposito F, Tramontano E. Past and future. Current drugs targeting HIV-1 integrase and reverse transcriptase-associated ribonuclease H activity: single and dual active site inhibitors. Antivir Chem Chemother 2014;23(4):129-44. DOI: 10.3851/IMP2690.
62. Li Y, Xuan S, Feng Y, Yan A. Targeting HIV-1 integrase with strand transfer inhibitors. Drug Discov Today 2015;20(4):435-49. DOI: 10.1016/j.drudis.2014.12.001.
63. Semenova EA, Johnson AA, Marchand C, Davis DA, Yarchoan R, Pommier Y. Preferential inhibition of the magnesium-dependent strand transfer reaction of HIV-1 integrase by alpha-hydroxytropolones. Mol Pharmacol 2006;69(4):1454-60. DOI: 10.1124/mol.105.020321.
64. DuBois RM, Slavish PJ, Baughman BM, et al. Structural and biochemical basis for development of influenza virus inhibitors targeting the PA endonuclease. Plos Pathog 2012;8(8):e1002830. DOI: 10.1371/journal.ppat.1002830.
65. Noshi T, Kitano M, Taniguchi K, et al. In vitro characterization of baloxavir acid, a first-in-class cap-dependent endonuclease inhibitor of the influenza virus polymerase PA subunit. Antiviral Res 2018;160:109-117. DOI: 10.1016/j.antiviral.2018.10.008.
66. Ianevski A, Giri AK, Aittokallio T. SynergyFinder 2.0: visual analytics of multi-drug combination synergies. Nucleic Acids Res 2020;48(W1):W488-W493. DOI: 10.1093/nar/gkaa216.

67. Lakdawala SS, Menachery VD. The search for a COVID-19 animal model. Science 2020;368(6494):942-943. DOI: 10.1126/science.abc6141.
68. Winkler ES, Bailey AL, Kafai NM, et al. SARS-CoV-2 infection of human ACE2-transgenic mice causes severe lung inflammation and impaired function. Nat Immunol 2020;21(11):1327-1335. DOI: 10.1038/s41590-020-0778-2.
69. Menachery VD, Gralinski LE, Baric RS, Ferris MT. New Metrics for Evaluating Viral Respiratory Pathogenesis. PLoS One 2015;10(6):e0131451. DOI: 10.1371/journal.pone.0131451.
70. Leist SR, Dinnon KH, 3rd, Schafer A, et al. A Mouse-Adapted SARS-CoV-2 Induces Acute Lung Injury and Mortality in Standard Laboratory Mice. Cell 2020;183(4):1070-1085 e12. DOI: 10.1016/j.cell.2020.09.050.
71. Rockx B, Baas T, Zornetzer GA, et al. Early upregulation of acute respiratory distress syndrome-associated cytokines promotes lethal disease in an aged-mouse model of severe acute respiratory syndrome coronavirus infection. J Virol 2009;83(14):7062-7074. (In eng). DOI: 10.1128/JVI.00127-09.
72. Graham RL, Becker MM, Eckerle LD, Bolles M, Denison MR, Baric RS. A live, impaired-fidelity coronavirus vaccine protects in an aged, immunocompromised mouse model of lethal disease. Nat Med 2012;18(12):1820-6. DOI: 10.1038/nm.2972.
73. Baang JH, Smith C, Mirabelli C, et al. Prolonged Severe Acute Respiratory Syndrome Coronavirus 2 Replication in an Immunocompromised Patient. J Infect Dis 2021;223(1):23-27. DOI: 10.1093/infdis/jiaa666.
74. Havlin J, Svorcova M, Dvorackova E, et al. Immunogenicity of BNT162b2 mRNA COVID-19 vaccine and SARS-CoV-2 infection in lung transplant recipients. J Heart Lung Transplant 2021;40(8):754-758. DOI: 10.1016/j.healun.2021.05.004.
75. Eberhardt CS, Balletto E, Cornberg M, Mikulska M. Coronavirus disease 2019 vaccination in transplant recipients. Curr Opin Infect Dis 2021;34(4):275-287. DOI: 10.1097/QCO.0000000000000739.
76. Ku Z, Xie X, Hinton PR, et al. Nasal delivery of an IgM offers broad protection from SARS-CoV-2 variants. Nature 2021;595(7869):718-723. DOI: 10.1038/s41586-021-03673-2.
77. Vanderheiden A, Thomas J, Soung AL, et al. CCR2-dependent monocyte-derived cells restrict SARS-CoV-2 infection. bioRxiv 2021. DOI: https://doi.org/10.1101/2021.05.03.442538.
78. Johnson BA, Hage A, Kalveram B, et al. Peptidoglycan-Associated Cyclic Lipopeptide Disrupts Viral Infectivity. J Virol 2019;93(22). DOI: 10.1128/JVI.01282-19.
79. Menachery VD, Gralinski LE, Mitchell HD, et al. Combination Attenuation Offers Strategy for Live Attenuated Coronavirus Vaccines. J Virol 2018;92(17). DOI: 10.1128/JVI.00710-18.
80. Menachery VD, Gralinski LE, Mitchell HD, et al. Middle East Respiratory Syndrome Coronavirus Nonstructural Protein 16 Is Necessary for Interferon Resistance and Viral Pathogenesis. mSphere 2017;2:e00346-17-12. (In English). DOI: 10.1128/mSphere.00346-17.
81. Menachery VD, Mitchell HD, Cockrell AS, et al. MERS-CoV Accessory ORFs Play Key Role for Infection and Pathogenesis. Mbio 2017;8:e00665-17. (In English). DOI: 10.1128/mBio.00665-17.
82. Menachery VD, Debbink K, Baric RS. Coronavirus non-structural protein 16: evasion, attenuation, and possible treatments. Virus Research 2014;194:191-199. (In English). DOI: 10.1016/j.virusres.2014.09.009.

SPECIFIC AIMS

Hendra virus (HeV) and Nipah virus (NiV) are members of the genus *Henipavirus* (family *Paramyxoviridae*) that can cause severe respiratory illness and encephalitis in animals and humans. Both viruses are among the deadliest human pathogens known to man with case fatality rates up to 100% for the Bangladesh strain of NiV (NiV$_B$). Significantly, multiple rounds of person-to-person transmission of NiV$_B$ is also possible. Cedar henipavirus (CedV) is the only other henipavirus isolate and also the only known non-pathogenic henipavirus, and has been recently demonstrated to be an effective surrogate platform for henipavirus antiviral drug discovery and tool for countermeasure research. There are no approved vaccines or therapeutics for disease caused by HeV or NiV. This proposal focuses on developing antiviral countermeasures to combat the pathogenic henipaviruses which today includes HeV and two distinct NiV strains, NiV$_B$ and NiV Malaysia (NiV$_M$). Genevant Sciences, Inc. has considerable experience developing RNA interference (RNAi) therapeutics against highly pathogenic viruses, with demonstrated efficacy in nonhuman primate (NHP) models of infection. The RNAi therapeutics approach utilizes Genevant's lipid nanoparticle (LNP) platform which stabilizes encapsulated small interfering RNA (siRNA) ensuring effective delivery to target cells. Aim 1 will focus on developing RNAi strategies to combat henipaviruses. A second antiviral discovery approach will focus on compounds that target the replication complex of henipaviruses. This approach is supported by the previous results that the polymerase inhibitor remdesivir can confer protection to animals against NiV$_B$ demonstrating the promise of this class of drugs. Here, Aim 2 will perform high-throughput screening (HTS) using a large library of compounds with an established HTS assay employing a replication competent authentic henipavirus platform (CedV) to identify additional lead antiviral candidates. Aim 3 will focus on combination therapy which is known to be highly effective for combating other viral pathogens, and also one that limits the potential emergence of drug resistance. This uniquely different and targeted combination approach will utilize the most potent antivirals identified in Aims 1 and/or 2 and could provide an effective and complementary treatment of henipavirus infection that is more effective than each therapeutic modality alone. Specifically, we will pursue the following three aims:

Aim 1. Develop and optimize siRNA for protection against Hendra and Nipah viruses. A battery of siRNAs against conserved regions of the L, N, P, M, G, and F genes of HeV, NiV$_M$, and NiV$_B$ will be designed and evaluated for antiviral activity *in vitro*. The most promising candidates will be formulated in LNP targeting different cell types. Pharmacokinetic and biodistribution studies will be performed in hamsters. Protective efficacy of lead candidates will be evaluated in hamster models of henipavirus disease when administered at various times after exposure and by different routes of delivery. Dosing and delivery regimens will be evaluated in hamsters, and the most promising siRNAs will be assessed in a proof-of-concept protective efficacy in African green monkey (AGM) models of HeV and NiV$_B$.

Aim 2. Develop and optimize replication complex inhibitors against Hendra and Nipah viruses. Libraries of drugs predicted to have activity against the replication complex of henipaviruses will be screened using a novel surrogate virus system based on the henipavirus Cedar virus (CedV) which has significant homology to both HeV and NiV. This system utilizes recombinant CedV encoding luciferase (rCedV-Luc) and will be used at BSL-2 for high-throughput screening (HTS) of a library of up to 100,000 compounds. Lead candidates will then be screened against wild type HeV and NiV *in vitro*. Pharmacokinetic and biodistribution studies will be performed in hamsters on inhibitors showing *in vitro* activity against wild type henipaviruses. Protective efficacy of lead drugs will be performed in hamster models of HeV and NiV infection. Antivirals showing strong protective efficacy may be optimized in collaboration with the Chemistry Core. Following optimization of dosing and delivery regimens in hamsters the most promising antivirals will be assessed for protective efficacy in AGM models of HeV and NiV$_B$. In order to leverage existing data showing that remdesivir protected a small cohort of AGMs when given very shortly after exposure to NiV$_B$, studies will be performed to determine the therapeutic window for remdesivir in AGMs exposed to HeV and NiV$_B$, i.e., can remdesivir be given at the onset of clinical disease or at advanced stages of illness and still have a beneficial effect?

Aim 3. Develop combination therapy of broad-spectrum siRNA-conjugate therapeutics and/or replication complex inhibitors against Hendra and Nipah viruses. The most promising antivirals from Aims 1 and/or 2 targeting different antigenic sites will be combined and assessed for enhanced efficacy in henipavirus-infected cells and hamsters. The objectives of these studies will be to determine if the combinatorial approach will confer 1) improved antiviral efficacy over that seen with each therapeutic alone; and 2) improved efficacy when treatment is initiated either at, or even after, the prodromal phase.

RESEARCH STRATEGY

(a) Significance. Hendra virus (HeV) and Nipah virus (NiV) are enveloped, single-stranded negative-sense RNA viruses and the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae*[1-3]. NiV and HeV are bat-borne paramyxoviruses that are distinguished from the many paramyxoviruses that cause human and animal diseases by virtue of their uniquely broad host tropism and impressive lethality. Henipaviruses are capable of infecting at least 18 animal species across six orders of mammals, including bats[4,5]. HeV was first recognized in 1994 as the cause of an outbreak that led to fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra, Australia. To date, HeV has appeared in Eastern Australia on 62 occasions causing the death or euthanasia of more than 100 horses, two HeV antibody-positive and euthanized dogs, and 4 fatalities of 7 human cases[6,7]. The horse is the predominant target host acquiring HeV infection from virus-shedding bats and is also the source of human infection. NiV emerged just a few years later in Malaysia and Singapore in 1998-99 causing a large outbreak of encephalitis with high mortality in people and also respiratory disease in pigs which served as amplifying hosts[8,9]. NiV was later recognized as the agent causing a large outbreak of encephalitis among pig farmers in Malaysia in 1998/1999. Although NiV from Malaysia (NiV_M) has not re-emerged in Malaysia, nearly annual outbreaks of human cases of NiV infection have been recorded since 2001 in Bangladesh and a few in India caused by NiV-Bangladesh (NiV_B)[10]. The outbreaks of NiV^B have had notably higher human fatality rates ranging from 75-100%, along with the direct food-borne transmission of NiV from bats to humans and significant human-to-human transmission of NiV infection[11]. Evidence of NiV has also been detected in cattle, goats, and pigs[12]. However, in 2014, an outbreak of NiV encephalitis (attributed to the NiV-Malaysia strain) occurred in the Philippines with 9 fatalities of 11 human encephalitic cases and an influenza-like illness in another 6 individuals, one also with meningitis[13]. The Philippines outbreak involved horse-to-human and human-to-human transmission of NiV infection as well as apparent lethal infections of both cats and dogs. A more recent 2018 NiV_B outbreak in Kerala, India, occurred in a new geographic region far from the locations in Bangladesh and India where all prior appearances have occurred and had a high case fatality - 91%, with 22 of 23 cases the result of human-to-human infection transmission at three different hospital locations[14]. A fatal case of NiV_B was reported in Kerala in September of 2021 causing concern of another potential outbreak[15]. Together, there have been over 650 cases of human NiV infection with a combined ~60% case fatality rate, in South Asia and Southeast Asia across five countries[16]. The observations that the NiV_M and NiV_B strains reportedly display differences in case fatality rates and human-to-human transmission are interesting as there is 91.8% nucleotide homology between the genomes[17].

HeV and NiV are classified as Biosafety Level (BSL)-4 pathogens because of the high mortality rates associated with infection, the lack of effective medical countermeasures, and the ease of transmission. In addition to causing morbidity and mortality as a naturally acquired infection, HeV and NiV are also categorized as Category C priority pathogens by several US Government agencies because of the concern for deliberate misuse. Along this line NIV_M and NiV_B cause lethal infection in nonhuman primates (NHPs) when delivered by small particle aerosol[18,19]. Importantly, NiV and henipaviral diseases were recently included on the World Health Organization's (WHO) Blueprint List of Priority Pathogens[20] while NiV was also included on the Coalition for Epidemic Preparedness Innovations (CEPI) list of Priority Diseases[21]. As a result of the unprecedented global pandemic of COVID-19 there is heightened concern regarding respiratory pathogens. Consequently, in March of 2020 the US CDC recommended that NiV be added to the list of Tier 1 Select Agents[22]. Characteristics of NiV enhancing its global pandemic potential include: humans are susceptible; NiV is capable of person-to-person transmission; it is an RNA virus with potential to mutate, and if a human-adapted strain were to infect communities in South Asia, high population densities and global interconnectedness would rapidly spread the infection[23].

Currently, there are no approved human vaccines or therapeutics for NiV or HeV disease. While substantial progress has been made in developing preventive vaccines against NiV and HeV[24-29], with a soluble glycoprotein-based vaccine in Phase I clinical trials[30], the development of postexposure treatments has lagged behind. A variety of antiviral therapies have been explored to combat NiV and HeV infection with some assessed in animal challenge models, including ribavirin, chloroquine, polyIC12U, and heptad peptide fusion inhibitors but all with none to limited effectiveness[31]. The most effective postexposure treatments identified to date that have been assessed in the gold standard African green monkey (AGM) models have been remdesivir[32] and the human monoclonal antibody (mAb) m102.4[33-35]. Remdesivir was shown to completely protect AGMs from lethal NiV_B disease when treatment was initiated 24 hours after virus exposure and before the onset of viremia or clinical disease. The human mAb m102.4, which targets the henipavirus G glycoprotein, has shown more promise in the AGM model. Specifically, m102.4 completely protected AGMs against HeV or NiV_B when treatment was initiated beginning 3 days after virus exposure[33,35] and against NiV_M when treatment was initiated as late as 5 days after virus exposure[34]. In addition, m102.4 has also been employed under compassionate use protocols to treat HeV patients in Australia and a potential exposure in the US[30]. However, while m102.4 completely protected AGMs when initiated beginning 5 days after exposure to NiV$_M$ it was unable to protect any AGMs when initiated beginning 5 days after exposure to NiV$_B$[35] supporting views that NiV$_B$ is more pathogenic in humans and AGMs. Importantly, while NiV$_M$ causes disease and lethality in AGMs it is not uniformly lethal while NiV$_B$ causes uniform lethality in AGMs under the same test conditions[35]. A current major gap in preclinical postexposure treatment studies is the lack of any antiviral modality to show protective efficacy in animal models when treatment is initiated at the onset of clinical signs for HeV and NiV$_B$ or at more advanced stages of clinical illness.

Henipaviruses have a negative sense, single-stranded RNA genome that is 18,234 nucleotides in length for HeV[36], 18,246 nucleotides in length for NiV$_M$[37] or 18,252 nucleotides in length for NiV$_B$[17]. The genome consists of six genes, namely, N, P, M, F, G and L[36,37]. The N gene encodes the nucleoprotein that is essential for genome replication and packaging[38]. The P gene encodes the phosphoprotein (P), which is essential for genome replication[38], and three additional proteins termed C, V, and W that appear to have pathogenic roles[39]. The M gene encodes the matrix protein that is required for viral particle formation and budding[40,41]. The F gene encodes the fusion protein that allows for fusion of the viral and host cell membranes in viral entry[42]. The G gene encodes the glycoprotein, which is the viral attachment protein that recognizes the host ephrin-B2 and ephrin-B3 receptors expressed on a variety of cell types including endothelial cells, neurons, pulmonary alveolar Type II epithelial cells, and dendritic cells that are highly conserved among many mammalian species[43-45]. The L gene encodes the large viral RNA-dependent RNA polymerase[9,37]. All of these genes and gene products serve as potential targets for therapeutic interventions. This proposal focuses on developing antivirals that can provide broad protection against all medically relevant henipaviruses by 1) developing and optimizing small interfering RNAs (siRNA) directed against genes important for henipavirus replication and assembly; 2) developing and optimizing small molecules inhibitors of the henipavirus replication complex; and 3) combining the most promising antivirals for improved antiviral efficacy over that seen with an individual therapeutic.

(b) Innovation.

siRNA and LNP. siRNAs are powerful, sequence-specific reagents able to suppress gene expression through the process of RNAi. The siRNA approach described in this proposal provides a significant new antiviral strategy that enables targeting of multiple viral genomes and virally-derived mRNA molecules. Development and optimization of this technology has the potential to lead to an effective "one-stop" treatment against NiV and HeV. Genevant's liver targeting LNP platform is a clinically validated technology that enabled the FDA approval of the first RNAi drug (Onpattro™)[46]. They have shown promising results in clinical trials targeting hepatitis B virus, and more recently been successfully deployed in clinical trials for mRNA-LNP vaccines designed against SARS-CoV-2 with Pfizer recently obtaining US FDA licensure. Moreover, in a previous collaboration with UTMB Genevant (formerly Arbutus) developed LNP-siRNA therapeutics that demonstrated complete protection against Ebola, Sudan, and Marburg viruses in guinea pigs and NHPs[47-53]. As with this proposal, that work began with siRNA screening and lead identification, progressing through to proof of concept studies in NHPs during a 5 year term. siRNAs have also shown *in vitro* efficacy against henipaviruses[54,55]. Together, these results strongly support the applicability of an siRNA-LNP approach to addressing pathogenic henipaviruses.

siRNA Delivery. Genevant's technology platform is based on specialized lipid nanoparticles (LNP) capable of efficient encapsulation and delivery of nucleic acids (**Figure 1**). LNP are comprised of a proprietary combination of various lipid components and novel cationic lipids[56]. The spontaneous vesicle formation process is highly efficient, and typically 90-95% of the siRNA are encapsulated within these lipid particles. The resulting LNP are filter-sterilized, and ready-for-injection vials can be stored refrigerated for many months. LNP particles are 50-90 nm in diameter and serum stable, rendering them suitable for parenteral administration. In addition, the feasibility of pulmonary delivery of nucleic acid payload with LNP has been demonstrated by our group and others[57-59]. The small size of LNP allows them to be entrapped in aerosolized droplets that can easily be deposited deep in the lungs. Thus, LNP provide a means of transporting nucleic acid drugs to the appropriate target tissues and target cells in a much more efficient and pharmacologically active manner than is achieved by administering the nucleic acid alone. It does this by: 1) providing protection from nuclease degradation in the bloodstream, 2) permitting administration via several different routes; 3) allowing accumulation in target tissues and 4) providing a means of siRNA entry into the cellular cytoplasm for RNAi activity. The LNP delivery platform

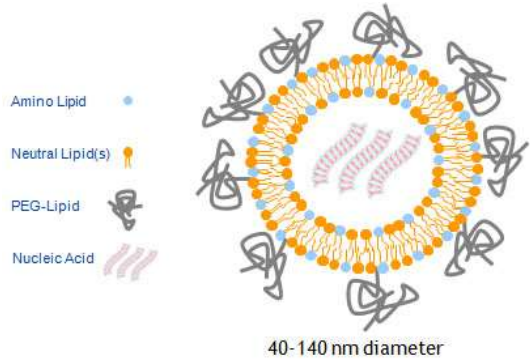


Figure 1. Schematic of LNP.

can be readily adapted as needed to target new or divergent agents by incorporation of different siRNAs, and the delivery of multiple siRNAs (a "cocktail") within a single LNP is a powerful tool for targeting different critical nodes within a single disease or to enable broad spectrum antiviral activity.

Henipaviruses have a broad cellular tropism. The early targets of NiV and HeV infection are respiratory epithelial and endothelial cells[60,61]. Multiple sources of data indicate that LNP are effectively delivered to these cells in preclinical species[57-59]. Genevant has demonstrated that LNP lipid components can be designed and combined in a rational manner to modulate the pharmacology of the resulting particle, allowing optimization of formulations for new applications[62,63]. LNP pharmacology is particularly influenced by the polyethylene glycol (PEG)-lipid and cationic lipid structures and content. Changes to the LNP bilayer have provided up to 10-fold improved target knockdown in rodents and NHPs[64]. Engineering of lipid components should also allow redirection of LNP to other cells, including extra hepatic. Genevant has already developed LNP formulations that can withstand nebulization for delivery to the airway epithelium, and further optimization should enhance delivery efficiency. **Immunostimulation.** Many types of nucleic acids, including siRNAs, are potent activators of the mammalian immune system[65]. Immune activation represents an undesirable side effect due to the toxicities induced by unwarranted cytokine expression and inflammation. The potential of siRNA-based drugs to activate the immune system and elicit downstream effects such as antibody responses is an area of concern in the evaluation of both safety and efficacy. These responses to nucleic acids may further be amplified through the use of delivery vehicles that facilitate the cellular uptake of the siRNA (such as LNP). Genevant's work developing siRNAs for therapeutic applications has shown that siRNA chemical modifications confer nuclease resistance and prevent recognition of siRNA by the innate immune system[66]. These chemically modified siRNAs have improved tolerability and reduced off-target effects (relative to siRNAs composed of native RNA chemistry) in an animal model of hepatitis B infection[67]. Genevant's "minimal modification" strategy involves the incorporation of 2'-OMe nucleotides into siRNAs - this approach has been demonstrated to abrogate the induction of the immune response and associated toxicities in primary human cell cultures, mice and NHPs, indicating the preservation of the inhibitory mechanism across species[68].

Preliminary studies.

b.1 Design of Specific siRNAs Using Bioinformatic Algorithms. Genevant is experienced in designing active and specific siRNAs for targets using the Whitehead site (http://jura.wi.mit.edu/bioc/siRNAext/) based on thermodynamic criteria, known to be a major factor in siRNA efficacy. We have successfully designed siRNAs targeting both endogenous and viral genes using this method[50-54,69,70]. Recently, we have improved our design by adopting a proprietary bioinformatic algorithm with more advanced off-target analysis for higher specificity. This algorithm allows off-target sequence alignment with all known transcripts for a given species. It also compares the seed region of siRNAs with known microRNAs for evaluating potential microRNA-like off-target effects. In addition, single nucleotide polymorphism (SNP) will also be investigated for each of the nucleotide in the siRNA to estimate potential loss of activity risk. Using this algorithm, we have successfully identified active siRNAs *in vivo* for multiple programs. In this research project, we will apply our siRNA design experience to identify specific siRNA candidates for NiV and HeV.

b.2 *In vitro Screen* of Virus Targeting siRNA with Dual Luciferase Reporter (DLR) Assay. *In vitro* screening of antiviral siRNAs is challenging with live viral infection models, particularly for viruses in Risk Groups 3 or 4 that requires specialized facilities for handling. The DLR assay has been identified as an effective strategy to enable early-stage siRNA screening that mitigates this risk. This nonviral screening system involves transfection of plasmid vectors for firefly and *Renilla* luciferase. The viral gene fragments containing the siRNA targeting sites will be inserted between the two luciferase genes. siRNA knock down of target mRNA is reflected by a concomitant decrease in the (downstream) *Renilla* luciferase luminescence, which is normalized to luminescence produced by firefly luciferase (itself a measure for cell transfection efficiency). Genevant harnesses this assay for multiple antiviral siRNA screen efforts such as HBV, Ebola, Marburg, etc. In this research proposal, we will synthesis luciferase reporter constructs containing the NiV and HeV viral gene targets and screen designed anti-NiV/HeV siRNAs using DLR assay.

b.3 Delivery of Luciferase mRNA LNP via Aerosolization. The aerosolized approach is complicated by the mucosal barrier of the respiratory epithelium, which facilitates ciliary clearance of foreign particulates. LNP typically comprise four lipid components: an ionizable lipid, a PEG-conjugated lipid, cholesterol, and a phospholipid. To identify an initial LNP for delivery to lung epithelium, we employed a nucleic acid reporter payload (luciferase mRNA) and tested different combinations of each for their ability to withstand nebulization (PennCentury microsprayer) and impart functional delivery and expression of the nucleic acid payload. Luciferase biodistribution was visualized by luminescence imaging (**Figure 2**) and activity was assessed with a luciferase enzymatic assay (**Figure 3**). We found that variations in the identity and proportions of the lipid

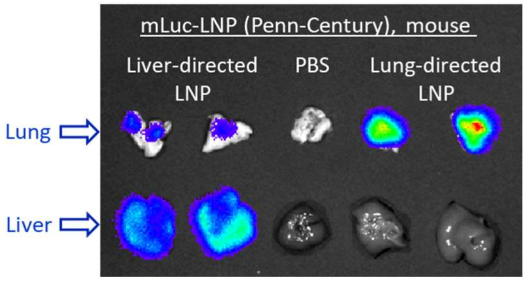


Figure 2. *Ex vivo* imaging of tissues from animals treated with PBS, a lung-directed, or liver-directed LNP.

components strongly influence the ability to mediate delivery. LNP F demonstrated localized expression in the lung, with high luciferase activity in this target organ compared to the liver. In later studies, we moved from intratracheal (i.t.) delivery to nebulization (Aerogen Aeroneb Solo device). Nebulizers are commonly used for the treatment of respiratory diseases because of their ability to disperse drug formulations into small aerosol droplets that are inhaled into the airway through a mouthpiece. Animals in these studies were exposed to LNP in a nebulization chamber. Luciferase expression was predominantly reported in the lung, whereas i.t. administration (Penn Century) often resulted in expression in the airway bifurcation. IVIS whole body imaging showed localized distribution of luciferase in the lung following nebulization (**Figure 4**). Although bioluminescence differed visually between left lung (1 lobe) and right lung (4 lobes), we confirmed that both lungs have similar luciferase activity. Furthermore, we hypothesized that rapid clearance would improve tolerability profile of the LNP formulation. Thus, we designed and used biodegradable lipids with structural motifs that are rapidly hydrolyzed to facilitate rapid clearance. As confirmed by Mass Spectrometry (MS) analysis, very low levels of the parent ionizable lipid were present in the lungs at 6h and 24h post-nebulization (**Figure 5**).

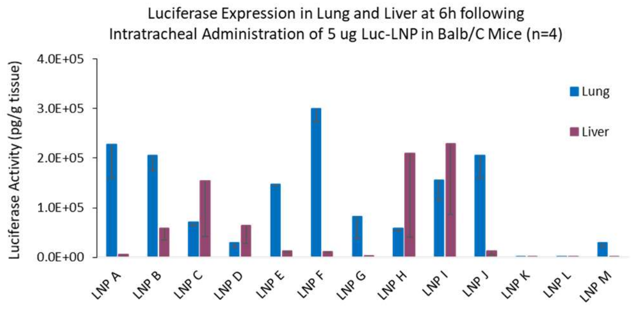


Figure 3. Example of a formulation screen in naïve mice.

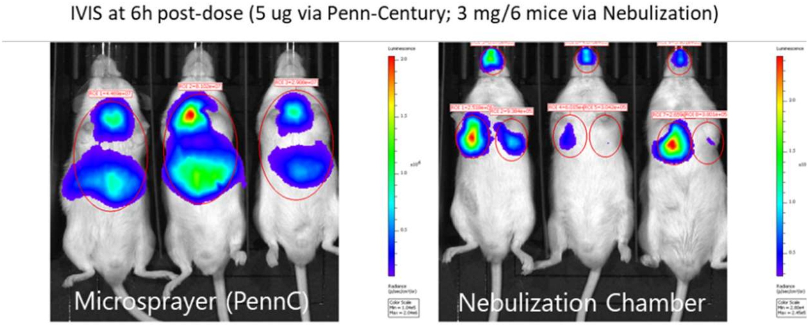


Figure 4. IVIS whole body imaging of mice at 6h following aerosolized delivery of luciferase mRNA LNP.

Delivery of siRNA LNP via intravenous administration to target specific cell types in the liver. Although our work with lung delivery has been focused on delivery to the lung epithelium, we have demonstrated in another application that LNPs can be designed to selectively target different cell types in the liver following intravenous (i.v.) administration. As an example, we have developed LNPs that target hepatic stellate cells (HSCs) in the liver and demonstrated that they accumulate to a much lesser degree in hepatocytes (the usual target of LNP in the liver). A sustained 12-month screening effort of our proprietary lipids and compositions resulted in the identification of LNP M3, a considerably more effective formulation for silencing the HSC-specific gene target RELN. It showed minimal activity against hepatocyte (TTR) or endothelial cell (TIE-2) targets (**Figure 6**). Conversely, LNP A is far more effective for targeting hepatocytes and LNP G was effective against all 3 cell-specific gene targets. A similar screening approach could be used to identify LNP compositions that target the lung endothelium, using TIE-2 siRNA.

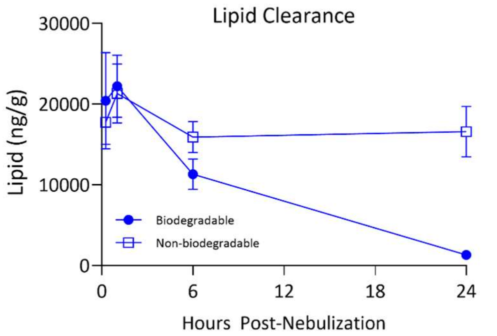


Figure 5. Clearance of parent ionizable lipid following aerosolized delivery to mice.

b.4. Antiviral drug discovery. Antiviral drug discovery for HeV and NiV has been significantly hampered due to requirements of BSL-4 containment. However, the recently discovered Cedar virus (CedV), which shares significant homology to both NiV and HeV and the third member of the *Henipavirus* genus, has allowed us to circumvent the BSL-4 requirement for henipavirus drug discovery, because CedV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease, and thus designated as a BSL-2 agent. We have recently optimized a recombinant CedV encoding luciferase (rCedV-Luc) for high-throughput screening (HTS) applications and performed a pilot HTS to identify small molecule inhibitors using the recombinant CedV[71]. Importantly, several small molecule inhibitors (**Figure 7**) have been validated *in vitro* using infectious HeV and NiV in the BSL-4 facility, demonstrating the proof-of-concept feasibility of using this CedV-based surrogate HTS platform for inhibitor screening against HeV and NiV, and providing a strong foundation for the current drug discovery project. Particularly, our furopyrimidine-based hit **Z2** was found to have an EC_{50} value of 0.5 µM. In Table 2 are shown additional inhibitors in the furopyrimidine series that we identified from a focused screening campaign, which allowed us to develop some initial structure-activity relationships (SARs). In the Research Plan we discuss methods for the preparation of new compounds in each series as well as specific plans for developing the SARs. Our recombinant CedV HTS system will be used to support Aim 2.

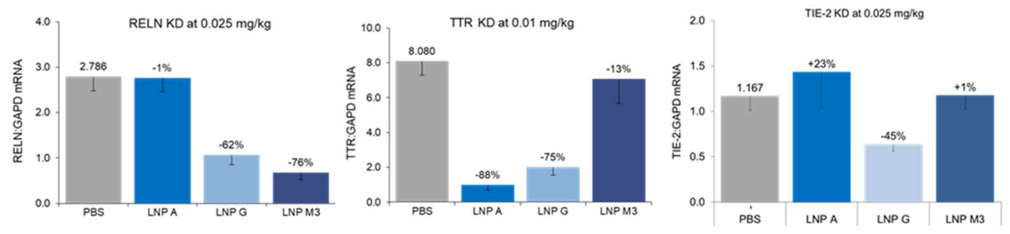


Figure 6. Gene silencing in different cell types following IV administration of LNPs.

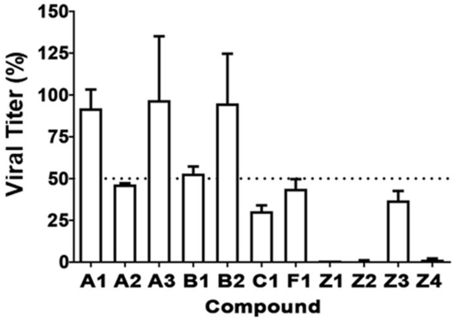


Figure 7. Inhibition of NiV_B replication Vero 76 cells measured by virus titer reduction assay (% infection relative to the DMSO control).

Table 1. Validation of rCedV-based HTS assay hits for inhibition of NiV_B.

Hit	CedV (293T)		CedV (Vero)		NiV_B (Vero)
	IC_{50} (µM)	CC_{50} (µM)	IC_{50} (µM)	CC_{50} (µM)	IC_{50}(µM)
Z1	0.70	100	2.1	100	3.3
Z2	0.78	37	13.2	100	0.5

b.5. Combination therapy. Combining antiviral drugs with different mechanisms of action is one possible strategy for limiting drug resistance and may have synergistic or additive effects that can improve outcome. Combination therapies became a standard of care for treating HIV and HCV infections and have been more commonly used to treat chronic viral infection. However, we recently showed that a combination of a polymerase inhibitor (remdesivir) with a human mAb targeting a surface glycoprotein had a substantial and statistically significant effect in extending the therapeutic window for treated Marburg virus infection of NHPs[72]. In brief, rhesus monkeys are protected against lethal Marburg virus infection when treatment with either a human mAb (MR186-YTE; 100%), or remdesivir (80%), is initiated 5 days post-inoculation (dpi) with Marburg while no animals survive when either treatment is initiated alone beginning 6 dpi. However, by combining MR186-YTE with remdesivir beginning 6 dpi, significant protection (80%) is achieved, thereby extending the therapeutic window. This new data shows that combination therapy may also have utility against acute viral infections.

(c) Approach

Research Strategy:

Respiratory epithelial cells, endothelial cells, and neurons are important cellular targets of NiV and HeV[73-88]. In addition, dendritic cells and macrophages can be infected and disseminate henipaviruses[79,86]. Temporal studies in animal models show that henipaviruses initially infect respiratory epithelium, spread to other cells such as endothelial cells, and eventually to neurons[75,76]. Once henipaviruses infect neurons therapeutic intervention is difficult. A goal of Aim 1 is to develop siRNAs that can target henipavirus genes associated with replication and assembly and deliver these siRNAs to primary cellular targets in order to slow the ability of henipaviruses to gain access to the CNS and improve survival. In addition to liver-based targets and indications, Genevant has been working to expand LNP delivery to extrahepatic tissues, including lung. Here we will seek to apply these advances, identifying potent and cross-species active NiV/HeV targeting siRNAs for pulmonary delivery to both endothelium and epithelium. Aim 2 is focused on replication complex inhibitors. Some of these inhibitors will be developed within this project while other antivirals will be provided by Arbutus and Gilead. Currently, Arbutus has actively engaged in the discovery and development of broad spectrum small-molecule inhibitors against the RNA-dependent RNA polymerase of coronaviruses, including that of SARS-CoV-2. Since the active site of these viral enzymes can be highly conserved, antiviral compounds discovered from this program may also exhibit inhibitory effect across multiple RNA viruses. Representative compounds from the different sub-series will be evaluated by the HTS Core for antiviral effect against henipaviruses. Confirmed hits will be elaborated to develop SAR, with assistance from structure-based computational modeling. Lead compounds with promising antiviral and pharmacokinetics profiles will be progressed into testing against viral infection in animal models to determine *in vivo* efficacy.

Aim 1. Develop and optimize siRNA for protection against Hendra and Nipah viruses. We have three Subaims under Aim 1 that together are focused on the development of broad spectrum, non-immunostimulatory siRNAs targeting NiV and HeV genes associated with replication and assembly. They will be encapsulated in a single LNP to form a postexposure therapeutic and tested in hamsters and NHPs for protection against NiV and HeV. We will first identify the most efficacious siRNAs against four different genes (L, N, P, M) of one or more NiV and/or HeV strains (**Subaim 1.1**). These will subsequently be combined into a tri- or quadripartite siRNA cocktail to confer activity against potential mutations of the virus. siRNAs displaying the strongest anti-NiV/HeV activity will be incorporated into selected novel LNP to enable their delivery to the lung endothelium and/or epithelium. In parallel, we will select and formulate a shortlist of novel lipid compositions for lung delivery (both intravenously to endothelium and aerosolized to the epithelium) (**Subaim 1.2**). Emphasis will be placed on those with proven high potency in other applications and biodegradable functionality to provide the best tolerability profile. LNP will be screened in naïve rodents using model siRNA (TIE2 for endothelium, e-Cadherin for epithelium). The best LNP will be combined with the NiV/HeV siRNA from Subaim1.1 and assessed in hamsters and NHPs challenged with NiV and HeV (**Subaim 1.3**).

1.1. Design and *In vitro* screening of siRNAs targeting NiV/HeV L, N, P and M proteins. The goal of Aim 1 is to design, screen, and select lead siRNAs targeting NiV and HeV viral genes. siRNAs targeting each viral gene will be designed using bioinformatic algorithms and screened through *in vitro* dual luciferase reporter (DLR) assays. 2’-OMe chemical modifications will be applied to the lead siRNA candidates to mitigate immunostimulatory effect while maintain the gene silencing potency. siRNAs targeting the conserved regions of viral genes will be prioritized for candidates down selection. For viral genes with short sequence length and low homology between NiV and HeV or among different viral strains in the same species, siRNAs targeting one or more dominant strains will also be screened as potential cocktail component. The viral gene targets we selected include the L pol (L), which provides the RNA-dependent RNA polymerase activity of the polymerase complex, and which is necessary for all viral RNA synthesis. The second target is the nucleocapsid (N) protein that facilitates the functional transition of the viral polymerase from a viral transcriptase to a viral replicase during viral replication. In addition, the polymerase cofactor phosphoprotein (P) and virion budding coordinator matrix protein (M) are also promising anti-NiV/HeV targets. ***Design of siRNAs targeting NiV/HeV L, N, P and M proteins.*** Using proprietary bioinformatic algorithms, siRNA will be designed targeting NiV/HeV L, N, P and M genes. Viral gene sequences of all clinically relevant NiV and HeV species will be obtained from NCBI Entrez viral genome sequence databases. If available, conserved regions of each viral gene will be identified for siRNA design. For viral genes with low cross-species homology, the sequence from dominant clinic species will be used. The siRNA targeting specificity will be assessed by aligning with human, NHP and rodent transcriptomes and seed sequence comparison with known human, NHP and rodent microRNAs. Specificity of both sense and antisense strand will be analyzed. ***In vitro screening of unmodified NiV/HeV siRNA.*** For each gene target, a pool of 48 of bioinformatically designed siRNA candidates with high specificity will be synthesized for *in vitro* activity screening using DLR assay. This nonviral screening system involves transfection of plasmid vectors for firefly and *Renilla* luciferase. The viral gene fragments containing the siRNA targeting sites will be inserted at the 3’ end of *Renilla* luciferase gene. siRNA knock down of target mRNA is reflected by a decrease in *Renilla* luciferase luminescence, which is normalized to luminescence produced by firefly luciferase (itself a measure for cell transfection efficiency). Two rounds of screening will be performed, including a 2-concentration initial screen for all candidates and a subsequent 10-concentration dose response comparison for the top 12 candidates. The most active siRNA candidates will be selected for chemical modification optimization. ***Chemical modification optimization for lead siRNA.*** Through our research at Genevant, we have determined that 2′-OMe modifications to both sense and antisense strands yields viable and effective siRNAs without any induced toxicity, cytokine induction or off-target effects[59,62]. In this Aim, we will modify 3-6 best unmodified siRNA performers (Subaim 1-2) for each viral gene. For each parental siRNA sequence, 5-10 different modification patterns will be tested. The activity of modified siRNAs will be tested using DLR assay. The suppression effect on immune stimulatory will be verified using human whole blood assay. The siRNAs (single siRNA or siRNA cocktails) will be formulated into the lung targeting LNP and incubated with heparinised human whole blood for 24 h, followed by plasma isolation. Cytokine and/or chemokine levels will be quantitated using commercially available assays. In addition to assessments in human whole blood, modified siRNAs will also be administered to ICR mice and assessed for the production of IFN-α and IFN-β and IFN-induced protein with tetratricopeptide repeats (IFIT1) mRNA as has been done previously[51]. ***Efficacy testing of siRNA candidates against live henipavirus infection.*** Antiviral activity of the LNP-encapsulated siRNAs will be evaluated against live NiV_B, NiV_M, and HeV infection. In brief, human airway epithelial and endothelial cells or Vero cells will be infected with different strains of NiV and HeV as described previously[80,83,85,88]. One hour post-infection, cells will be treated with individual anti-NiV/HeV siRNAs. Culture fluids will be harvested at various times after challenge (e.g., 24, 48, 72, 96 hours) and assayed for the presence of infectious NiV and HeV by conventional plaque assay and by quantitative RT-PCR[73,74]. Non-targeting luciferase siRNA will be used as a control in all assays.

1.2. Identify novel LNP compositions for lung specific-siRNA delivery in naïve mice. In parallel with Subaim 1-1, we will screen and select one or more LNP lipid compositions with potent and specific delivery to the lung endothelium and/or epithelium in naïve mice. Through our previous research, we have demonstrated functional nucleic delivery (luciferase mRNA) to the lung epithelium via nebulization, with minimal expression in the liver. Our current benchmark lung formulation exhibits rapid clearance and great stability, withstanding shear forces generated during aerosolization. Furthermore, we have demonstrated the ability to design LNP compositions that specifically target different cell types in the liver following intravenous administration. We will leverage this combined experience to identify a potent LNP formulation for i.v. delivery of siRNA to lung endothelium, and/or for aerosolized delivery to the lung epithelium. Genevant has designed a library of advanced lipid components that have been engineered to clear shortly after administration. This biodegradable functionality results in improved tolerability, while maintaining high potency *in vivo*. Initial screening of these novel lipids for lung specific delivery will utilize model siRNAs (e.g., TIE-2 to target endothelial cells and CDH1 to target epithelial cells) and will take place in a naïve mouse model. ***Screen novel lipids for lung siRNA delivery in naïve mice.*** The goal is to design, screen, and select novel lipids and LNP compositions to be used for delivery of a cocktail of model siRNAs (e.g., TIE-2 and CDH1) to the lung endothelium via i.v. administration, or to the lung epithelium via aerosolized delivery. Initial screening of these novel LNP will take place in a single dose naïve mouse model and will comprise four rounds of LNP screening; each round will consist of PBS + 9 novel LNPs. Since there are two routes of administration, the same 9 LNP formulations in each round of screening (encapsulating both TIE-2 and CDH1) will be administered i.v. (in one study) and aerosolized (in a separate study). In total eight mouse studies conducted in this phase. LNP specificity will be evaluated using model siRNA specific to endothelial cells (e.g., TIE-2) or epithelial cells (e.g., CDH1). Activity of these formulations in the lung tissue (versus other tissues) will be determined by measuring mRNA knockdown using the QuantiGene 2.0 Assay (QG 2.0) assay. After four rounds of screening, the top 5 LNP compositions will advance to a dose response study (at 3 dose levels) to rank potency and assess tolerability. Tolerability of these formulations will be assessed by cytokine (e.g., MCP-1, IL-6) ELISA assays and clinical pathology analysis. The top three formulations will advance to the next phase based on their activity and tolerability profiles. ***Dose response/tolerability studies.*** The three lead LNP candidates selected from the initial LNP screens will be evaluated for dose responsiveness and tolerability at three dose levels. Activity will be measured by mRNA knockdown and tolerability will be assessed by clinical pathology, cytokine induction, and anti-PEG antibody responses. ***Duration of action/repeat dose tolerability studies.*** The three lead LNP candidates selected from the initial LNP screens will also be evaluated for duration of activity and repeat dose tolerability at an appropriate dose level selected from the dose response study above. Duration of activity (measured by mRNA knockdown over a time course) will be determined following single dose i.v. administration or aerosolization in naïve mice. Repeat dose tolerability will be assessed by clinical pathology, cytokine induction, and anti-PEG antibody responses. Rate of lipid clearance/accumulation will also be determined in this study. Frequency of siRNA LNP administration will depend on duration of activity results and will provide informative data for subsequent studies in viral challenge models where LNPs may require repeat dose administration. The best formulations (based on their activity and tolerability profiles) will advance to further assessment in naïve hamsters. ***Evaluate translation of results in naive hamster model.*** To ensure translation of results from the mouse to hamster model, we will perform a final LNP evaluation of the top three LNP candidates in naïve hamsters. Based on the activity and tolerability results in naïve hamsters, three lung targeted LNP compositions will be selected and combined with lead siRNAs (Subaim 1-1) for evaluation in the HeV and NiV hamster models at UTMB in the **Geisbert Lab** (Subaim 1-3).

1.3. Assessing anti-NiV/HeV activity of LNP-siRNA cocktail therapeutics in preclinical species. Lead siRNA sequences and LNP compositions resulting from Subaims 1 and 2 will be tested for efficacy in a series of hamster viral challenge studies against NiV and HeV. Cocktails of different siRNA candidates will be formulated to lead LNP compositions. Infected hamsters will receive treatment of LNP-siRNA cocktails via i.v. injection and/or aerosol delivery, starting at different time points post-infection to assess protective efficacy. ***In vivo* testing of anti-NiV/HeV LNP-siRNA cocktails in NiV/HeV infected hamsters.** Cocktails of chemically optimized siRNA leads from Subaim 1-1 will be formulated to LNP composition leads from Aim 1-2. The *in vivo* activity of these LNP-siRNAs will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) and intranasally (i.n.) inoculated with ~ 5x10^6 PFU of NiV_B or HeV[75,89-91]. Animals will then be treated with anti-NiV/HeV LNP-siRNAs by i.v. injection (starting at 1 mg/kg) and/or aerosol delivery, beginning ~ 1 hour after virus challenge, and continuing daily for up to 6 days after exposure. The control animals will be treated in parallel with an equivalent dose of a non-targeting Luciferase LNP-siRNA. siRNA antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. siRNA candidates showing the most protection will be selected for assessment in NHPs. ***In vivo* testing of anti-NiV/HeV LNP-siRNA cocktails in NiV/HeV infected nonhuman primates.** A final proof of concept study will be performed in NHPs using the LNP-siRNA candidates providing best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated i.t. and i.n. with a uniformly lethal challenge dose (~ $2.5x10^5$ PFU per route, ~ $5x10^5$ PFU total dose) of NiV or HeV as described previously[29,35]. The treatment groups (consisting of five animals each) will be treated with an anti-NiV/HeV LNP-siRNA candidate (1 mg/kg) by bolus i.v. infusion up to 5 days after viral challenge (initiation time informed by hamster studies) while a control animal will receive an equivalent dose of non-targeting Luciferase LNP-siRNA. Animals will receive six additional daily treatments of the anti-NiV/HeV LNP-siRNAs. The siRNA antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

Aim 2. Develop and optimize replication complex inhibitors against Hendra and Nipah viruses. Toward this Aim we will 1) further develop remdesivir in NHPs; 2) perform large scale screening of small molecule libraries (up to 100,000 compounds) using an optimized HTS platform; 3) confirm, analyze, and validate hit compounds with infectious HeV and NiV *in vitro*, and rank according to their antiviral potency and cytotoxicity; 4) chemically optimize the lead compounds and construct useful Structure-Activity Relationships (SAR); 5) evaluate the most potent lead compounds with infectious HeV and NiV both *in vitro* (5-10) and *in vivo* (2-5); 6) perform mechanism of action (MOA) studies to determine the targets of these lead compounds using an integral approach of biochemical, biophysical, biological, and structural techniques; and 7) identify the best lead compounds with good oral bioavailability as candidates for an IND study. In addition, antivirals from Arbutus and Gilead funneled in from the HTS Core will be assessed *in vitro* and *in vivo* against infectious HeV and NiV.

2.1. Optimization of remdesivir in AGMs. A previous study showed that administration of remdesivir beginning one day after exposure of AGMs to NiV_B completely protected the animals against lethal disease[32]. While the results of this work are encouraging the treatment was initiated very shortly after infection before any animals showed clinical signs of illness. The goal of this Subaim is to better define the therapeutic window of remdesivir in AGMs, i.e., can remdesivir still protect AGMs against henipavirus disease if administered at or after the onset of clinical illness? A group of six AGMs will be challenged by combined i.t. and i.n. routes with a lethal challenge dose (~ $2.5x10^5$ PFU per route, ~ $5x10^5$ PFU total dose) of a low passage (p2) NiV_B isolate as previously described[29,35]. At day 4 after NiV_B infection (when AGMs begin to show signs of clinical disease) five of the animals will be given a 10 mg/kg i.v. loading dose of remdesivir, followed by 5 mg/kg i.v. daily maintenance doses 5-15 dpi, for a total of 12 consecutive days of treatment, as previously described[32] while a control AGM will be treated in parallel with sterile saline. Based on the results, the study will be repeated to further define the therapeutic window, e.g., if all five animals treated with remdesivir survive the follow up study will begin treatment at day 5 after NiV_B infection. Conversely, if any of the remdesivir treated AGMs succumb the follow up study will begin treatment at day 3 after NiV_B infection. A third study will be done to assess the protective efficacy of remdesivir for treating HeV-infected AGMs; test conditions for the HeV study will be the same as the test conditions showing the best protection against NiV_B. Defining the therapeutic window is not only important in terms of translating preclinical animal data to humans but also in regard to combination treatment studies where it is important to establish breakthrough for each individual drug to be used in combination approaches in order to be able to show a benefit of combining drugs.

2.2. HTS assay. We will employ our recently published optimized HTS protocol utilizing the recombinant CedV system[71]. Cells preseeded in a 96-well plate at a density of 2.5 x 10^4 cells per well will be treated with growth medium containing either DMSO or selected compound at predetermined nontoxic concentrations for 1 hour at 37 °C, 5% CO_2. Cells will be infected with rCedV at multiplicity of infection (MOI) of 0.01 and the plate incubated for 48 h. Luciferase activity will be examined by reading the luminescent signal using the ENVISION JANUS automated workstation (PerkinElmer, Waltham, MA) as per the manufacturer's instructions. This plate reader is integrated directly with the robotic deck so that readings can be made unattended. The data will be normalized by the average signal of the negative control wells in each plate. The best inhibitors will be evaluated in HEK293T cells and Vero cells in 96-well plates. Cells will be incubated with compounds at a 3-fold serial dilution ranging from 30 nM to 200 μM followed by the addition of rCedV-Luc (MOI: 0.01). After incubation at 37 °C for 48 h, luciferase activity will be examined by reading the luminescent signal as described above. Sample signals will be normalized by signals from the DMSO control wells. EC_{50} and CC_{50} values will be calculated by dose-response curve fitting with Graphpad prism. These assays will be conducted in **the Rong Lab.**

Screening Libraries. These libraries will be provided by the UIC Research Resource Center (RRC). Multiple ready-to-use chemical libraries to facilitate drug discovery and drug target identification are curated at the RRC. They are available in 96- or 384-well plate formats suitable for HTS.

AI-Aided Screens for Anti-Henipaviral (Nipah/Hendra) Drug Discovery In collaboration with the MCC at Southern Research, artificial intelligence (AI)-aided screens will be utilized for the efficient discovery of novel anti-henipaviral agents (**Figure 8**). Cheminformatics analysis will be performed on the UIC HTS library to remove the pan-assay interference compounds (PAINS) based on an in-house developed PAINS structure filter. A machine learning (ML) model will be used to perform AI-aided predictions of the phenotypical anti-henipaviral activity of non-PAINS compounds. Taking NiV as an example, an ML model (*via* BIOVIA Pipeline Pilot platform) will be constructed and trained with the already-available phenotypical anti-NiV data of 10K compounds from Southern Research database[92] as well as other anti-henipaviral compounds previously identified in PIs' labs[71]. Based on our experience of the successful development of a SARS-CoV-2 ML model using similar algorithm and data size (**Core Approach Section C1**), we expect a NiV ML model of at least 80% prediction accuracy to be developed. Subsequently, protein-structure-based virtual screens (VS) will be performed on the AI-predicted active compounds to evaluate their target specificity. For example, two of our top interests are the NiV G glycoprotein (G-gp) and polymerase. The G-gp VS will utilize a crystal structure of NiV G-gp[93] and a validated VS protocol that we previously applied in other antiviral projects[94]. The VS site will be defined as a pocket at the Ephrin-B3 (entry receptor) recognition region on G-gp that is suitable for small molecule binding. To tackle the lack of NiV and HeV polymerase crystal structures, the viral polymerase VS will utilize homology models built through an AI-aided protein structure prediction algorithm[95,96]. Both the RNA binding cleft and allosteric binding regions will be defined as VS sites. Finally, the library for actual HTS will be tailored down to 10K−100K according to both the ML and VS predictions. Overall, the AI-aided screens would significantly increase the HTS hit rate of novel antiviral chemical matters that are both phenotypically potent and target-specific.

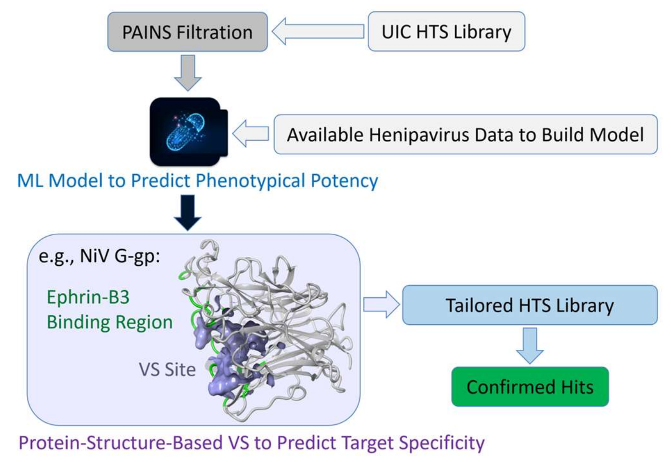


Figure 8. Workflow of AI-aided screens

There are several possible outcomes from the proposed HTS: 1) the majority of the compounds do not exert any inhibition on rCedPV infection. That is, the target cells infected with the virions in the presence of these compounds give the same levels of luciferase activity as the no compound control (DMSO). These compounds will not be further considered; 2) some compounds may enhance entry and infection (Higher Luc). These compounds will be noted but will not be characterized further since they are not the focus of this project. Nevertheless, we realize that these compounds may have added value in elucidating further details of the entry and replication of henipaviruses; 3) some compounds can "inhibit" and these compounds could be active during replication (post-entry), on the membrane fusion step during viral entry, or may be toxic to cells. These compounds will be the focus of this application, and their effects will be further evaluated.

Hit Selection Algorithm. A compound which displays more than 90% inhibition (one log decrease in the luciferase activity in the presence of the compound, 10 μM final concentration) will be considered a "hit" compound after the initial round of screening. To further determine the specificity, a secondary screening (counter screen) for the "hit" compounds will be performed, where the VSV-G pseudotyped HIV virions will be used again as a specificity control. We expect that the majority of the "hit" compounds will not inhibit the VSVG-mediated viral entry. The third round or "tertiary" screening will focus on a selected group of compounds, and here classical dose-dependent inhibition assays will be carried out to estimate EC_{50} values. Multiple cell lines will also be used in these experiments to avoid cell line bias. The compounds that do not exert effective inhibition (non-potent) will not be actively pursued, while the more potent compounds (potent inhibitor, EC_{50} less than 10 μM) will be analyzed further. It is expected that through multiple rounds of screening, we can select the most potent compounds for optimization and validation studies. **Hit Validation, Cytotoxicity Assessment and Ranking.** We will measure the direct toxicity of compounds to human cells to assess the overall cytotoxicity of the potent inhibitors. A variety of human cell lines, including HeLa, Jurkat, HepG2, Huh-7 and Hep3B, will be tested using the "CellTiter 96 aqueous nonradioactive cell proliferation assay" (Promega, Madison, WI) as described previously[97] in a 96-well format. The ratio of CC_{50}/EC_{50} is the selectivity index (SI). The results from the screening will be analyzed and the compounds ranked according to antiviral potency and cytotoxicity. **Hit Quality Control and Identity Assurance.** The most potent and selective compounds will be analyzed for chemical purity (HPLC) to confirm that the compounds have the expected structure (LC-MS, NMR spectroscopy). Compounds found to be <80% pure will be purified and retested; compounds found to have incorrect structures assigned will be corrected. We will eliminate compounds that are known PAINS[98] (e.g., rhodanines aralkyl pyrroles, hydroxyphenylhydrazones), or that have reactive functional groups (e.g., Michael acceptors, alkylating agents).

2.3. Evaluation of protective properties of the advanced candidates in the henipavirus infectious assays will be done as previously described[71]. Vero cells will be seeded in a 96-well plate at a density of 2 × 10^4 cells per well and incubated at 37 °C overnight. Cells will be incubated with a 3-fold dilution series of the selected compounds diluted in cell culture growth medium (concentrations ranging from 100 μM to 0.01 μM) with or without NiV and HeV (200 PFU/mL). Infected cells treated with DMSO will serve as controls. At 48 hpi, viral supernatant will be collected and titrated by plaque assay to determine infectious viral titer. Compound only cells will be examined microscopically for cytopathic effects (CPE) induced by compound treatment. The percent reduction in PFU/mL titers in the samples containing compounds will be compared to that from parallel cultures with virus and DMSO vehicle only. This work will be performed in the BSL-4 facility at UTMB by the **Geisbert Lab**.

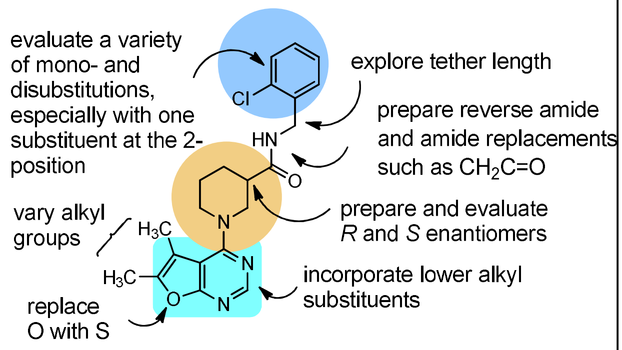


Figure 9. SAR development plan for furopyrimidine **Z2**.

2.4. Chemical optimization and SAR studies. In the initial stages, we will keep most of the molecule of interest constant while making well-defined changes in substituents. We will initially probe steric and electronic factors of the substituents, but more substantial changes in the scaffold will be investigated as the structure is optimized. By an iterative process of design, synthesis, and assay, we will improve the structure, and ultimately produce a fully optimized structure. We will also assess the cytotoxicity and construct Structure-Toxicity Relationships (STR). For potent, specific inhibitors, the SAR and STR will diverge at critical points, and we will synthesize novel inhibitors with maximized activity and minimized cytotoxicity. The SAR plan for optimization of the HTS hit Z2 is exemplified in **Figure 9**. Substituent changes on the core fused scaffold will involve variation of the alkyl groups on the furan ring, as well as adding alkyl groups at the 2-position of the pyrimidine ring, to explore the optimum overall size for the core. Note that we will be able to use these substituents as "ADME handles" to control the overall hydrophobicity of the molecules and the cLogP values. We will also replace the oxygen atom in the furan with a sulfur atom to compare the effect of changing the furan ring to the larger

Scheme 1. Preparation of analogs of furopyrimidine **Z2** with optimized amide *N*-substituent.

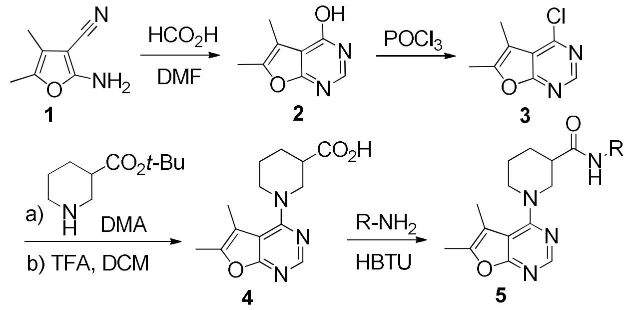
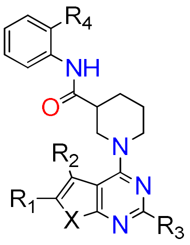


Table 2. Actelion OSIRIS calculated parameters for **Z2** (compound A) and proposed analogs.



ID	R_1	R_2	R_3	R_4	R_5	cLogP	Sol	MW	Druglike-ness	Drug Score
A	Me	Me	H	Cl	O	4.0	-6.7	384	-0.95	0.23
B	Me	Me	H	F	O	3.5	-6.3	368	-2.53	0.27
C	Me	Me	H	Cl	S	4.4	-6.8	400	2.01	0.32
D	Me	Me	H	F	S	3.9	-6.3	384	0.46	0.39
E	H	H	H	Cl	O	3.3	-6.0	356	3.04	0.42
F	H	H	H	F	O	2.8	-5.6	340	1.47	0.55
G	H	H	H	Cl	S	3.6	-6.0	372	3.70	0.41
H	H	H	H	F	S	3.1	-5.5	356	2.14	0.54
I	H	H	Me	F	O	3.0	-5.2	354	2.82	0.61
J	H	H	Me	F	S	3.3	-5.2	370	3.48	0.60

thiophene ring. Importantly, we will explore the biological activities of the *R*- and *S*-stereoisomers of this racemic molecule to determine whether there is differential recognition of these antipodes at the site of interaction in the inhibition assays. In addition to evaluating a variety of mono- and disubstituted aromatic rings, we will also explore length of the tether connecting these aromatic units to the amide nitrogen atom, knowing that both benzyl groups and phenyl groups give rise to active compounds.

The synthetic route that we envision for the preparation of the furopyrimidines is shown in Scheme 1, as illustrated for the optimization of the amide *N*-substituent for furopyrimidine analogs of compound **1**. Starting with the commercially available 2-amino-4,5-dimethyl-3-furancarbonitrile (**1**), treatment with formic acid in dimethylformamide will produce the 5,6-dimethylfuro[2,3-*d*]pyrimidine (**2**). Conversion of **2** to the corresponding chloro compound **3** is accomplished by treatment with phosphoryl chloride. Displacement of the chloro group with piperidine-3-carboxylic acid *t*-butyl ester, followed by removal of the *t*-butyl ester with trifluoroacetic acid, gives key intermediate **4**, which can then be elaborated to amides **5**[99]. In Table 2 is shown a set of proposed compounds related to **Z2** (compound **A**) for which we show parameters calculated using Actelion OSIRIS software[100]. All of the proposed compounds (**B** through **J**) show improved druglikeness and drug scores with respect to **Z2**, and therefore represent promising new targets for synthesis and evaluation.

2.5. *In vivo* testing of lead compounds NiV/HeV infected hamsters. The *in vivo* activity of the most potent compounds from 2.5 will be tested in a series of hamster infection studies. In brief, animals will be randomized to multiple study groups (n = 5 per group) inoculated i.n. with ~ 5x10^6 PFU of NiV_B or HeV[75,89-91]. Animals will then be treated by i.v. injection ~ 1 hour after virus challenge. Additional treatments may be given based on PK data. The control animals will be treated in parallel with sterile saline. Antiviral efficacy will be assessed by several methods, including weight loss, temperature, viremia (nasal swabs and blood), and survival. The treatment initiation time points will also be optimized in later studies. Antiviral candidates showing the most protection will be selected for assessment in NHPs.

2.6. *In vivo* testing of lead compounds in NiV/HeV infected nonhuman primates. A final proof of concept study will be performed in NHPs using the lead candidates providing best protection against NiV/HeV infection in hamsters. Healthy, NiV/HeV-seronegative AGMs will be inoculated i.t. and i.n. with a uniformly lethal challenge dose (~ $2.5\text{x}10^5$ PFU per route, ~ $5\text{x}10^5$ PFU total dose) of NiV or HeV as described previously[29,35]. The treatment groups (consisting of five animals each) will be treated with the lead antivirals by bolus i.v. infusion up to 5 days after viral challenge (initiation time informed by hamster studies) while a control animal will receive sterile saline. Additional treatments may be given. Antiviral efficacy will be assessed by several methods, including respiration quality, physical appearance, clinical pathology, viremia (nasal swabs and blood), and survival.

2.7. Mechanism of action (MOA) studies. Understanding the mechanism of action of the novel inhibitors of henipaviruses will help us to prioritize the lead compounds. The following experiments will be performed: 1) **Determine the impact of compound treatment on viral protein expression and viral RNA levels in rCedV infected cells.** Confluent HEK293T cells in a 6-well plate will be treated in duplicate with cell growth medium containing either DMSO or selected furopyrimidines at a predetermined concentration for 1 h, followed by infection with rCedV at MOI of 0.1. At 4, 8, 16 and 24 h post infection (hpi) cells will be collected to determine viral protein expression by standard western blot techniques or viral RNA levels by quantitative real time polymerase chain reaction assays (q-RT-PCR). Subsequent immunoblot with CedV specific antibodies against the attachment (G) and fusion (F) that were generated in the **Broder Lab**. In addition, antibodies to CedV-N and CedV-P will be utilized. To determine changes in viral protein expression at the different time points, densitometric counts of these bands will be calculated using the ImageJ Software and normalized against β-actin and calculated as a fold change compared to the infected untreated cells (DMSO control). Total RNA will be extracted and 500 ng of total DNase I digested RNA will be used in q-RT-PCR assays with CedV specific TaqMan primer/probe pair targeting the P gene. The one-step reaction will be performed using the Applied Biosystems (ABI) 7500 thermocycler. The ABI 7500 software will be used to analyze all q-RT-PCR results. Fold changes will be calculated relative to 18S using the ΔΔCt method and normalized to respective DMSO control samples. We expect to observe a decrease in intracellular viral RNA levels expression with compound treatment when compared to the DMSO control. Results from this data will indicate which viral protein(s) is affected by the compounds and the impact of the compounds on viral RNA levels over time; 2) **Determine the stage(s) of inhibition of furopyrimidines (and quinazolines) in the viral lifecycle.** For time of addition studies, HEK293T cells will be infected with rCedV at MOI of 0.1 diluted in cell growth medium for 1 hour at 37 °C, at which time the viral inoculum will be removed and cells washed three times with PBS. This will be considered as 0 hpi. Compounds diluted in cell growth medium will be added to the cells at either 0, 2, 4, 6, 8 or 12 hpi and remain on the cells for the duration of the assay. All supernatants will be collected at 24 hpi and analyzed for infectious viral titers (PFU/mL) by plaque assay[37,38]. Results from this data will indicate at which stage of the viral life cycle the compounds are affecting; 3) **Analyze escape mutants against the advanced lead compounds with Cedar virus.** Escape mutants provide information on potential targets of small molecule inhibitors as well as the potential for development of drug resistance in the field. We will generate escape mutant rCedV viruses that are resistant to the identified lead compounds and determine the rate at which resistance is developed to our inhibitors. In so doing, we will also identify protein coding as well as regulatory sequences that are altered. HEK293T cells will be infected with rCedV virus at MOI of 1 in the presence of the lead compounds at 10 times the EC_{50} values of each of the compounds. At 72 hpi, viral supernatant will be passaged in Vero cells at MOI of 0.01 in the presence of increasing concentrations of compound for >5 passages to obtain the escape mutants. The escape mutants will be plaque purified and subjected to whole-genome sequencing. The identified mutation(s) will be introduced into the rCedV anti-genome cDNA and upon successful incorporation of the mutation, the mutant rCedV will be rescued and sequenced to ensure retention of the introduced mutation. The rescued mutant rCedV will be fully characterized as described.

Aim 3. Develop combination therapy of broad spectrum siRNA-conjugate therapeutics and/or replication complex inhibitors against Hendra and Nipah viruses. Different combinations of broad spectrum siRNA therapeutics and small molecule therapeutics will be evaluated in hamsters infected with HeV and NiV_B. The objectives of these studies will be to determine if the combinatorial approach will confer 1) improved antiviral efficacy over that seen with each therapeutic alone; and 2) improved efficacy when treatment is initiated either at, or even after, the prodromal phase. A combinatorial therapy approach to treat henipaviral infection has several advantages. The application of multiple modalities ensures against the development of viral escape mutants, as each of the therapeutics target different aspects of viral infection biology. In addition, therapeutic activities may synergize and offer greater efficacy in treating henipavirus infection when compared to a therapeutic used alone.

3.1. Define breakthrough conditions for anti-henipavirus inhibitors in hamsters. This Subaim will employ the most promising antivirals from Aims 1 and 2 that are shown to completely protect hamsters against lethal henipavirus infection. Aims 1 and 2 assess protective efficacy at times shortly after henipavirus exposure. The goal of this Subaim is to determine the time points of treatment initiation when the antivirals fail to confer protective efficacy. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^6 PFU of NiV or HeV[75,89-91]. Treatments will be initiated at various times after henipavirus infection, e.g., days 1, 2, 3, etc. to determine the initiation time point at which most hamsters succumb to disease.

3.2. Combination therapy of anti-henipavirus inhibitors in hamsters. This Subaim will employ the most promising antivirals from Aims 1 and 2 where breakthrough conditions have been defined in 3.1. Hamsters will be randomized to multiple study groups (n = 5 per group) and inoculated i.n. with ~ 5x10^5 PFU of NiV or HeV[75,89-91]. Treatment with at least two different antivirals will be administered beginning at a time point where substantial breakthrough was shown in 3.1 for each individual antiviral in 3.1 to determine if combining at least two different antivirals improves survival. Groups may be repeated or group sizes increased as needed to obtain appropriate statistical power to show the survival benefit of combination therapy.

General Methods, Scientific Rigor, and Biological Variables Criteria: All animal studies will be blinded to all staff performing animal procedures and assays, and to the veterinary pathologist performing gross and/or histological analysis of tissues, i.e., antiviral drugs will be coded so that researchers will not know which compounds were given to which animals until experiments and analysis are completed. We will employ equal or near equal numbers of male and female animals and general age (infant or geriatric animals will not be used).

Pitfalls and Alternative approaches: A theoretical (although unlikely) pitfall for siRNA design In Aim 1 would be the inability to identify a potent siRNA against one or more of the four viral gene targets. However, we have previously shown that LNP bearing siRNA against just one or two viral genes constitute effective antiviral therapeutics in the context of Marburg virus[50], and Sudan virus[52]. Another possible pitfall would the inability to identify a LNP formulation that provided sufficient siRNA delivery to the lung. Here, we would switch to an alternate siRNA delivery strategy, using a ligand-siRNA conjugate approach. Other groups have shown gene silencing in the airway using this type of delivery system[101]. For Aim 2, the proposed synthetic derivatives will be synthesized, under the guidance of Dr. Gaisina, by **MedChem Core.** If we encounter a synthesis-related problem with our primary scaffold compounds, we will use an alternate strategy based on a different reaction sequence. Should we encounter a scaffold-related problem with our furopyrimidines, we will switch to the additional HTS hits[71] shown in **Figure 10** and use them as our backup scaffolds for defining lead compounds for advancement.

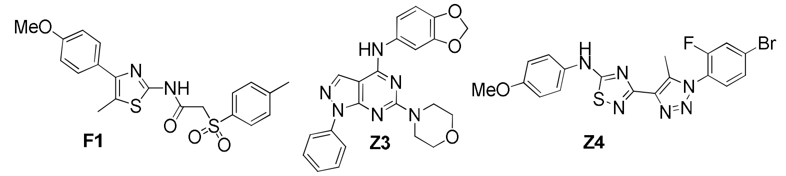


Figure 10. Additional HTS hits.

SPECIFIC AIMS

The Administrative Core of the Antiviral Drug Discovery and Development Center-2 (AD3C-2) has a history of providing organizational leadership for the current Antiviral Drug Discovery and Development Center (AD3C), a NIAID Center of Excellence for Translational Research (CETR). The Administrative Core of AD3C-2 will continue to play a key role in leadership, communication, coordination and oversight of the proposed projects and cores in this application. Importantly, it will promote collaboration and synergy between the projects. Operationally, it is responsible for the fiscal and contractual management of the Center. Further, it organizes such activities as meetings among the Scientific Advisory Board (SAB), members composed of at least 5 non-conflicted external advisors, the Executive Committee (EC, lead PIs) and, and an annual meeting of all Projects and Cores. In addition, it will manage the inter-institutional consortium/contractual agreements. The broad objectives of the Core are as follows:

1. **Providing programmatic and administrative leadership**
   a. Make decisions, specifically “go versus no-go” per discussions with the Executive Committee (EC).
   b. Track and encourage research productivity
   c. Promote interactions and collaboration between Projects and Cores, in particular facilitate overarching synergy to pursue orally bioavailable broad-spectrum antivirals as well as sharing of reagents
   d. Monitor the direction and overall priorities of the Center
   e. Directly interface with NIH staff, consultants, and the Scientific Advisory Board (SAB)
2. **Fiscal and administrative management of the center**
   a. Oversee expenditures, budget information, fiscal reports
   b. Manage contracts and the consortium agreement
   c. Establish and monitor compliance with federal and NIH regulations
3. **Develop, support and monitor progress of projects**
   a. Manage projects through regular virtual and in-person meetings
   b. Organize quarterly project reviews (face-to-face or virtual) by the EC
   c. Monitor overall Center research quality and progress annually with the SAB
   d. Solicit additional regulatory guidance on an as-needed basis
   e. Assist with identification and management of intellectual property developed by projects
   f. Manage the ‘Developmental Research’ and ‘Mentored Projects.’ The later will train young investigators in antiviral drug discovery.
4. **Stimulate collaboration and synergy**
   a. Identify potential areas or topics of collaboration between Projects and Cores
   b. Provide and facilitate access to resources needed in the Projects
   c. Ensure that all active compounds are cross-evaluated in other Projects
   d. Manage a data sharing and project tracking web-based system
   e. Encourage public/private partnerships and alternative sources of funding to expedite project development and translation to INDs
5. **Facilitate meetings**
   a. Host monthly virtual meetings among project teams
   b. Organize virtual and in-person meetings of the Project and Core PIs (EC)
   c. Organize and implement an annual face-to-face meeting with all involved personnel, including the SAB
   d. Facilitate consulting and other scientific and professional meetings
   e. Attend and assist with coordination of the NIH reverse site visit
6. **Outreach to the public**
   a. Maintain a website
   b. Manage press releases
   c. Assist with scientific publications and ensure grant acknowledgement

We firmly believe that a strong Administrative Core will be crucial to the success of the AD3C-2. We have brought together leading virologists, chemists, scientific advisors, and projects and cores from around the country and are confident that the Administrative Core will facilitate communication and collaboration among the PIs. Accomplishing the objectives set out above will ensure focus and synergy among the projects, accelerating the development of new, and potentially broad-spectrum therapeutics for emerging infections caused by coronaviruses, henipaviruses, flaviviruses, bunyaviruses and alphaviruses. With the new Developmental and Mentored projects to expand the scope and flexibility of the overall center, we will have new and exciting paths to follow.

I ADMINISTRATIVE CORE STRATEGY:

I.1 RELATIONSHIP OF THE ADMINISTRATIVE CORE TO THE PROGRAM'S GOALS

The first iteration of AD3C was established as a CETR in 2014 and is driven toward product development of broad-spectrum antivirals. Over the years of its existence, we have explored novel targets and defined their role in viral biology. Furthermore, we have utilized state-of-the-art screening mechanisms and first-rate medicinal chemistry resources to develop novel hit and lead molecules for unmet medical needs. Essential to this process has been the involvement of individuals with basic, translational, clinical and regulatory experience, and bringing them together in a structured way to ensure progress down the drug discovery and development.

AD3C and now this iteration, AD3C-2, was modeled after the Alabama Drug Discovery Alliance (ADDA), a collaboration initiated in 2008 between the University of Alabama at Birmingham (UAB) and Southern Research (SR), and directed by Dr. Richard Whitley – the proposed Program Director/Principal Investigator for this AD3C-2 application and also Lead of this Administrative Core. Similar to the AD3C-2, the ADDA aims to support the discovery and development of therapeutic molecules that address unmet medical needs. It has built on the expertise present at both UAB and SR, with several in preclinical lead development and one compound in phase I clinical trials. The ADDA has succeeded by emphasizing the use of interdisciplinary teams to achieve the project goals. These teams consist of investigators with a wide variety of expertise from both UAB and SR and contribute to the program's success. In addition, individuals from the respective technology transfer offices are an integral part of the team in order to guide intellectual property development and any commercial opportunities that may arise. Another key member of this Administrative Core is Dr. Stephanie Moore who plays a critical role in orchestrating the ADDA. She will be an important go-to for the AD3C-2 teams to facilitate communication among teams and directly work with each member of the team for communications, meetings, and progress. She has assisted the current AD3C by managing progress and communication among projects and cores as well as the NIH. The Core's Project Oversight Director, Dr. Shi from University of Texas Medical Branch (UTMB), is the proposed Co-Program Director/Principal Investigator for this AD3C-2 application. He is an expert in virology, drug discovery, and vaccine development with both translational and basic research expertise from academia, a public health laboratory, and the pharmaceutical industry. He has extensive experience in leadership, mentoring, and has played a major role in the response to the SARS-CoV-2 by quickly developing critical assays that enabled Pfizer's COVID-19 vaccine development. With the experience of these core members, and those introduced below, the administrative core will be in a position to support all of the program's Core and Project teams.

For this new iteration, Antiviral Drug Discovery and Development Center-2, we have structured our program in a similar manner as AD3C; however, we have added important new members. The focus of our drug discovery screening is biochemical in nature. Briefly, we will continue to use the structure of an interdisciplinary model to manage the five proposed research projects and their interactions with the Screening, Medicinal Chemistry, and Structural Biology Core. The Administrative Core will organize regular team meetings in which the projects' progress will be discussed, along with the identification of logical next steps. These meetings will provide an opportunity to identify potential solutions to encountered obstacles, whether those are resource or expertise related. In addition, closely monitoring projects will ensure clarity about when assays are completed and compounds of interest have emerged, particularly those of potential interest to all the projects. The expectations of each of the cores will be ensured by the Administrative Core. The Screening Core will provide the expertise and platform to develop high-through put screening and assay development for each Project. Further, the Medicinal Chemistry Core is one of the most critical aspects of this application. Chemistry from SR, University of Southern California, and UTMB with consultation and collaboration of industry partners, Arbutus, Genevant and Gilead, through iterative design and AI-assisted modeling, will design compounds optimized to inhibit viral replication among all project viral families and also decrease non-specific interactions with host-targets. Compounds must have drug-like properties for oral delivery. Since multiple medical chemistry resources are involved, efforts will be co-ordinated by Corinne Augelli-Szafran at SR and monitored by the Administrative Core. A critical asset of this program is the new Structural Biology Core, consisting of structural biologists who are also virologists. The Administrative Core ensures that each project utilizes the resources within the cores by holding monthly meetings among project and Core members.

Together, our Project PIs and Core leaders have an impressive track record in research productivity, collaboration and expertise in drug discovery and development. With our Administrative Core, we plan on maximizing utilization of these groups by providing a structured framework in which projects and advanced compounds with *in vivo* antiviral activity can progress as expeditiously and efficiently as possible.

I.2 ADMINISTRATIVE CORE STRUCTURE

There are three components to the planned Administrative Core: 1) the Core Personnel who manage day to day operations of the Center; 2) the Executive Committee (EC), which is responsible for monitoring progress of the Center's research projects as well as functionality of the Center's Cores and consists of all project PIs and core directors and 3) the Scientific Advisory Board (SAB) consisting of external advisors who will provide additional expertise and a high level review of the Center's productivity and direction on, at minimum, an annual basis; they will also be responsible for oversight and selection of the Developmental and Mentored Research Projects. Importantly, the SAB will provide 'go-no-go' insight on all projects. Regularly scheduled communications between these three components ensure timely dissemination of materials, information, data and advice – a detailed management plan can be found below under section I.3.

A key role of the Administrative Core is to support the project teams to guide the planning and progress of each individual research Project. In general, the Project teams include the project PI and listed co-investigators, but also key individuals from other projects with appropriate expertise, as well as personnel situated in the Screening Core, the Medicinal Chemistry Core, and Structural Biology Core. Involving individuals and expertise throughout the process help facilitate transferring compounds in and out of the Cores as well as between Projects, since personal relationships have been developed over time and individuals will be intimately familiar with the goals and details of the respective project. A diagram detailing the relationship of the Administrative Core with the Projects and Cores can be found in Figure 1.

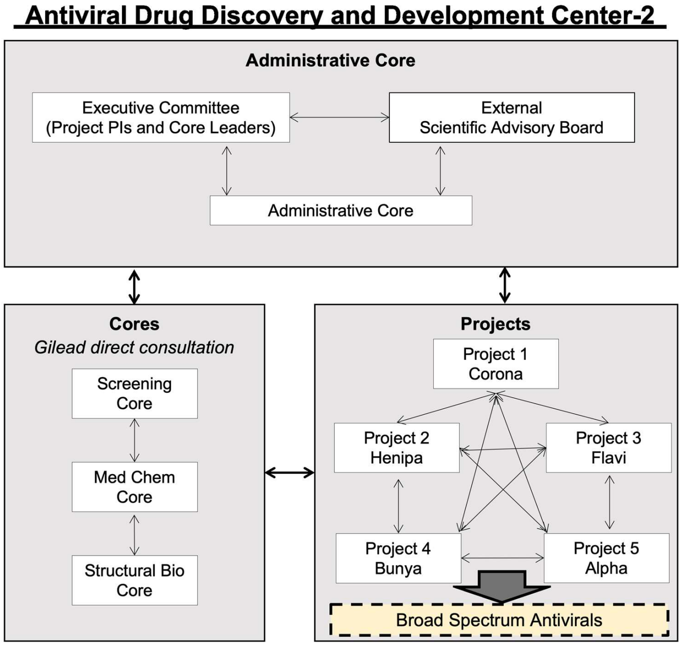


Figure 1 Structure of AD3C-2. Overall oversight will be provided by Drs. Richard Whitley and Pei-Yong Shi.

I.2.1 Role of Administrative Core Personnel

Richard J. Whitley, MD – Core Lead (2.4 calendar months)

Dr. Whitley is currently Distinguished Professor, Professor of Pediatrics, Microbiology, Medicine and Neurosurgery, and holds the Loeb Chair in Pediatrics in the Heersink School of Medicine at the University of Alabama at Birmingham (UAB). He has performed translational research for more than 40 years at UAB and has focused on antiviral drug development ranging from molecule selection, preclinical toxicology, ADME, animal models, resistance assessment, to human studies. Through his contracts from the National Institute of Allergy and Infectious Diseases' (NIAID) Collaborative Antiviral Study Group (CASG), he has directed multi-institutional Phase I-III trials in both adults and children that have led to seven NDAs. In addition, his team has performed detailed pharmacokinetic and pharmacodynamic studies on drugs advancing toward licensure for pediatric indications (acyclovir for the neonatal HSV and herpes simplex encephalitis, valganciclovir for congenital cytomegalovirus infection and oseltamivir for influenza in babies < 2 years of life. As the PI of AD3C-2, Dr. Whitley is also the Administrative Core Lead, and will utilize his expertise in managing consortia with multiple institutions to ensure appropriate communications between projects and cores and will aid in conflict resolution, should any conflicts arise.

Pei-Yong Shi, PhD – Core Project Oversight Director (1.8 calendar months effort)

Dr. Shi is the Director for the Institute for Drug Discovery, Vice President for Research Innovation, and Associate Chief Research Officer at UTMB. He is an expert in virology, drug discovery, and vaccine development with both translational and basic research expertise from academia, public health laboratory, and pharmaceutical industry. He served as Executive Director at Novartis Institute for Tropical Diseases from 2008 to 2015 and has a track record in leading multifunctional teams to develop therapeutic and vaccine products. Dr. Shi's team developed the first infectious cDNA clone and replicon systems for Zika virus and the epidemic strain of West Nile virus. His team also developed the first peer-reviewed infectious cDNA clone for SARS-CoV-2. Together with Dr. Richard Whitley (PI of the AD3C-2 Center), Dr. Shi will be responsible for the overall operation of the AD3C-2 Center as well as the oversight of the projects at UTMB.

Stephanie Moore, PhD – Associate Administrative Core Director (3.6 calendar months effort)

Dr. Moore is Instructor in the Department of Pediatrics, Heersink School of Medicine at the University of Alabama at Birmingham. She is also the Associate Director with the Alabama Drug Discovery Alliance (ADDA). In that capacity, she interfaces with a wide range of scientists involved in the drug discovery and development process, with a focus on projects in oncology, infectious diseases and neurodegenerative disorders. She is trained in biochemistry and protein biology and has a broad range of publications in protein post-translational modifications and translational studies regarding oxidative lung injury. For both collaborative efforts of the ADDA and AD3C, she aids in building Project teams for funded drug discovery grants, facilitates regular meetings and helps with communications between UAB and SR. Importantly, she will organize the necessary project teams, ensuring the appropriate expertise is represented and will organize and chair the various project team meetings. In addition, she will work with AD3C-2's PIs and Core Lead (Rich Whitley and Pei-Yong Shi – see above) and Business Officer (Sarah Dowdy – see below) to achieve the Administrative Core's objectives. These include the organization of all in-person meetings, communication with the EC and SAB, communication with NIAID, preparation of relevant documents such as annual progress reports, solicitation and organization of scientific review of supplemental research projects.

Sarah Dowdy – Administrative Core Business Officer (2.4 calendar months effort)

Ms. Dowdy has 15 years of experience in research administration managing research grants and contracts. She is the current AD3C program manager and will oversee the financial and contractual administration of the proposed AD3C-2. She has extensive experience with coordination of subcontracts, institutional invoicing, federal guidelines related to both grants and contracts and large meeting planning. She will be responsible for all budgetary issues including institutional consortium/cooperative agreements, subawards, amendments to the budget, invoicing, monitoring spending, and keeping the PI and Executive Committee informed about the status of the annual budget. She will be responsible for grants management issues and will aid Dr. Moore (see above) with meeting organization, the annual noncompetitive progress report preparation, and all other proposals related to AD3C funding. She will oversee all compliance and federal regulatory requirements of AD3C.

Other Administrative Staff

Other Administrative Staff includes Ms. Sara Davis (4.2 calendar months) who is the administrative coordinator for Dr. Rich Whitley. Ms. Davis will assist Drs. Whitley and Moore with logistical aspects of meeting scheduling, travel arrangements, and organization as well as maintenance of the AD3C-2 website. An additional administrative support person (4.2 calendar months) will be on-boarded to assist with the complex program management logistics involved in this large program. Support person would be selected for basic skills in note taking, time management, communication, and assisting with online submissions.

I.2.2 Executive Committee

The Executive Committee will be composed of all Project PIs and Core Leaders and will be responsible for monitoring the progress and direction of each project, as well as AD3C-2 as a whole. The EC will meet quarterly: one meeting will be held at the time of the annual AD3C-2 meeting in Birmingham, AL, and a second, six months following the annual meeting, at a rotating project site or in conjunction with related national scientific meetings such as International Conference on Antiviral Research (ICAR). Two additional meetings will be virtual. During these meetings, the EC will review each project and core for scientific progress as measured against the proposed timelines and milestones, as well as the utilization of financial resources. Additional opportunities for collaboration between the projects will be identified.

EC members will be familiar with the Projects since each member will participate in monthly conference calls where all Projects provide a status update. This frequency of communication will ensure that EC members are well informed and can make appropriate decisions during their quarterly EC meetings.

EC members, in addition to the PI's, Drs. Whitley and Shi, include Drs. Alec Hirsch, Dan Streblow (Oregon Health & Science University), Michael Diamond and Sean Whelan (Washington University), Mark Denison (Vanderbilt University), Mark Heise (University of North Carolina, Chapel Hill), Bob Bostwick and Corinne Augelli-Szafran, (SR), Charles McKenna (University of Southern California), Vineet Menachery, Jai Zhou, Alex Freiberg, Tom Geisbert, and Shinji Makino (UTMB), Jamil Saad, and Stephanie Moore (UAB). Our pharmaceutical partners will be invited to attend.

I.2.3 Scientific Advisory Board (SAB)

We will recruit a Scientific Advisory Board of at minimum 5 non-conflicted advisors with experience in molecular virology and biochemistry as well as, importantly, drug discovery and development. Since our Center is engaged ultimately in product development, we will have at least two advisors with industry-level expertise in antiviral therapeutics and especially those with experience at all levels of drug development. Considering the multiple contacts that the various Project and Core Leaders have had with industry throughout their respective careers, we do not anticipate any hurdles in identifying appropriate individuals. All proposed members will be vetted by NIAID staff. The SAB will complement the EC by providing a high-level evaluation of AD3C-2's progress and successes and will help refine and focus the Center's activities and direction, particularly as to those projects that should be discontinued. The SAB will perform this evaluation annually and will be invited to the EC meeting in conjunction with the annual AD3C-2 meeting, in Birmingham, AL. Additionally, their advice will be sought at quarterly intervals for appropriate projects. Of note, appropriate NIAID staff will also be invited to join this meeting, should they be able to attend. Prior to these meetings, Projects and Cores will prepare annual reports that will be submitted to the SAB for evaluation. Scheduling the SAB meeting at the end of the annual AD3C-2 meeting will allow the SAB members to attend scientific presentations of the projects that will complement the written reports, thus fully informing these individuals about the Projects' status and direction.

Annually, NIAID requires a reverse site visit at which time the progress of each Project is presented. Such meetings will be organized by the Administrative Core. Importantly, in addition to Project PIs, at least one member of the SAB will be in attendance in order to present an external, objective evaluation of the projects to NIH Program Officers and staff.

The SAB will also be engaged on an ad-hoc basis if the EC deems this appropriate. EC members participate in updates about each Project on a monthly basis via teleconference in addition to a more thorough review during the semi-annual meeting, providing important touch points to evaluate the need to engage the SAB. Should this need be identified, the Administrative Core will mediate a virtual meeting to discuss the matter.

Finally, we expect that several SAB members to have extensive regulatory expertise and will help guide the projects to an appropriate preclinical development plan in collaboration with the EC.

I.2.4 Regulatory Expertise with Respect to the Challenges Facing Infectious Diseases

The Center will draw upon the regulatory knowledge and experience of the PI, Project and Core Leaders and SAB to meet the regulatory requirements related to research in emerging infection diseases. As past president of the Infectious Diseases Society of America (IDSA) and a member of the Working Group on Influenza for the Presidential Council of Advisors on Science and Technology during the 2009 influenza pandemic and the vaccine advisory board for the current COVID-19 pandemic, Dr. Whitley is intimately familiar with challenges of conducting research in infectious diseases in the existing regulatory environment. He has led numerous UAB research projects that included both *in vitro* and *in vivo* work related to orthopox drug discovery in BSL3 certified labs. In addition, he was instrumental in the preparation and successful submission of the funding application for the BSL3 facility, SEBLAB, which opened at UAB in 2009 and is aware of strict oversight, certifications, and regulations required for select agent research lab including CDC, IACUC and Occupational and Safety review and approval for projects. Preclinical toxicology studies have been overseen by him in preparation for human Phase I studies. He has been the PI for the NIAID Collaborative Study Group that was responsible for all phases of clinical trial development from protocol design, IRB approval, including the establishment of a universal IRB, biostatistical coordination, investigator recruitment, clinical trial monitoring (by FDA standards), addressing FDA audits, and data analysis and reporting. Data tapes were provided to the NIH, FDA and pharmaceutical sponsors for registrational purposes. His studies have led to the definition of the standard of care for herpes encephalitis, neonatal herpes, congenital cytomegalovirus infections, and influenza in children, among others.

Dr. Shi also has vast experience related to navigating the challenges of regulatory requirements for research in emerging infectious diseases. As discussed above, he has unique expertise within the context of academia and industry, but he also has worked in the realm of public health too. As a key leader at Novartis institute for Tropical Diseases, he has been responsible for developing antiviral strategies and has executed drug discovery and development for this purpose. A recent example is his team at UTMB that delivered Phase I and II neutralization data for Pfizer/BioNTech’s COVID-19 vaccine, which led to the first FDA EUA and final approval.

In addition to regulatory challenges pertinent to infectious diseases, several individuals on the EC have extensive experiences in regulatory expertise appropriate for lead development activities in general, including patent issues and IND submissions.

I.3 MANAGEMENT PLAN

It is imperative that AD3C-2 is appropriately managed to ensure that projects can flourish and be supported by high-functioning cores. Timely and clear communication will be critical to achieve the objectives listed under the Specific Aims (above), and further elaborated on below. The Administrative Core will oversee and manage the Center activities by implementing the following:

1. **Providing programmatic and administrative leadership**
    a. Make decisions, specifically “go versus no-go” decisions per discussions with the EC and SAB.

        Quarterly in person meetings will provide an opportunity for the EC to be updated in-depth on progress made in each project, and provide feedback to adjust Projects’ direction and focus, as needed. At key points, more frequent meetings may be required and organized. Of note, EC members will also attend monthly conference calls.

    b. Track and encourage research productivity

        Project teams have conference calls at least monthly in which an update of the projects will be presented. This is summarized by the Administrative Director, Stephanie Moore. Meeting minutes, including action items, will be distributed to the Project teams using a ShareFile site managed at SR. Minutes will include a list of metrics, including Intellectual Property disclosures filed with respective technology transfer offices, (provisional) patents filed, patents awarded, presentations at meetings (oral or poster), and those publications submitted and accepted. These metrics will be used to assist in preparing annual progress reports to the EAB and NIAID staff.

    c. Promote interactions and collaboration between Projects and Cores

        The evaluation of active compounds in multiple projects to identify broad-spectrum antivirals will promote interactions and collaboration between Projects. In addition, the EC will meet quarterly and have a discrete agenda item for identification of additional opportunities for collaboration. Finally, the monthly conference calls for the Project teams regularly inform team members about status of all projects. Along with promoting interactions, the core will utilize the structure to identify and manage the resolution of problems in a timely fashion.

   d. Monitor the direction and overall priorities of the Center

      This will occur during the EC and SAB meetings immediately following the annual AD3C-2 meeting. We will invite NIAID staff to attend these meetings as well, who can provide feedback on how the Institute sees AD3C-2 evolving.

   e. Directly interface with NIH staff and consultants

      As mentioned above under 1.d, NIAID staff will be formally invited to attend the annual AD3C-2 meeting. In addition, the Administrative Core will regularly communicate with NIAID to answer questions that may arise and provide informal updates about AD3C-2's progress. Further, this Core will facilitate the reverse site visit at the NIH.

2. **Fiscal and administrative management of AD3C**
   a. Finances: oversee expenditures, budget information, fiscal reports

      Project leaders and administrators at each institution will be provided with quarterly financial reports, which will provide current balances and projected balances based on rate of expenditures, to ensure that projects are not over/under budget for the year. Project leaders will be notified should it appear that funds are not being expended as outlined, or if it appears re-budgeting is needed.

   b. Manage contracts and the consortium agreement

      Our team has navigated the implementation of an institutional consortium/contractual agreement among many of the members of this current proposal. Under this proposal, the Administrative Core will facilitate the development of a new consortium agreement with all members and with requirements specific to this application. We have provided the consortium agreement plan to the PIs for this application and they are committed to ensure the development of this agreement (see letters of support). The core also facilitates preparation and processing of other agreements or forms such as confidential disclosures (CDAs), material transfer agreements (MTAs) or invention statements that may be needed by investigators.

   c. Establish and monitor compliance with federal and NIH regulations

      The Administrative Core will ensure compliance with the financial and administrative aspects of the award. AD3C-2's Business Officer, Ms. Dowdy, will work closely with UAB's Office of Sponsored Programs as well as with similar offices at the participating institutions and will create and maintain necessary documentation, such as technical and administrative reports, preparing justifications, appropriately acknowledging NIAID support of research findings in publications, announcements, news programs, and other media, and ensuring compliance with other federal and organizational requirements.

3. **Develop, support and monitor progress of Projects**
   a. Manage Projects by having regular conference calls and in-person meetings

      As noted above, Project teams will have monthly conference calls with progress being tracked and to-do-items clearly delineated by preparing and distributing minutes by the Administrative Director. Quarterly EC meetings will provide regular feedback to the Projects, allowing rapid realignment, should Projects veer off course.

   b. Organize quarterly Project reviews (face-to-face) by the EC

      As elaborated above, the EC will meet quarterly, in addition to attending the monthly conference calls. This will provide the opportunity to review the Projects in greater depth.

   c. Monitor overall Center research quality and progress annually by the SAB

      Annual SAB meetings will provide feedback and advice on a high, project portfolio level. Preparation of progress reports in advance of these meetings will also help in preparing documents to be submitted NIAID.

   d. Solicit additional regulatory guidance on an as-needed basis

      As noted in above in section II.2.4, several investigators in AD3C-2 have regulatory expertise that can be brought to bear on the individual research projects. These individuals include the

PI, Drs. Whitley, Shi, and Geisbert, who have direct experience and track records in antiviral and vaccine product development.

e. Assist with identification and management of Intellectual Property developed by projects

As mentioned under Point 2.b, AD3C-2 will establish an umbrella inter-institutional consortium agreement, part of which describes how Intellectual Property (IP) is managed. Existing IP will not be affected by the agreement completed under this proposal, allowing collaborators to contribute exciting compounds to the consortium, but all inventions, developments and discoveries resulting from AD3C-2 efforts will be promptly disclosed to the employer of the associated scientists. Most likely, there will be joint inventions, as, for example, novel chemical scaffolds may be identified that have potent antiviral activity. In these cases, the Administrative Core will aid in the formation of appropriate inter-institutional agreements regarding this specific invention, which will describe aspects such as patent filing, prosecuting, patent maintenance, marketing and revenue sharing, among others, taking into account the relative contributions of the inventors.

f. Manage the Developmental Research and Mentored Project Programs

Developmental Research Projects – The Administrative Core will at the end of years 1 and 3 release a RFA to each Project and Core for Developmental Research Projects. The purpose will be to expand the scope of the current platform - develop inhibitors of viral replication – as well as to increase the range of research, investigators, and institutions involved in the program. Applicants will be directed to submit a 6-page description regarding the scope of the proposed studies and must describe how the work supports the platform, the innovation, and significance of the project, along with a biosketch and a budget and justification. The EC and SAB will be responsible for selection of applications that are potentially most scientifically fruitful and fit the Center platform. The selection committee, consisting of senior scientists in the program (TBD), will select applications to initiate studies in years 2 and 4 of the project period and must be completed by the end of year 5. The selection committee will be instructed to focus on expanding the scope of the current platform. Progress for the selected projects will be tracked by the Administrative Core and will be assisted as needed or ended with oversight from the SAB and EC. The awardees will be invited to attend monthly meetings (with appropriate agreements added as needed). The budgets will be limited for one or two years, with a range of $200,000 to $250,000 per project per year, direct costs. The Developmental Project Leader must commit at least 1.2 (CY) person months effort per year. Budgets for Developmental Research Projects can be requested in years 2-5 of the project period and must be completed by the award end date.

Mentored Projects – The Administrative Core will release, on a rolling basis during years 2-5, an RFA for mentored projects. The goal of this program will be to develop junior scientists on a career path of drug discovery. The application and the materials requested (2-page application, biosketch, and budget/justification) will be accepted via an online application form. The applicant will be required to define the scope and career goals. Once received, the Administrative Core will determine the potential fit and utilize the EC and SAB as the selection committee. Applicants must be postdoctoral fellows, early career investigators or senior investigators new to the field. The intention will be to increase the availability of qualified researchers and other personnel for antiviral discovery and to offer opportunities to advance the applicant's professional career. Furthermore, selection will focus on promoting diversity in the biomedical research workforce by making efforts to welcome underrepresented racial and ethnic groups, persons with disabilities and women. Once selected the awardee will be assigned a mentor and progress reports will be requested annually. No more than $600,000 (total) would be awarded per year; awardee(s) must devote 9.6 (CY) to the project. In addition to the financial support, the trainee would be offered the opportunity to travel to our core sites including SC, MCC, and SBC to get specialized support and training experience in drug discovery and development.

Commented [MS(1]: Cores please comment on whether this is feasible or if you have opinion on this.

4. **Stimulate collaboration and synergy**
    a. Identify potential areas of collaboration between Projects and Cores

As noted, monthly conference calls will allow Project teams to benefit from the expertise of all other scientists and will lead to additional collaborations. The quarterly EC meeting will specifically evaluate this topic during each of its get-togethers.

b. Provide and facilitate access to resources needed in the projects

   Although we have mapped out a reasonable timetable and order in which Projects will be queued up for assay support and medicinal chemistry, adjustments will undoubtedly have to be made as Projects progress and unexpected delays occur. The EC will evaluate adjustments and change resource allocation accordingly.

c. Ensure that active hits in one Project with potential against other viruses are evaluated in other Projects

   The SR Screening Core will perform a targeted evaluation of a novel 1000 compound library of nucleoside analogues against all viruses in the program. The goal is to identify hits with broad-spectrum activity. Further, all Project leads will be tested across the spectrum of virus families. These hits will be prioritized for further evaluation and optimization. The Administrative Core, in collaboration with the Medicinal Chemistry and Lead Development Core, will continue to manage a data sharing and project tracking website at SR.

   To avoid sending data files back and forth as email attachments, we will continue to utilize a password protected, web-based software system, Citrix ShareFile, to share data and track projects. All screening data on compounds will be available to all the Project team members. The data will be organized according to viral target; the database will be maintained by the SR Cores. SR has extensive experience in data management of large cooperative projects, including ones funded by NIAID. All the AD3C-2 and SAB members will have access to this secured and encrypted site.

d. Encourage public/private partnerships and alternative sources of funding to expedite project development and translation to INDs

   From the previous iteration, AD3C, we successfully competed for a complementary Partnerships for Countermeasures against Select Pathogens (R01) grant (R01AI132178). This grant has been awarded to Vanderbilt University, the University of North Carolina at Chapel Hill and Gilead Sciences, to facilitate (pre-) clinical development of an antiviral against coronavirus infections. Within, this new proposal, AD3C-2, we will encourage our projects to look for such opportunities to further support IND-enabling studies for our newly discovered direct acting antivirals.

5. Facilitate meetings

a. Host meetings among project and core teams

   Monthly virtual meetings will be held for the Project and Core teams.

b. Organize in-person meetings of the Project and Core PIs (EC)

   The EC will meet during the annual AD3C-2 meeting in Birmingham AL, and 6 months thereafter in person, at rotating project sites. Any additional meetings will be held virtually.

c. Organize and implement Annual face-to-face meeting with all involved personnel, including the SAB

   AD3C-2 will host an annual scientific meeting, in which all involved personnel will be invited to get together in Birmingham, AL, if the pandemic abates. The goal of this meeting is to learn about the status of each of the Projects. The SAB will convene at this time as well, to review the science and progress of each project. This will not only provide an opportunity to present an in-depth update of all Projects to the EC, SAB and NIAID staff, but will also provide an educational opportunity for involved trainees.

d. Facilitate consulting and other scientific and professional meetings

   As described under Point 3.d, we will solicit additional regulatory expertise on an ad-hoc basis, should this be necessary to move projects forward down the development pathway. The Administrative Core will make the necessary arrangements to orchestrate such consulting meetings and will invite the appropriate personnel to attend. Furthermore, if it is deemed appropriate to have Project teams meet face-to-face in addition to the annual meeting, the Administrative Core will facilitate such travel. This might occur for example when the chemists need to meet with the virologists to focus on the most attractive chemical series. A conference call may not allow a comprehensive interrogation of the data.

e. Attend the reverse site visit

The Administrative Core will help organize the attendance of Dr. Whitley along with selected key Project leaders and one SAB member at the annual NIAID reverse site visit. We have included funds in our travel budget for these meetings.

6. **Outreach to the public**
   a. Maintain a website

      The Administrative Core will develop a website for the AD3C-2. The website will list the Projects and the Cores, key personnel and publications, among others. This website will be updated regularly.

   b. Manage press releases

      UAB has an active and well-staffed Media Relations Office in place which includes science writers. It also maintains a web address exclusively for research related news (http://www.uab.edu/news/research). The Administrative Core will utilize the expertise of the staff to ensure that Center research findings and related news is disseminated in a timely and appropriate manner.

   c. Assist with scientific publications and ensure grant acknowledgement

      The Center will follow all current guidelines related to the NIH Public Access Policies for NIH funded research. It will assist project investigators with final review of publications resulting from research and ensure that it is submitted to PubMed Central and a PMCID number obtained. In addition, the Administrative Core staff will assist investigators with obtaining My NCBI lists of publications as part of the annual progress reports.

I.4 BSL3/4 ACTIVITIES AND ACCESS

Several Projects and Cores require access to BSL3/4 biocontainment facilities. The table below lists the various activities within AD3C that require such access, and which facilities will likely be used. Detailed descriptions of the BSL3/4 facilities can be found in the Resources sections of the respective projects.

Table I

Activity	Facility
Assay activity of compounds against SARS-Urbani Coronavirus with a nanoluc reporter (Screening Core)	Testing of antiviral activity of compounds against this virus will be performed at **Southern Research's** HTS BLS3 biocontainment facility. The Freiberg, Menachery, and Shi labs (**UTMB**) will be able to perform SARS-CoV, MERS-CoV, SARS-CoV-2, Nipah virus, and/or other BSL3/4 viruses for antiviral spectrum study.
Determine mechanism of action of compounds with activity against SARS-Coronavirus and in vivo evaluation of compounds' activity against SARS-Coronavirus (Project 1)	Both the Denison (**Vanderbilt University**) and Menachery and Shi (**UTMB**) labs have SA-registered BSL3 suites capable of working with SARS-CoV, and the Baric lab is well equipped to perform in vivo pathogenesis studies at SA-BSL3 conditions.
Compounds against Henipavirus replication (Project 2)	Both Geisbert and Freiberg labs (**UTMB**) will be able to perform Hendra virus and Nipah virus.
In vitro and in vivo evaluation of compounds' activity against West Nile Virus (Project 3)	Both the Hirsh (**OHSU)** and the Diamond (**Wash U**) labs have access to BSL3 facilities for cell culture and animal work at their respective institutions. Mice used in the in vivo evaluation will be housed either at Washington University or at OHSU in an A-BSL3 animal facility that has been approved by the USDA.
In vitro and in vivo evaluation of compounds' activity against Bunyavirus (Project 4)	The BSL-3 laboratory at SR will be utilized for testing of lead compounds against HPAI both in vitro and in vivo.

In vitro and in vivo evaluation of compounds' activity against VEEV and/or Chikungunya (CHIKV) (Project 5)	Dr. Streblow (**OHSU**) has access to the Vaccine and Gene Therapy Institute **(VGTI**) at Oregon Health and Science University, which contains 2 BSL-3 suites comprising 4 BSL-3 tissue culture laboratories of 800 sq. ft. each. One suite is registered with the Federal Select Agent Program. In addition, Dr. Streblow has access to the Oregon National Primate Research Center (ONPRC), one of eight national primate centers supported by the National Institutes of Health, located on the West Campus of the OHSU with the VGTI. Animals are housed in ABSL-2, 2+ or 3 containment housing as appropriate for the agent under study. Dr. Heise (**UNC-Chapel Hill**) supervises a secure BSL-3+ facility (approximately 1500 square feet), registered with the CDC for select agent use, with dedicated animal housing where animals will be housed in negative pressure caging.

I.5 ***<u>TRAVEL BUDGET</u>***

The Administrative Core has a travel budget that will allow for the following meetings:

- Reverse site visit to NIAID – Dr. Whitley along with 5 other Project and/or Core Leaders and 1 SAB member - Years 1-4
- In-person semi-annual EC meeting not associated with AD3C-2's annual meeting – all Years
- Annual AD3C-2 scientific meeting for all AD3C-2 personnel, in conjunction with the EC and SAB meeting – all Years
- Ad-hoc travel, such as project personnel traveling in between project sites, meeting with external consultants, etcetera.

In order to manage AD3C-2 travel budgets, the Administrative Core will utilize procedures developed as part of the meeting management and planning practices for the multi-institutional clinical trials group meetings organized by staff at UAB. As part of the meeting planning process, a budget will be set and expenses estimated for all costs including a "per traveler" estimate. Prior to any Center meetings, travelers will be provided with information related to both UAB and NIH travel guidelines, including federal rates for mileage, lodging and per diem to ensure that policies are followed. As required by UAB, travelers will be required to provide original receipts for all travel related expenses and reimbursement requests will be reviewed by Core staff prior to submission for processing. We are planning to host the annual AD3C meeting in Birmingham, AL, to keep meeting costs as low as possible. Personnel from UAB and SR would not need to travel, and Birmingham is only a few hours' drive from Nashville, TN, thus making travel for Vanderbilt personnel easy as well. Both UAB and SR have conference rooms available and have negotiated favorable rates with hotels in the vicinity. In addition, Birmingham is an airport serviced by Southwest Airlines, an airline with very favorable airfares which also services cities where the additional project sites are located (Portland, OR; Galveston (Houston), TX; Raleigh/Durham, NC).

The Screening Core (SC) will support Antiviral Drug Discovery & Development Consortium-2 (AD3C-3) for identification of novel antiviral drug-leads. SC will employ the unique set of Antiviral Assays based on rational selection of druggable viral targets and functional integration of these therapeutic targets into the molecular network of interrelated biological pathways that are linked to unique viral transcriptional machinery and associated functions. The integrated cellular models that we will construct will monitor the functions of molecular networks that are linked to viral transcription and proliferation. Before initiation of High Throughput Screening (HTS) campaigns, the SC will work with individual projects **(Figure 1)** to assess the candidate target across a common set of selection criteria such as genetic evidence, druggability, assay viability, availability of crystal structures, and availability of known ligands and essential role of the target in viral transcription and proliferation.

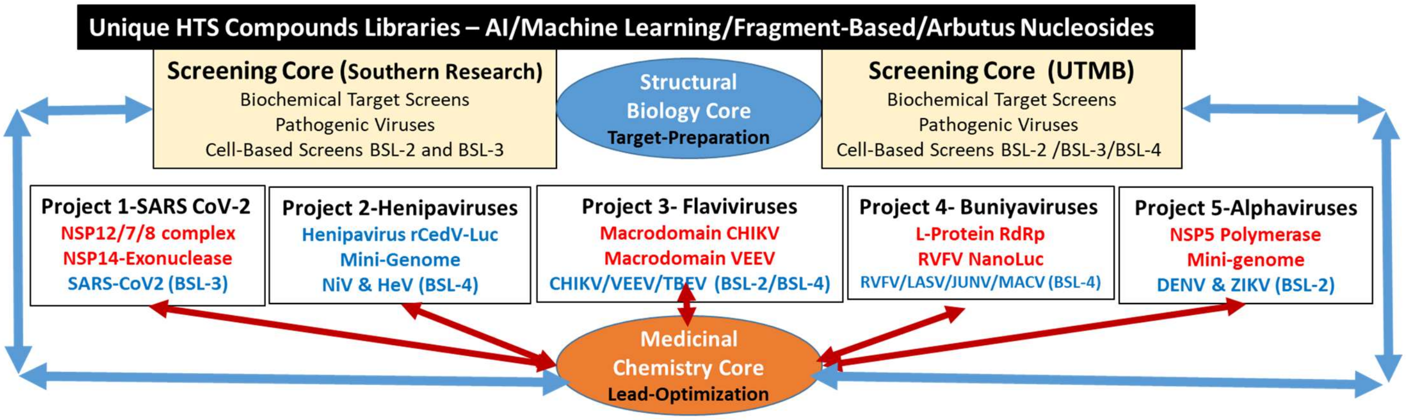


Figure 1- Overview of Antiviral Screening Core and Relationship with Other Cores and Individual Projects. The target screens shown in **Red** have been selected for HTS campaigns and will also be used in SAR analysis and reiterative screening for lead optimization. The target screens shown in **Blue** will be employed to supplement SAR, lead optimization and target identification **The target screens have been selected based on objectives of the individual research projects. The Screening and SAR Assays may be tailored based on dynamic project needs.**

The SC shall capitalize on state-of-the-art infrastructures and a highly qualified team of researchers at Southern Research (SR) and the University of Texas Medical Branch (UTMB) with complimentary experience on the development and validation of antiviral assays and a thorough understanding of pathogenesis, biology and pharmacology of antiviral drugs. This also includes experience in the constructions of transgenic cellular models. The SC will be responsible for achieving the following **Specific Aims**:

Aim 1. Develop, optimize and miniaturize assays for use in HTS to identify hit compounds and in secondary screening to support the Medicinal Chemistry Core and individual projects. Unique reagents will be required to enable development and performance of assays for RdRp and associated targets functions identified by the individual projects. The SC will work with the Structural Biology Core and Project Leaders to develop these unique reagents. Several BSL2/3/4/antiviral assays with multiple variants have already been established and optimized by the SC. Biochemical, molecular and cell-based assays will be established based on the functionality and selectivity of the antiviral targets identified by individual projects.

Aim 2. Identify novel-hit compounds for each unique viral targets by executing HTS campaign. A diverse collection of ~212K novel compounds, selected through a Machine-Learning Process from non-proprietary libraries assembled from various commercial vendors, targeted fragments library at SR and a focused set of nucleosides, will be screened to identify novel-compounds engaging each viral target. Hit compounds will be validated by determining potency and efficacy for on-target activity and antiviral functions in cell-based assays.

Aim 3. Support hit development, structure-activity relationship studies (SAR) and lead optimization for advancing the leads to preclinical & clinical development. Newly synthesized compounds originating in the Medicinal Chemistry Core will be tested in dose-response assays to provide biological data for the development of SAR for the discovery of chemical probes. Testing will be performed in an iterative fashion to help drive the design-make-test cycle in medicinal chemistry. Lead compounds will also be profiled for activity against multiple-variants, more virulent viral species as well as in secondary assays for early cytotoxicity, organ-specific toxicity and other adverse-reactions.

A comprehensive IT infrastructure with secure Oracle database will be used for screening data storage, analysis, retrieval and follow-up analyses performed by the SC. A Laboratory Management Information System (LIMS) will be used to track and manage reagent and resource consumption.

Introduction

The Screening Core (SC) will develop, optimize, and conduct biochemical and cell-based assays for High & Medium Throughput Screening (HTS) and subsequent testing of compounds synthesized to support hit identification and hit-to-lead efforts for the multiple viral-targets identified by the individual projects in the Antiviral Drug Discovery & Development Center-2 (AD3C-2) (**Figure 1**). The SC will optimize the research reagents needed for functional cell-based and target-based antiviral bioassays for use in HTS campaigns with the goal to identify the leads for target-engagement and to complement medicinal chemistry efforts for lead-optimization for enhanced activity and individual projects to investigate molecular pharmacology of novel-antiviral drug leads. The SC has worked with the individual research projects and identified the targets and assays for initial HTS campaigns. The SC will work with Structural Biology Core (SBC) to produce functionally active recombinant target proteins and also acquire engineered reporter cell-lines for the target viruses. SC will develop and validate assays for HTS and will subsequently conduct all of the HTS campaigns as well as provide data management and analysis of the hits. The SC will also perform the assays needed by the Medicinal Chemistry Core (MCC) to develop the structure-activity relationships (SAR) for each project to ensure a timely turnaround of the biological data necessary to the chemists. By screening a unique, common compound collection against multiple targets across the Projects, the program will seek to identify selective as well as broad-based inhibitors of the targets-linked to viral replication in accordance with the theme of the program. This will require an integrated approach and information sharing between the project centers the SC, SBC and MCC (**Figure 1-Specific Aims**). The SC will also provide resources to assist with specific assays that may be needed by the individual investigators and tailor these assays to support specific viral targets identified by individual projects. This core group has extensive experience in the development and miniaturization of cell based and biochemical assays as well as automation, compound management and bioinformatics experience across a wide variety of assay types. The combined SC at Southern Research (SR) and the University of Texas Medical Branch (UTMB) has the ability to conduct assays and HTS at all levels of biosafety containment (BSL1-4). Several of the key assays needed, such as SARS-CoV-2 cell-based assays using multiple strains of live virus, are already established, validated and are routinely run under BSL2/BSL3 condition at SR[1-6] and BSL2-4 conditions at UTMB[7-10].

Screening Core (SC) Overview The SC shall capitalize on state-of-the-art infrastructure for HTS as well as highly experienced team of researchers at SR and UTMB with complimentary experience on antiviral drug discovery including HTS, transgenic cell-lines, pharmacology, bioassays, target-identification, target-engagement, target-validation and HTS data analysis & management.

The HTS Center at Southern Research has advanced robotic equipment and a well-trained staff to ensure the highest quality of screening for research projects. The Center maintains a collection of over 750,000 compounds and has IT capabilities to enable large scale data analysis and management. Since 2006 the HTS Center at SR has tested over 30 million compound samples in more than 100 screening campaigns which included over 200 assays to obtain quantitative results for hit identification, confirmation, and validation. During this time the HTS Center has supported dozens of grants and contracts originating from both private organizations, academic research institutions and government agencies. Historically, the HTS Center has performed HTS campaigns for several viruses including an *in vitro* antiviral screening program for SARS-CoV[1-6], highly pathogenic (H5N1) avian influenza, and other respiratory viruses (NIAID, N01-AI-30047)[11-14], has served as the Specialized Biocontainment Screening Center for the Molecular Libraries Program during its pilot program (U54 HG003917) and further production phase (U54 HG005034). In addition, the HTS Center has performed ten HTS campaigns as assigned Task Orders under an NIAID/DAIDS contract (N01-AI-1400010I) "In Vitro Testing Resources for HIV Therapeutics and Topical Microbicides", and seven HTS campaigns for various viruses (SARS-CoV, DENV-2, WNV, ZIKV, CHIKV, VEEV, and H1N1/H3N2) as the Screening Core for the Antiviral Drug Discovery and Development (AD3C) program funded under an NIAID supported multi-institute CETR program titled "Identification of New Antiviral Therapies" (1U19AI109680). Currently, the SR HTS Center functions as the Assay Core for the AD3C funded under NIAID CETR grant (U19AI142759).

The Screening Core at University of Texas Medical Branch (UTMB), an internationally recognized institution with strong expertise and resources in the global fight against infectious diseases, is part of the Keiller Building-Galveston National Laboratory (GNL) research complex. GNL is a sophisticated high containment select agent research facility that houses approximately 36,000 sqft of BSL3/ABSL3 and 14,500 sqft of BSL4/ABSL4 space. The latter is split up between the Robert E. Shope BSL4 and the GNL BSL4 laboratories. The GNL is located on the campus of the UTMB and operates under the umbrella of UTMB's Institute for Human Infections and Immunity. Since 2011, UTMB has performed antiviral screens as part of the NIH/NIAID B task order focusing on Ebola, Marburg, Lassa, and Nipah viruses (HHSN2722010000401, B07; HHSN272201100019I, B09, B12, B16, B20, B22, B24, B27, B28, B05, and B07). Several compounds identified through these screening efforts have been further characterized in rodent models for Filoviruses through the NIH/NIAID A task orders

(HHSN272201000040I, A64, HHSN272201700040I A07, A35). In addition, UTMB is home to the World Reference Center for Emerging Viruses and Arboviruses (WRCEAV)[15,16], a collection of over >8,000 arbovirus strains, encompassing 21 viral families. These viruses, along with associated antigens and antibodies, represent a critical resource that the WRCEVA distributes to the research and surveillance community free of charge to facilitate the response to new and continuing outbreaks.

Innovation

The SC will engage novel research reagents (functionally active target recombinant proteins and uniquely engineered reporter cell lines e.g., RVFV NLuc Reporter virus) and employ innovative complex bioassays [e.g., Fluorescence Resonance Energy Transfer(FRET) SARS CoV-2 RdRp complex assay; AlphaScreen assay for CHIV/VEEV macrodomain function; Nipah Virus RdRp mini-genome bioluminescence assay) to screen a new and unique compound library assembled through machine learning algorithm, target-based fragment analysis and unique set of nucleosides for the discovery of new therapeutic approaches for antiviral drug discovery against pathogens with pandemic potential. Novel therapeutic targets have been selected based on evidence of functional integration into the molecular network of interrelated biological pathways linked to unique viral transcriptional machinery of individual viral pathogens. The HTS centers at SR and UTMB have several unique screening platforms, an unparalleled battery of BSL3/BSL4 antiviral screens, streamlined HTS data analysis and management systems as well as exceptionally experienced team of researchers & technicians that have developed and validated multiple types of antiviral assays for use in HTS, SAR and lead optimizations.

Research Strategy

The SC will follow an industry accepted hit-to-lead strategy in early phase drug discovery. Assays suitable for HTS will be developed and employed to identify active compounds which can be used as starting points for medicinal chemistry efforts to develop structure activity relationships for synthesis of new compounds and discovery of leads for further development. The overall research strategy of SC has been outlined in **Figure 2**. Additional details on individual target screens are and an overview of SC are presented in **Figure 1** (Specific Aims). A new unique library composed of compounds with structures designed and synthesized within the last three years will be screened in HTS assays for each proposed target. As such, the compounds in this collection have had very little exposure to human pathogen drug discovery programs, and have a great chance of providing novel, chemically-tractable hits for lead optimization. The unique design of the library is not only based on optimizing diversity in terms of chemical composition and spatial orientation, but also on appropriate physicochemical properties (Lipinski Rules of Five, i.e. MW) to ensure hits are appropriate starting points for medicinal chemistry efforts.

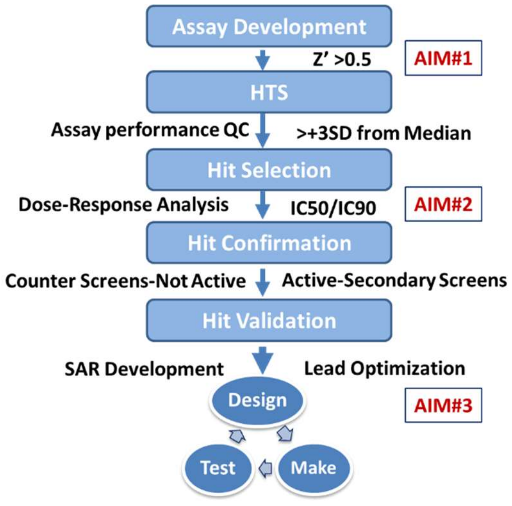


Figure 2-Overall Research Strategy of SC: Outlines

Aim 1. Develop, optimize and miniaturize assays for use in HTS to identify hit compounds and in secondary screening to support the MCC and individual projects.

Although several assays are already available for screening, others will need to be developed and optimized depending on the molecular targets (**Figure 1; Tables 1-3**). Prior to assay development it will be necessary to design and develop required reagents and tools, particularly for the RdRp target assays.

Reagent production. Unique reagents may be required to enable development and performance of an assay for new targets. The SC will work with the SBC to ensure that these reagents can be produced and that they meet the reproducibility and rigor needed to advance a discovery campaign. Also, it will be important to ensure that reagents can be produced in sufficient quantities for screening and to support SAR-driving assays. SARS-CoV-2 NSP12/8/7 RdRp complex[17-21], SARS-CoV-2 NSP14/NSP10 exonuclease function[22-26], alphaviruses macrodomain interactions[27-28], flaviviruses NSP4/NSP5 RdRp activities[29-31] and relevant proteases from target viruses have been identified as potential targets for reagent generation. Additionally, cells-based 'mini-replicon/mini-genome complex' models[32-36] may be established and tailored to investigate functions of individual components of virus replication/transcription machinery.

Development of biochemical and cell-based assays. Once the appropriate reagents are obtained the assays will be constructed and optimized. Since compound samples in the screening collection are dissolved in 100% DMSO, we will initially measure the limit of DMSO tolerance in the assay. The assay will be further developed at the DMSO concentration required for screening to determine appropriate plate type, optimized reagent concentrations, order of addition of reagents, and incubation times that give an optimal signal to background ratio and %CVs.

Assay implementation and optimization. Each assay used in HTS will be optimized to provide a positive signal above background that is of sufficient magnitude and of low enough variability to allow for reliable detection of an active compound when tested once at a single concentration. Overall assay quality will be assessed by several factors during assay implementation and validation and must meet the criteria specified below to be considered for an HTS campaign. A battery of statistical methods (***See Box- Equations 1-3)*** are used at this stage to characterize the suitability of the assay with regard to sensitivity, accuracy, precision and robustness.

Equation 1: $\boldsymbol{Coefficient\ of\ Variation\ (\%CV) = 100 \times \frac{SD}{m}}$
SD = Standard Deviation
m = mean of maximum or minimum control response values
Equation 2: $\boldsymbol{Signal\ to\ Background\ Ratio\ (SBR) = \frac{m_t}{m_b}}$
m_t = Mean of the maximum controls
m_b = Mean of the minimum controls
Equation 3: $\boldsymbol{Z' - factor\ value = 1 - \frac{3(SD_t+SD_b)}{|m_t-m_b|}}$
SD_t = Standard deviation of the maximum controls
SD_b = Standard deviation of the minimum controls

Assay sensitivity. The maximum assay signal should be sufficiently above background to allow for identification of active compounds and minimize the number of false negatives. Assays will be optimized to give SBR ≥ 5 prior to implementation in HTS.

Assay accuracy. Concentration-response assays will be performed for reference compounds, if available, on three different days and the calculated IC_{50} or EC_{50} values will be compared between runs and with those obtained in the originating investigator laboratory or as reported in the literature. The acceptance criteria is that the obtained values agree within 3-fold.

Assay precision. Inter-plate, intra-plate and inter-run reproducibility of the assay will be assessed by calculating the %CV. Acceptance criteria are %CV < 10% for Max and Min signals with SBR > 5. The assay should also exhibit plate spatial uniformity devoid of drift or edge effects. This parameter can be assessed by visual heat maps of the plate data and by calculating ratios of average signals in left/right, top/bottom, inner wells/outer wells, Quad1/Quad4, Q2/Q3, Q1/Q2, Q3/Q4, Q1/Q3 and Q2/Q4 for plates with constant signals in each. Ratios should = 1 for all comparisons of average minimum or average maximum signals.

Assay robustness. When all of the conditions are optimized, three Z-plates consisting of positive and negative controls will be run on three separate days to assess inter- and intra-day variability. For reliability under these conditions, the Z'-factor should be ≥ 0.5, indicating that the minimum and maximum signals are sufficiently separated, and that the variation of these signals do not overlap.

HTS assay validation. The final step prior to undertaking a full screening campaign is to validate the performance of the assay under screening conditions[37-40]. A subset of 10K compounds, selected to represent the chemical diversity of the full collection, will be screened at 10 μM in two independent runs on different days. The results from the two assay runs will be compared statistically to determine whether "hits" and non-hits reproduce on both days. It also provides an initial estimate of the expected hit rate for the full campaign. If the hit rate (using provisionally defined threshold of 50% effect) is very high (>2%) or very low (<0.2%), then the screening concentration can be adjusted for the full campaign. These validation runs must demonstrate that the assay is both robust (Z' ≥ 0.5) and reproducible (correlation P value < 0.001) when performed using the automated platform under conditions to be used in the full HTS campaign. This step is also used to evaluate parameters associated with running large batches of plates such as timing of reagent additions and reagent stability.

Quality control of biological reagents for HTS. As a quality control measure, cell lines will be tested for mycoplasma. Biological reagents amenable to freezing will be stored in frozen aliquots sufficient to supply a single screening run which will avoid repeated freeze/thaw cycles. We will also determine that the stability of all reagents used for an assay will be sufficient to allow for their use in an HTS format in which they may be exposed to suboptimal storage conditions for several hours during the processing of hundreds of assay plates

Aim 2. Identify novel-hit compounds for each target by executing HTS campaigns.

HTS campaigns will be implemented only when each assay is validated as described above. Assay runs will be scheduled in advance depending on the number of runs allowable within a work week to accommodate necessary incubation times. The primary HTS of the compound library will be performed by testing each compound once at a single concentration, typically 10 μM. Positive and negative controls used to assess assay quality will be placed in the outside columns of each plate to accommodate automation of liquid dispensing systems. The data generated during a screening campaign will be evaluated for consistency and bias as described above. Plates with non-uniform spatial patterns will also be scrutinized for possible effects due to liquid handling errors and scheduled for retesting. Raw values for positive and negative controls will be tracked by plate over the course of the screen and control CV values and Z'-values calculated for each plate. The trends observed in the controls are helpful in troubleshooting the automation process to minimize artifacts or reagent failures. Controls also serve a quality control purpose. Plates that do not demonstrate acceptable Z'-values, positive control compound response inconsistent with historical values or spatial-non-uniformity will be rejected and scheduled for retesting.

Upon completion of a screen, a statistical analysis of the HTS data will be performed to determine overall distribution and variability of data and to set the activity threshold or “hit” criteria at 3xSD above the average value for all of the compound wells. This is accepted as the value which is statistically outside the population of no effect data. The database will be queried to correlate the selected hits with the specified source plate and well locations for each compound. The “hit” compounds will be cherry picked from the source plates and retested in a 10-point concentration response series from 0.2 -100 µM to confirm activity and determine the potency of the compound to inhibit or enhance the assay signal. A counter-screen will be performed to exclude false positives resulting from off-target effects. In biochemical assays compounds will be tested in assays omitting the molecular target in order to exclude compounds causing interference with the assay signal. In cell-based assays compounds will be tested for cytotoxic effect on uninfected host cells. Concentration-response assays are set up as serial two-fold dilutions in an inter-plate rather than intra-plate dilution scheme to enable more efficient and cost-effective use of liquid handling systems. This method also allows for many more compounds to be selected and tested in a concentration-response assay, thus maximizing potency and efficacy data available to chemists for selecting scaffolds to advance through the hit-to-lead process.

The biological single concentration and concentration response data will be uploaded into an ActivityBase database. The technical lead scientist who conducted the screen will review the data and accept or reject the assay plates based on defined assay criteria. Once verified in ActivityBase, the data is then accessible to the chemistry team through Dotmatics and available for additional analysis such as a cluster analysis, analysis of structure–activity relationship (SAR) trends and property predictions using sophisticated software such as Pipeline Pilot, ScaffoldHunter, Star Drop, Dotmatics-Vortex, StarVision, Sybyl and Schrödinger-QikProp. The ActivityBase administrator will prepare a report describing overall assay statistics for the HTS campaign as well as a report containing concentration-response data and graphs for each individual hit compound.

The medicinal-computational chemistry team will perform cluster analysis of the hits to organize the small molecules into groups based on common core structural features referred to as clusters containing a common substructure or scaffold. In some cases, a compound may have structural features in the molecule that permits the molecule to be grouped into more than one clustered group of molecules. The hits will be analyzed and clustered based on Tanimoto distances using the predefined FCFP_4 fingerprint property of Pipeline Pilot software (Accelrys, San Diego, CA). The cluster analysis and scaffold enrichment analysis that compares the distribution of members of each scaffold within the hit set versus their distribution in the entire screening set provides knowledge regarding privileged scaffolds and structural features present in the hits. The chemists will also utilize property predictions such as solubility and metabolic liabilities of compound clusters of interest to permit prioritization of the active compounds/scaffolds based on lead-like characteristics.

Unique HTS Compounds Collections. To address the need for novel, drug-like, and target-specific antiviral chemicals, a new HTS library of 200K compounds has been assembled from recent drug-like commercial collections (**Figure 3**). A major part of this new library is a 150K diversity set from ChemDiv. This customized selection aided by our cheminformatics approaches processes multiple merits. Specifically, it only contains new chemical structures that have been synthesized within the last three years thus making this a less explored and more novel collection. It does not contain duplicates to our current HTS collection for pan-assay interference structures (PAINS)[41-45]. The library has high structural diversity (~7,500 clusters with average size of 20 compounds per cluster) but no singletons (thus every hit will have initial structure-activity-relationship information without testing additional analogs). The compounds are also pre-rated by ChemDiv to ensure synthetic feasibility. The molecular property distribution analysis indicates that the compounds in the new library are drug-like (molecular weight ≤ 500; heteroatom count ≤ 10; rotatable bonds ≤ 8; aromatic rings ≤ 4; predicted log P ≤ 5; polar surface area ≤ 125; H-bond acceptors ≤ 7; H-bond donors ≤ 3). Moreover, according to our in-house developed machine learning prediction model for SARS-CoV-2, 20K of the 150K diversity set are likely to show phenotypical anti-SARS-CoV-2 activity, which makes it suitable to meet the special need for SARS-CoV-2 drug discovery. An additional 25K compounds have been selected

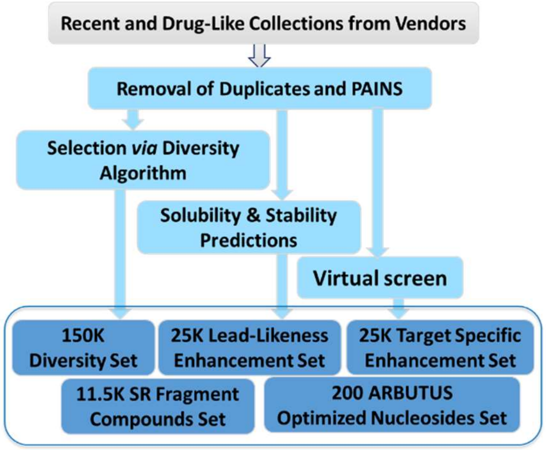


Figure 3- Unique HTS Compounds Library Sets

according to solubility and metabolic stability predictions (*via* cheminformatics software BIOVIA Pipeline Pilot) to increase the chance of finding hits with good pharmacological properties and facilitate the hit-to-lead pipeline. A 25K target specificity set selected by in-house virtual screens against viral targets nsP2 and nsP3 and virtual screens performed by vendors (e.g. viral RdRP inhibitor collections from OTAVA Chemicals and TargetMol) has been added to enhance target focus. Since the SBC will be able to crystalize several targets we will also include a library of 11,350 fragment compounds existing at SR and a library of 200 nucleotides provided by Arbutus. As a whole, these collections comprise a 211,550 HTS compound library suitable to discover novel, drug-like, and target-specific antiviral matters (**Figure 3**).

Compound Storage, Replication and Distribution: The compound library dissolved at 10 mM in 100% DMSO will be stored in deep well polypropylene 384-well plates containing 200 µL samples. This plate set is called a “mother” set. The “mother” set is then used to create copies of 384-well “daughter” plates in 384-well polypropylene Echo-qualified flat-bottom plates to allow for creation of assay ready plates (ARPs). All plates are barcoded and the compound IDs with plate and well locations are loaded to IDBS ActivityBase where they are tracked during all processes including compound plate replications, production of ARPs for single concentration and concentration response screening campaigns, distribution of ARPs to the screening labs at SR and UTMB as well as recording compound storage locations and maintaining inventory information.

HTS Assays. The HTS assays to identify compounds for each virus target are presented in Table 1. The HTS targets have been selected based on individual project objectives.

Project #	Virus	Target	Assay Type	Assay Technology	Assay Signal
1	SARS CoV-2	Nsp12/7/8 RdRp	biochemical	FRET	fluorescence
1	SARS CoV-2	Nsp14 ExoN	biochemical	Quanti-it RiboGreen	fluorescence
2	NIPAV	Mini-genome	cell-based	NLuc Reporter	luminescence
3	CHIKV	Macrodomain	biochemical	AlphaScreen	luminescence
3	VEEV	Macrodomain	biochemical	AlphaScreen	luminescence
4	RVFV	Phenotype	cell-based	NLuc Reporter Virus	luminescence
4	Bunya V	L Protein RdRp	biochemical	FP	fluorescence
5	DENV	NS5 polymerase	biochemical	AttoPhos	fluorescence

<u>SARS-CoV-2</u>: A FRET-based assay identifies RdRp inhibitors in which SARS-CoV-2 RdRp (NSP12/7/8) synthesizes RNA on a primer strand labeled with Cy3 and displaces a quencher strand resulting in an increase in Cy3 fluorescent signal[17]. SARS CoV-2 NSP14 protein is a bi-functional enzyme with an N-terminal 3-5-exoribonuclease (ExoN) domain and a C-terminal SAM-dependent N7-Methyltransferase[23],[46]. NSP14/NSP10 Exo-N function may be measured with intercalating Quant-it TM RiboGreen RNA reagent, which shows specific binding with dsRNA and generates strong fluorescent signal[47].

<u>DENV</u>: RdRp (NS5): Fluorescence-based alkaline phosphatase–coupled polymerase assay utilizes a modified nucleotide analogue (2′-[2-benzothiazoyl]-6′-hydroxy- benzothiazole) conjugated adenosine triphosphate (BBT-ATP) and 3′UTR-U30 RNA as substrates. After the polymerase reaction, treatment with alkaline phosphatase liberates the BBT fluorophore from the polymerase reaction byproduct[48, 49]. The assay can be performed with recombinant NS5 from DENV, WNV and ZIKV [50-53]

<u>NiV-</u> Small molecules inhibiting the NiV RNA synthesis machinery[54]. We will develop and optimize a HTS assay based on a NiV minigenome (MG) system. Synthesis of NiV RNA is dependent on the complex of several viral proteins, including the nucleoprotein (N), the phosphoprotein (P), and the viral RdRP[55]. Reconstitution of a functional NiV RdRp complex can be achieved by transfection of these three proteins into mammalian cells[56,57].

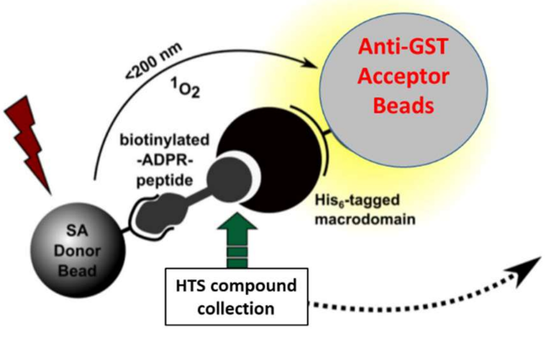


Figure 4. CHIKV Macrodomain AlphaScreen- Employs GST-tagged CHIKV NSP3, Biotinylated ADP-ribose attached peptide, Biotinylated-GST (Counter Screen) and GST Detection kit

<u>CHIKV and VEEV</u>: An AlphaScreen binding displacement assay to screen for compounds that inhibit the interaction of an ADP-ribose attached peptide to the nsP3 macrodomain[58-60] has been modified and validated for HTS at SR **(Figure 4**)

<u>RVFV:</u> A cell based nanoluciferase reporter virus assay measuring inhibition of virus replication[61,62]. We have already employed a similar assay for screening against SARS CoV-2[2,3].

Additional target assays will be included to enable dynamic individual project needs.

Aim 3. Support hit development, structure-activity relationship studies (SAR) and lead optimization for advancing the leads to preclinical & clinical development. The biochemical assays measuring target engagement of compounds will be used to drive hit-to-lead SAR. In most cases, these will be the same assays employed for HTS. However, it will be necessary to demonstrate that compounds showing target engagement in biochemical assays also exhibit antiviral activity against the live virus in a secondary cell-based assay. Also, the cell-based assays can be used to assess if compounds have pan-virus effects. These assays already exist in the SC and have been used to the support ongoing medicinal chemistry efforts of the current AD3C program. **The assays available for each virus are shown in Table 2** The MOA of compounds identified in the cell-based HTS for RVFV will be determined by the project team and the relevant target based assay will be subsequently established in the SC to drive SAR.

Virus	Assay type	Host cell
SARS CoV-2	Cytopathic Effect Reduction	VeroE6
SARS CoV-2	Virus linked nanoluciferase reporter	Calu-3 and A549 expressing ACE-2
SARS CoV-1	Cytopathic Effect Reduction	Vero E6
SARS CoV-1	Virus linked nanoluciferase reporter	Calu-3 and A549 expressing ACE-2
MERS	Cytopathic Effect Reduction	Vero CCL81
CHIKV	Virus linked nanoluciferase reporter	THF
VEEV	Cytopathic Effect Reduction	THF
DENV-2	Immunofluorescence assay	HEK293
DENV-4	Virus linked nanoluciferase reporter	HEK293
ZIKV	Immunofluorescence assay	Vero CCL81

The SC at UTMB will primarily focus on performing antiviral screens utilizing infectious viruses at BSL4 to identify small molecules that target viral replication. Previous efforts between SR and UTMB have established an automated HST platform, which was capable of testing up to 10,000 compounds per day within the BSL4 laboratory[63,64]. Using a cell-based HTS screen, compounds were identified for their ability to inhibit virus-induced cytopathic effect. However, since successfully establishing this HTS capability under BSL4 conditions, the platform has not been utilized since then. We propose to re-establish the screening capabilities at both BSL3 and BSL4 and then utilize to perform the screens through this project. Assays at BSL4 will focus on NiV[63,64], but will also be established for tick-borne flaviviruses (such as Central European Encephalitis virus) and *Nairoviridae* (Crimean-Congo hemorrhagic fever virus) to evaluate leads identified in Projects 2 and 4. At BSL3, assays will also be established for the viruses relevant to this application (SARS-CoV-1, SARS-CoV-2, MERS, CHIKV, VEEV) using cytopathic effect reduction and virus linked nanoluciferase reporter assays, as described in **Table 2**. Furthermore, antiviral screens may be employed expanding the screening of lead compounds based on individual projects. This could for example cover antiviral compounds identified in Project 4 for members of the *Bunyavirales* order and could be tested against additional human pathogenic members (such as Severe Fever with Thrombocytopenia Syndrome virus and Oropouche virus). In addition to wild-type viruses, certain recombinant viruses expressing fluorescent or luminescent reporter genes are available. **Table 3: Screening Core UTMB- Antiviral assays for BSL-4 viruses available for this program** and may be employed for expanding the screening of leads based on individual Project needs.

Virus	Assay type	Cell system
Filovirus		
Ebola Zaire (EBOV)	Cytopathic Effect Reduction	Vero CCL81, HeLa
Sudan virus (SUDV)	Cytopathic Effect Reduction	Vero CCL81
Marburg virus Angola (MARV)	Cytopathic Effect Reduction	Vero CCL81
Henipavirus		
Nipah virus (NiV)	Cytopathic Effect Reduction	Vero CCL81
rNiV-Gluc-eGFP	Luciferase Assay	Vero CCL81
Hendra virus (HeV)	Cytopathic Effect Reduction	Vero CCL81
Bunyavirales		
Rift Valley Fever virus ZH501 (RVFV)	Cytopathic Effect Reduction	Vero CCL81, A549
Crimean-Congo Hemorrhagic fever virus (CCHFV)	Cytopathic Effect Reduction	SW-13
Lassa virus (LASV)	Cytopathic Effect Reduction	Vero CCL81, Vero 76
Machupo virus (MACV)	Cytopathic Effect Reduction	Vero CCL81
Junin virus (JUNV)	Cytopathic Effect Reduction	Vero CCL81

Additional viruses that can be screened for at BSL-4 include tick-borne flaviviruses, as well as additional ebolavirus strains and arenaviruses.

Initial efforts will focus on assay implementation, optimization (as needed), and validation as described above. During this phase, the UTMB SC will work closely with colleagues at SR utilizing their HTS expertise, compound libraries, and bioinformatic support. For actual screens at UTMB and to simplify the assay performance, assay plates will be prepared at SR, sealed, and frozen plates send to UTMB. Screening will be performed against selected viruses listed in **Table 3**. Compounds that demonstrate antiviral activity in the primary screen, will then be confirmed in concentration dose-response, cell toxicity, viral yield reduction, and/or time-of-addition assays. Characterization of the MOA of identified small molecules will be further characterized by the individual Research Projects.

References Cited

1. Ahmed SK, Haese NN, Cowan JT, Pathak V, Moukha-Chafiq O, Smith VJ, Rodzinak KJ, Ahmad F, Zhang S, Bonin KM, Streblow AD, Streblow CE, Kreklywich CN, Morrison C, Sarkar S, Moorman N, Sander W, Allen R, DeFilippis V, Tekwani BL, Wu M, Hirsch AJ, Smith JL, Tower NA, Rasmussen L, Bostwick R, Maddry JA, Ananthan S, Gerdes JM, Augelli-Szafran CE, Suto MJ, Morrison TE, Heise MT, Streblow DN, Pathak AK. Targeting Chikungunya Virus Replication by Benzoannulene Inhibitors. J Med Chem. 2021 Apr 22;64(8):4762-4786. doi: 10.1021/acs.jmedchem.0c02183. Epub 2021 Apr 9. PMID: 33835811.
2. Chen CZ, Shinn P, Itkin Z, Eastman RT, Bostwick R, Rasmussen L, Huang R, Shen M, Hu X, Wilson KM, Brooks BM, Guo H, Zhao T, Klump-Thomas C, Simeonov A, Michael SG, Lo DC, Hall MD, Zheng W. Drug Repurposing Screen for Compounds Inhibiting the Cytopathic Effect of SARS-CoV-2. Front Pharmacol. 2021 Jan 25;11:592737. doi: 10.3389/fphar.2020.592737. PMID: 33708112; PMCID: PMC7942396.
3. Gorshkov K, Chen CZ, Bostwick R, Rasmussen L, Tran BN, Cheng YS, Xu M, Pradhan M, Henderson M, Zhu W, Oh E, Susumu K, Wolak M, Shamim K, Huang W, Hu X, Shen M, Klumpp-Thomas C, Itkin Z, Shinn P, Carlos de la Torre J, Simeonov A, Michael SG, Hall MD, Lo DC, Zheng W. The SARS-CoV-2 Cytopathic Effect Is Blocked by Lysosome Alkalizing Small Molecules. ACS Infect Dis. 2021 Jun 11;7(6):1389-1408. doi: 10.1021/acsinfecdis.0c00349. Epub 2020 Dec 21. PMID: 33346633; PMCID: PMC7771250.
4. Chen CZ, Shinn P, Itkin Z, Eastman RT, Bostwick R, Rasmussen L, Huang R, Shen M, Hu X, Wilson KM, Brooks B, Guo H, Zhao T, Klump-Thomas C, Simeonov A, Michael SG, Lo DC, Hall MD, Zheng W. Drug Repurposing Screen for Compounds Inhibiting the Cytopathic Effect of SARS-CoV-2. bioRxiv [Preprint]. 2020 Aug 18:2020.08.18.255877. doi: 10.1101/2020.08.18.255877. Update in: Front Pharmacol. 2021 Jan 25;11:592737. PMID: 32839771; PMCID: PMC7444282.
5. Gorshkov K, Chen CZ, Bostwick R, Rasmussen L, Xu M, Pradhan M, Tran BN, Zhu W, Shamim K, Huang W, Hu X, Shen M, Klumpp-Thomas C, Itkin Z, Shinn P, Simeonov A, Michael S, Hall MD, Lo DC, Zheng W. The SARS-CoV-2 cytopathic effect is blocked with autophagy modulators. bioRxiv [Preprint]. 2020 May 28:2020.05.16.091520. doi: 10.1101/2020.05.16.091520. Update in: ACS Infect Dis. 2020 Dec 21;: PMID: 32511355; PMCID: PMC7259466.
6. Goebel S, Snyder B, Sellati T, Saeed M, Ptak R, Murray M, Bostwick R, Rayner J, Koide F, Kalkeri R. A sensitive virus yield assay for evaluation of Antivirals against Zika Virus. J Virol Methods. 2016 Dec;238:13-20. doi: 10.1016/j.jviromet.2016.09.015. Epub 2016 Oct 3. PMID: 27678028
7. Terasaki K, Kalveram B, Johnson KN, Juelich T, Smith JK, Zhang L, Freiberg AN, Makino S. Rift Valley fever virus 78kDa envelope protein attenuates virus replication in macrophage-derived cell lines and viral virulence in mice. PLoS Negl Trop Dis. 2021 Sep 13;15(9):e0009785. doi: 10.1371/journal.pntd.0009785. PMID: 34516560; PMCID: PMC8460012.
8. Escaffre O, Freiberg AN. Polyphenylene carboxymethylene (PPCM) microbicide repurposed as antiviral against SARS-CoV-2. Proof of concept in primary human undifferentiated epithelial cells. Antiviral Res. 2021 Oct;194:105162. doi: 10.1016/j.antiviral.2021.105162. Epub 2021 Aug 9. PMID: 34384825; PMCID: PMC8349736.
9. Tigabu B, Ramanathan P, Ivanov A, Lin X, Ilinykh PA, Parry CS, Freiberg AN, Nekhai S, Bukreyev A. Phosphorylated VP30 of Marburg Virus Is a Repressor of Transcription. J Virol. 2018 Oct 12;92(21):e00426-18. doi: 10.1128/JVI.00426-18. PMID: 30135121; PMCID: PMC6189487.
10. Atkins C, Freiberg AN. Recent advances in the development of antiviral therapeutics for Rift Valley fever virus infection. Future Virol. 2017 Nov;12(11):651-665. doi: 10.2217/fvl-2017-0060. Epub 2017 Oct 23. PMID: 29181086; PMCID: PMC5696620
11. Noah JW, Severson W, Chung DH, Moore B, Jia F, Xu X, Maddox C, Rasmussen L, Sosa MI, Tower NA, Ananthan S, White EL, Jonsson C, Matharu DS, Golden JE, Prisinzano TE, Aubé J. A Cell Based HTS Approach for the Discovery of New Inhibitors of RSV. 2011 Apr 15 [updated 2013 Feb 28]. In: Probe Reports from the NIH Molecular Libraries Program [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2010–. PMID: 23658949.
12. Chung DH, Jonsson CB, Maddox C, McKellip SN, Moore BP, Heil M, White EL, Ananthan S, Li Q, Feng S, Rasmussen L. HTS-driven discovery of new chemotypes with West Nile Virus inhibitory activity.

Molecules. 2010 Mar 12;15(3):1690-704. doi: 10.3390/molecules15031690. PMID: 20336008; PMCID: PMC4839297.

13. Li Q, Maddox C, Rasmussen L, Hobrath JV, White LE. Assay development and high-throughput antiviral drug screening against Bluetongue virus. Antiviral Res. 2009 Sep;83(3):267-73. doi: 10.1016/j.antiviral.2009.06.004. Epub 2009 Jun 24. PMID: 19559054; PMCID: PMC2727572.
14. Noah JW, Severson W, Noah DL, Rasmussen L, White EL, Jonsson CB. A cell-based luminescence assay is effective for high-throughput screening of potential influenza antivirals. Antiviral Res. 2007 Jan;73(1):50-9. doi:10.1016/j.antiviral.2006.07.006. Epub 2006 Jul 28. PMID: 16904762.
15. Zhao G, Krishnamurthy S, Cai Z, Popov VL, Travassos da Rosa AP, Guzman H, Cao S, Virgin HW, Tesh RB, Wang D. Identification of novel viruses using VirusHunter --an automated data analysis pipeline. PLoS One. 2013 Oct 22;8(10):e78470. doi: 10.1371/journal.pone.0078470. PMID: 24167629; PMCID: PMC3805514.
16. Popov VL, Tesh RB, Weaver SC, Vasilakis N. Electron Microscopy in Discovery of Novel and Emerging Viruses from the Collection of the World Reference Center for Emerging Viruses and Arboviruses (WRCEVA). Viruses. 2019 May 25;11(5):477. doi: 10.3390/v11050477. PMID: 31130629; PMCID: PMC6563235.
17. Bertolin AP, Weissmann F, Zeng J, Posse V, Milligan JC, Canal B, Ulferts R, Wu M, Drury LS, Howell M, Beale R, Diffley JFX. Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of nsp12/7/8 RNA-dependent RNA polymerase. Biochem J. 2021 Jul 16;478(13):2425-2443. doi: 10.1042/BCJ20210200. PMID: 34198323; PMCID: PMC8286815
18. Pruijssers AJ, George AS, Schäfer A, Leist SR, Gralinksi LE, Dinnon KH 3rd, Yount BL, Agostini ML, Stevens LJ, Chappell JD, Lu X, Hughes TM, Gully K, Martinez DR, Brown AJ, Graham RL, Perry JK, Du Pont V, Pitts J, Ma B, Babusis D, Murakami E, Feng JY, Bilello JP, Porter DP, Cihlar T, Baric RS, Denison MR, Sheahan TP. Remdesivir Inhibits SARS-CoV-2 in Human Lung Cells and Chimeric SARS-CoV Expressing the SARS-CoV-2 RNA Polymerase in Mice. Cell Rep. 2020 Jul 21;32(3):107940. doi: 10.1016/j.celrep.2020.107940. Epub 2020 Jul 7. PMID: 32668216; PMCID: PMC7340027
19. Jochheim FA, Tegunov D, Hillen HS, Schmitzová J, Kokic G, Dienemann C, Cramer P. The structure of a dimeric form of SARS-CoV-2 polymerase. Commun Biol. 2021 Aug 24;4(1):999. doi: 10.1038/s42003-021-02529-9. PMID: 34429502; PMCID: PMC8385044.
20. Biswal M, Diggs S, Xu D, Khudaverdyan N, Lu J, Fang J, Blaha G, Hai R, Song J. Two conserved oligomer interfaces of NSP7 and NSP8 underpin the dynamic assembly of SARS-CoV-2 RdRP. Nucleic Acids Res. 2021 Jun 4;49(10):5956-5966. doi: 10.1093/nar/gkab370. PMID: 33999154; PMCID: PMC8191759.
21. Wang Q, Wu J, Wang H, Gao Y, Liu Q, Mu A, Ji W, Yan L, Zhu Y, Zhu C, Fang X, Yang X, Huang Y, Gao H, Liu F, Ge J, Sun Q, Yang X, Xu W, Liu Z, Yang H, Lou Z, Jiang B, Guddat LW, Gong P, Rao Z. Structural Basis for RNA Replication by the SARS-CoV-2 Polymerase. Cell. 2020 Jul 23;182(2):417-428.e13. doi:10.1016/j.cell.2020.05.034. Epub 2020 May 22. PMID: 32526208; PMCID: PMC7242921.
22. Khater S, Kumar P, Dasgupta N, Das G, Ray S, Prakash A. Combining SARS-CoV-2 Proofreading Exonuclease and RNA-Dependent RNA Polymerase Inhibitors as a Strategy to Combat COVID-19: A High-Throughput <i>in silico</i> Screening. Front Microbiol. 2021 Jul 20;12:647693. doi: 10.3389/fmicb.2021.647693. PMID: 34354677; PMCID: PMC8329495.
23. Yan L, Yang Y, Li M, Zhang Y, Zheng L, Ge J, Huang YC, Liu Z, Wang T, Gao S, Zhang R, Huang YY, Guddat LW, Gao Y, Rao Z, Lou Z. Coupling of N7-methyltransferase and 3'-5' exoribonuclease with SARS-CoV-2 polymerase reveals mechanisms for capping and proofreading. Cell. 2021 Jun 24;184(13):3474-3485.e11. doi: 10.1016/j.cell.2021.05.033. Epub 2021 May 24. PMID: 34143953; PMCID: PMC8142856.
24. Lin S, Chen H, Chen Z, Yang F, Ye F, Zheng Y, Yang J, Lin X, Sun H, Wang L, Wen A, Dong H, Xiao Q, Deng D, Cao Y, Lu G. Crystal structure of SARS-CoV-2 nsp10 bound to nsp14-ExoN domain reveals an exoribonuclease with both structural and functional integrity. Nucleic Acids Res. 2021 May 21;49(9):5382-5392. doi: 10.1093/nar/gkab320. PMID: 33956156; PMCID: PMC8136770.

25. Moeller NH, Shi K, Demir Ö, Banerjee S, Yin L, Belica C, Durfee C, Amaro RE, Aihara H. Structure and dynamics of SARS-CoV-2 proofreading exoribonuclease ExoN. bioRxiv [Preprint]. 2021 Apr 4:2021.04.02.438274. doi: 10.1101/2021.04.02.438274. PMID: 33821277; PMCID: PMC8020977.
26. Ogando NS, Zevenhoven-Dobbe JC, van der Meer Y, Bredenbeek PJ, Posthuma CC, Snijder EJ. The Enzymatic Activity of the nsp14 Exoribonuclease Is Critical for Replication of MERS-CoV and SARS-CoV-2. J Virol. 2020 Nov 9;94(23):e01246-20. doi: 10.1128/JVI.01246-20. PMID: 32938769; PMCID: PMC7654266
27. Alhammad YMO, Fehr AR. The Viral Macrodomain Counters Host Antiviral ADP- Ribosylation. Viruses. 2020 Mar 31;12(4):384. doi: 10.3390/v12040384. PMID: 32244383; PMCID: PMC7232374.
28. Eckei L, Krieg S, Bütepage M, Lehmann A, Gross A, Lippok B, Grimm AR, Kümmerer BM, Rossetti G, Lüscher B, Verheugd P. The conserved macrodomains of the non-structural proteins of Chikungunya virus and other pathogenic positive strand RNA viruses function as mono-ADP-ribosylhydrolases. Sci Rep. 2017 Feb 2;7:41746. doi: 10.1038/srep41746. PMID: 28150709; PMCID: PMC5288732.
29. Lim SP, Noble CG, Nilar S, Shi PY, Yokokawa F. Discovery of Potent Non-nucleoside Inhibitors of Dengue Viral RNA-Dependent RNA Polymerase from Fragment Screening and Structure-Guided Design. Adv Exp Med Biol. 2018;1062:187-198. doi: 10.1007/978-981-10-8727-1_14. PMID: 29845534.
30. Lim SP, Noble CG, Seh CC, Soh TS, El Sahili A, Chan GK, Lescar J, Arora R, Benson T, Nilar S, Manjunatha U, Wan KF, Dong H, Xie X, Shi PY, Yokokawa F. Potent Allosteric Dengue Virus NS5 Polymerase Inhibitors: Mechanism of Actio and Resistance Profiling. PLoS Pathog. 2016 Aug 8;12(8):e1005737. doi: 10.1371/journal.ppat.1005737. PMID: 27500641; PMCID: PMC4976923.
31. Lim SP, Noble CG, Shi PY. The dengue virus NS5 protein as a target for drug discovery. Antiviral Res. 2015 Jul;119:57-67. doi: 10.1016/j.antiviral.2015.04.010. Epub 2015 Apr 24. PMID: 25912817
32. Jérôme H, Rudolf M, Lelke M, Pahlmann M, Busch C, Bockholt S, Wurr S, Günther S, Rosenthal M, Kerber R. Rift Valley fever virus minigenome system for investigating the role of L protein residues in viral transcription and replication. J Gen Virol. 2019 Jul;100(7):1093-1098. doi: 10.1099/jgv.0.001281. Epub 2019 Jun 6. PMID: 31169489.
33. Teng MN, Tran KC. Use of Minigenome Systems to Study RSV Transcription. Methods Mol Biol. 2016;1442:155-64. doi: 10.1007/978-1-4939-3687-8_11. PMID: 27464693.
34. Uebelhoer LS, Albariño CG, McMullan LK, Chakrabarti AK, Vincent JP, Nichol ST, Towner JS. High-throughput, luciferase-based reverse genetics systems for identifying inhibitors of Marburg and Ebola viruses. Antiviral Res. 2014 Jun;106:86-94. doi: 10.1016/j.antiviral.2014.03.018. Epub 2014 Apr 5. PMID: 24713118.
35. Klemm C, Reguera J, Cusack S, Zielecki F, Kochs G, Weber F. Systems to establish bunyavirus genome replication in the absence of transcription. J Virol. 2013 Jul;87(14):8205-12. doi: 10.1128/JVI.00371-13. Epub 2013 May 22. PMID: 23698297; PMCID: PMC3700186.
36. Kohl A, Hart TJ, Noonan C, Royall E, Roberts LO, Elliott RM. A bunyamwera virus minireplicon system in mosquito cells. J Virol. 2004 Jun;78(11):5679-85. doi: 10.1128/JVI.78.11.5679-5685.2004. PMID: 15140965; PMCID: PMC415823.
37. Genick CC, Wright SK. Biophysics: for HTS hit validation, chemical lead optimization, and beyond. Expert Opin Drug Discov. 2017 Sep;12(9):897-907. doi: 10.1080/17460441.2017.1349096. Epub 2017 Jul 6. PMID: 28658992.
38. Judson R, Kavlock R, Martin M, Reif D, Houck K, Knudsen T, Richard A, Tice RR, Whelan M, Xia M, Huang R, Austin C, Daston G, Hartung T, Fowle JR 3rd, Wooge W, Tong W, Dix D. Perspectives on validation of high-throughput assays supporting 21st century toxicity testing. ALTEX. 2013;30(1):51-6. doi: 10.14573/altex.2013.1.051. PMID: 23338806; PMCID: PMC3934015.
39. Brooks HB, Geeganage S, Kahl SD, Montrose C, Sittampalam S, Smith MC, Weidner JR. Basics of Enzymatic Assays for HTS. 2012 May 1 [updated 2012 Oct 1]. In: Markossian S, Grossman A, Brimacombe K, Arkin M, Auld D, Austin CP, Baell J, Chung TDY, Coussens NP, Dahlin JL, Devanarayan V, Foley TL, Glicksman M, Hall MD, Haas JV, Hoare SRJ, Inglese J, Iversen PW, Kales SC, Lal-Nag M, Li Z, McGee J, McManus O, Riss T, Saradjian P, Sittampalam GS, Tarselli M, Trask OJ Jr, Wang Y, Weidner JR, Wildey MJ, Wilson K, Xia M, Xu X, editors. Assay Guidance Manual [Internet]. Bethesda

(MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004–. PMID: 22553875.

40. Iversen PW, Beck B, Chen YF, Dere W, Devanarayan V, Eastwood BJ, Farmen MW, Iturria SJ, Montrose C, Moore RA, Weidner JR, Sittampalam GS. HTS Assay Validation. 2012 May 1 [updated 2012 Oct 1]. In: Markossian S, Grossman A, Brimacombe K, Arkin M, Auld D, Austin CP, Baell J, Chung TDY, Coussens NP, Dahlin JL, Devanarayan V, Foley TL, Glicksman M, Hall MD, Haas JV, Hoare SRJ, Inglese J, Iversen PW, Kales SC, Lal-Nag M, Li Z, McGee J, McManus O, Riss T, Saradjian P, Sittampalam GS, Tarselli M, Trask OJ Jr, Wang Y, Weidner JR, Wildey MJ, Wilson K, Xia M, Xu X, editors. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004–. PMID: 22553862
41. Bolz SN, Adasme MF, Schroeder M. Toward an Understanding of Pan-Assay Interference Compounds and Promiscuity: A Structural Perspective on Binding Modes. J Chem Inf Model. 2021 May 24;61(5):2248-2262. doi: 10.1021/acs.jcim.0c01227. Epub 2021 Apr 26. PMID: 33899463.
42. Dantas RF, Evangelista TCS, Neves BJ, Senger MR, Andrade CH, Ferreira SB, Silva-Junior FP. Dealing with frequent hitters in drug discovery: a multidisciplinary view on the issue of filtering compounds on biological screenings. Expert Opin Drug Discov. 2019 Dec;14(12):1269-1282. doi: 10.1080/17460441.2019.1654453. Epub 2019 Aug 16. PMID: 31416369.
43. Jasial S, Gilberg E, Blaschke T, Bajorath J. Machine Learning Distinguishes with High Accuracy between Pan-Assay Interference Compounds That Are Promiscuous or Represent Dark Chemical Matter. J Med Chem. 2018 Nov 21;61(22):10255-10264. doi: 10.1021/acs.jmedchem.8b01404. Epub 2018 Nov 13. PMID: 30422657.
44. Siramshetty VB, Preissner R, Gohlke BO. Exploring Activity Profiles of PAINS and Their Structural Context in Target-Ligand Complexes. J Chem Inf Model. 2018 Sep 24;58(9):1847-1857. doi: 10.1021/acs.jcim.8b00385. Epub 2018 Aug 27. PMID: 30105913.
45. Chakravorty SJ, Chan J, Greenwood MN, Popa-Burke I, Remlinger KS, Pickett SD, Green DVS, Fillmore MC, Dean TW, Luengo JI, Macarrón R. Nuisance Compounds, PAINS Filters, and Dark Chemical Matter in the GSK HTS Collection. SLAS Discov. 2018 Jul;23(6):532-545. doi: 10.1177/2472555218768497. Epub 2018 Apr 26. PMID: 29699447.
46. Basu S, Mak T, Ulferts R, Wu M, Deegan T, Fujisawa R, Tan KW, Lim CT, Basier C, Canal B, Curran JF, Drury LS, McClure AW, Roberts EL, Weissmann F, Zeisner TU, Beale R, Cowling VH, Howell M, Labib K, Diffley JFX. Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of Nsp14 RNA cap methyltransferase. Biochem J. 2021 Jul 16;478(13):2481-2497. doi: 10.1042/BCJ20210219. PMID: 34198328; PMCID: PMC8286817.
47. Canal B, McClure AW, Curran JF, Wu M, Ulferts R, Weissmann F, Zeng J, Bertolin AP, Milligan JC, Basu S, Drury LS, Deegan TD, Fujisawa R, Roberts EL, Basier C, Labib K, Beale R, Howell M, Diffley JFX. Identifying SARS-CoV-2 antiviral compounds by screening for small molecule inhibitors of nsp14/nsp10 exoribonuclease. Biochem J. 2021 Jul 16;478(13):2445-2464. doi: 10.1042/BCJ20210198. PMID: 34198326; PMCID: PMC8286829.
48. Duan Y, Zeng M, Jiang B, Zhang W, Wang M, Jia R, Zhu D, Liu M, Zhao X, Yang Q, Wu Y, Zhang S, Liu Y, Zhang L, Yu Y, Pan L, Chen S, Cheng A. <i>Flavivirus</i> RNA-Dependent RNA Polymerase Interacts with Genome UTRs and Viral Proteins to Facilitate <i>Flavivirus</i> RNA Replication. Viruses. 2019 Oct 10;11(10):929. doi: 10.3390/v11100929. PMID: 31658680; PMCID: PMC6832647.
49. Niyomrattanakit P, Abas SN, Lim CC, Beer D, Shi PY, Chen YL. A fluorescence- based alkaline phosphatase-coupled polymerase assay for identification of inhibitors of dengue virus RNA-dependent RNA polymerase. J Biomol Screen. 2011 Feb;16(2):201-10. doi: 10.1177/1087057110389323. Epub 2011 Jan 10. PMID: 21220550.
50. Arora R, Liew CW, Soh TS, Otoo DA, Seh CC, Yue K, Nilar S, Wang G, Yokokawa F, Noble CG, Chen YL, Shi PY, Lescar J, Smith TM, Benson TE, Lim SP. Two RNA Tunnel Inhibitors Bind in Highly Conserved Sites in Dengue Virus NS5 Polymerase: Structural and Functional Studies. J Virol. 2020 Nov 23;94(24):e01130-20. doi: 10.1128/JVI.01130-20. PMID: 32907977; PMCID: PMC7925201.

51. Li XD, Shan C, Deng CL, Ye HQ, Shi PY, Yuan ZM, Gong P, Zhang B. The interface between methyltransferase and polymerase of NS5 is essential for flavivirus replication. PLoS Negl Trop Dis. 2014 May 22;8(5):e2891. doi: 10.1371/journal.pntd.0002891. PMID: 24852307; PMCID: PMC4031075.
52. Van Slyke GA, Ciota AT, Willsey GG, Jaeger J, Shi PY, Kramer LD. Point mutations in the West Nile virus (Flaviviridae; Flavivirus) RNA-dependent RNA polymerase alter viral fitness in a host-dependent manner in vitro and in vivo. Virology. 2012 May 25;427(1):18-24. doi: 10.1016/j.virol.2012.01.036. Epub 2012 Feb 23. PMID: 22365326; PMCID: PMC3299857.
53. Liu L, Dong H, Chen H, Zhang J, Ling H, Li Z, Shi PY, Li H. Flavivirus RNA cap methyltransferase: structure, function, and inhibition. Front Biol (Beijing). 2010 Aug 1;5(4):286-303. doi: 10.1007/s11515-010-0660-y. PMID: 21927615; PMCID: PMC3172701
54. Ravichandran L, Venkatesan A, Febin Prabhu Dass J. Epitope-based immunoinformatics approach on RNA-dependent RNA polymerase (RdRp) protein complex of Nipah virus (NiV). J Cell Biochem. 2018 Nov 11. doi: 10.1002/jcb.27979. Epub ahead of print. PMID: 30417438
55. Halpin K, Bankamp B, Harcourt BH, Bellini WJ, Rota PA. Nipah virus conforms to the rule of six in a minigenome replication assay. J Gen Virol. 2004 Mar;85(Pt 3):701-707. doi: 10.1099/vir.0.19685-0. PMID: 14993656.
56. Freiberg A, Dolores LK, Enterlein S, Flick R. Establishment and characterization of plasmid-driven minigenome rescue systems for Nipah virus: RNA polymerase I- and T7-catalyzed generation of functional paramyxoviral RNA. Virology. 2008 Jan 5;370(1):33-44. doi: 10.1016/j.virol.2007.08.008. Epub 2007 Sep 27. PMID: 17904180; PMCID: PMC2716073.
57. Edwards MR, Pietzsch C, Vausselin T, Shaw ML, Bukreyev A, Basler CF. High- Throughput Minigenome System for Identifying Small-Molecule Inhibitors of Ebola Virus Replication. ACS Infect Dis. 2015 Aug 14;1(8):380-7. doi: 10.1021/acsinfecdis.5b00053. Epub 2015 Jun 24. PMID: 26284260; PMCID: PMC4537067
58. Rack JGM, Zorzini V, Zhu Z, Schuller M, Ahel D, Ahel I. Viral macrodomains: a structural and evolutionary assessment of the pharmacological potential. Open Biol. 2020 Nov;10(11):200237. doi: 10.1098/rsob.200237. Epub 2020 Nov 18. PMID: 33202171; PMCID: PMC7729036.
59. Nandi I, Gupta A, Chaudhary VK, Gupta V, Gabrani R, Gupta S. Expression, purification and functional characterization of recombinant hypervariable region (HVR) of Chikungunya virus nsP3 protein. 3 Biotech. 2019 Jun;9(6):235. doi: 10.1007/s13205-019-1759-8. Epub 2019 May 27. PMID: 31139550; PMCID: PMC6536559.
60. Abraham R, McPherson RL, Sreekumar E, Leung AKL, Griffin DE. Preparation of Recombinant Alphaviruses for Functional Studies of ADP-Ribosylation. Methods Mol Biol. 2018;1813:297-316. doi: 10.1007/978-1-4939-8588-3_21. Erratum in: Methods Mol Biol. 2018;1813:E1. PMID: 30097877; PMCID: PMC6382469.
61. Jérôme H, Rudolf M, Lelke M, Pahlmann M, Busch C, Bockholt S, Wurr S, Günther S, Rosenthal M, Kerber R. Rift Valley fever virus minigenome system for investigating the role of L protein residues in viral transcription and replication. J Gen Virol. 2019 Jul;100(7):1093-1098. doi: 10.1099/jgv.0.001281. Epub 2019 Jun 6. PMID: 31169489.
62. Klemm C, Reguera J, Cusack S, Zielecki F, Kochs G, Weber F. Systems to establish bunyavirus genome replication in the absence of transcription. J Virol. 2013 Jul;87(14):8205-12. doi: 10.1128/JVI.00371-13. Epub 2013 May 22. PMID: 23698297; PMCID: PMC3700186.
63. Tigabu B, Rasmussen L, White EL, Tower N, Saeed M, Bukreyev A, Rockx B, LeDuc JW, Noah JW. A BSL-4 high-throughput screen identifies sulfonamide inhibitors of Nipah virus. Assay Drug Dev Technol. 2014 Apr;12(3):155-61. doi: 10.1089/adt.2013.567. PMID: 24735442; PMCID: PMC3994909.
64. Rasmussen L, Tigabu B, White EL, Bostwick R, Tower N, Bukreyev A, Rockx B, LeDuc JW, Noah JW. Adapting high-throughput screening methods and assays for biocontainment laboratories. Assay Drug Dev Technol. 2015 Jan-Feb;13(1):44-54. doi: 10.1089/adt.2014.617. PMID: 25710545; PMCID: PMC4340648.

From: Moore, Stephanie B on behalf of Moore, Stephanie B <stephaniemoore@uabmc.edu>
To: Denison, Mark; Menachery, Vineet; Weller,Sandra K.; Wright, Dennis L.; Geisbert, Thomas W.; lijun@uic.edu; igaysina@uic.edu; CHRISTOPHER BRODER; James Heyes; Diamond, Michael; Alec Hirsch; Makino, Shinji; Freiberg, Alex; Whelan, Sean; Daniel Streblow; Heise, Mark T; Morrison, Thomas; Hanks, Lynae J.; Bostwick, Bob; Augelli-Szafran, Corinne; Zhou, Jia; Chappell, Jim; Charles Mc Kenna; Saad, Jamil S; Prevelige, Peter Edward, Jr; Moukha-Chafiq, Omar; Wu, Mason; Evans, Carrie W.; Suto, Mark J.; Inah Kang; Tekwani, Babu; Ballinger, Carol A
Cc: Whitley, Richard; Pei-Yong Shi
Subject: Re: Project Order, PDP, and Project drafts
Date: Friday, October 8, 2021 3:40:30 PM
Attachments: AD3C.2 Project_1_10.8.21_Aims_Plan_Biblio.docx
SA and Research Strategy- Henipavirus-UAB-7October21.docx
Admin Core SA Research Strategy Oct 7 Final.docx
P21.0410 Screening Core (Specific Aims-Research Strategy-References) FinalV2.docx

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Hello all,

I'm sending drafts for Project 1: coronavirus and Project 2: Henipavirus. As well as the screening core (SC) and admin core for your input. I will send drafts from Overall and Med Chem Core, once they can incorporate the remaining two project. If you can get comments back by Tuesday, Oct. 12 at the latest, that would be helpful.

Also, just to make sure we are consistent with our core titles:
Administrative Core (Admin Core)
Screening Core (SC)
Medicinal Chemistry Core (MCC)
Structural Biology Core (SBC)

Thank you,

Stephanie

From: "Moore, Stephanie B" <stephaniemoore@uabmc.edu>
Date: Tuesday, October 5, 2021 at 2:55 PM
To: "Denison, Mark" <mark.denison@vumc.org>, "Menachery, Vineet" <vimenach@utmb.edu>, "Weller,Sandra K." <weller@uchc.edu>, "Wright, Dennis L." <Dennis.Wright@uconn.edu>, "Geisbert, Thomas W." <twgeisbe@UTMB.EDU>, "lijun@uic.edu" <lijun@uic.edu>, "Gaisina, Irina N" <igaysina@uic.edu>, CHRISTOPHER BRODER <christopher.broder@usuhs.edu>, James Heyes <james.heyes@genevant.com>, "Diamond, Michael" <mdiamond@wustl.edu>, Alec Hirsch <hirschal@ohsu.edu>, "Makino, Shinji" <shmakino@UTMB.EDU>, "Freiberg, Alex" <anfreibe@UTMB.EDU>, "Whelan, Sean" <spjwhelan@wustl.edu>, Daniel Streblow <streblow@ohsu.edu>, "Heise, Mark T" <mark_heisem@med.unc.edu>, "Morrison, Thomas" <THOMAS.MORRISON@CUANSCHUTZ.EDU>, "Hanks, Lynae J." <lhanks@southernresearch.org>, "Bostwick, Bob" <bbostwick@southernresearch.org>, "Augelli-Szafran, Corinne" <caugelli-szafran@southernresearch.org>, "Zhou, Jia" <jizhou@UTMB.EDU>, "Chappell, Jim" <jim.chappell@vumc.org>, Charles Mc Kenna <mckenna@usc.edu>, "Saad, Jamil S" <saad@uab.edu>, "Prevelige, Peter Edward, Jr"

<prevelig@uab.edu>, "Moukha-Chafiq, Omar" <omoukha-chafiq@southernresearch.org>, "Augelli-Szafran, Corinne" <caugelli-szafran@southernresearch.org>, "Wu, Mason" <mwu@southernresearch.org>, Carrie Evans <cevans@southernresearch.org>, "msuto@southernresearch.org" <msuto@southernresearch.org>, Inah Kang <inahkang@usc.edu>, Babu Tekwani <btekwani@southernresearch.org>, "Ballinger, Carol A" <caball@uab.edu>
Cc: "Whitley, Richard" <rwhitley@uabmc.edu>, Pei-Yong Shi <peshi@UTMB.EDU>
Subject: Re: Project Order, PDP, and Project drafts

Hello all,

Please find the current draft of the Structural Biology Core from Dr. Jamil Saad and team. The file is quite large - please find the file here.

Please let me know if you have questions or need help sending edits back to him.

Thank you,

Stephanie

From: Moore, Stephanie B
Sent: Monday, October 4, 2021 3:57:21 PM
To: Denison, Mark; Menachery, Vineet; Weller,Sandra K.; Wright, Dennis L.; Geisbert, Thomas W.; lijun@uic.edu; igaysina@uic.edu; CHRISTOPHER BRODER; James Heyes; Diamond, Michael; Alec Hirsch; Makino, Shinji; Freiberg, Alex; Whelan, Sean; Daniel Streblow; Heise, Mark T; Morrison, Thomas; Hanks, Lynae J.; Bostwick, Bob; Augelli-Szafran, Corinne; Zhou, Jia; Chappell, Jim; Charles Mc Kenna; Saad, Jamil S; Prevelige, Peter Edward, Jr; Moukha-Chafiq, Omar; Augelli-Szafran, Corinne; Wu, Mason; Evans, Carrie W.; Suto, Mark J.; Inah Kang
Cc: Whitley, Richard; Pei-Yong Shi
Subject: Project Order, PDP, and Project drafts

Hello all project and core leaders,

Thank you for your continued progress. We have a few immediate items to bring to your attention.

While working on the overall specific aims, it became necessary to reorder the projects. Therefore, here is the new project order:

1. Coronavirus
2. Henipavirus
3. Flavivirus
4. Bunyavirus
5. Alphavirus

Stephanie can help address the change of numbers on the documents and will change the component order in ASSIST to reflect the new project order.

Another point we want to address is that we would like to use a product development profile (PDP) within the "overall" section to apply to all projects. **We would like the attached paragraph that references the PDP to be added to all projects and for you to include a timeline for your project please refer to Bunya write up for an example timeline**.

Finally, please see the attached current versions of Alpha and Bunya projects. We would appreciate any comments or edits by **Thursday, Oct. 7** that we can share with them. The other project drafts will be sent as they are received.

Thank you,

Stephanie, Rich, and Pei-Yong

CEPI Task Force on Nipah Standards, Assays & Animal Models

CEPI

September 2021

Agenda

Topic	Presenter	Allocated time	Objective
Introduction & welcome	Raúl Gómez Román, CEPI	5 min	
Updates from henipavirus-affected countries	Glenn Marsh, Australia Syed Satter, Bangladesh Pragya Yadav, India LiYen Chang, Malaysia	10 min each + 5 min Q&A	To gain visibility on recent henipavirus work and on how COVID-19 has affected R&D in country
NiV antibody standard development I	LiYen Chang, University of Malaya	10 min	To share information on objectives, including NiV assays in the pipeline
NiV antibody standard development II	Mark Hassall, NIBSC	10 min	To gain visibility on the plans and timelines for the feasibility study leading to the development of an international NiV antibody standard
		10 min	Q&A on antibody standard development
Natural history studies	Carolyn Clark/Amy Shurtleff , CEPI	10 min	To share information/updates on objectives, status and expected timelines.
Updates from CREID network	Sara Woodson, NIAID	10 min	
Updates from CEPI Epi RfP	Maïna L'Azou Jackson, CEPI	10 min	
		5 min	Q&A on CEPI & NIAID initiatives
Action items & closing	Raúl Gómez Román, CEPI	5 min	

Affected country updates

Australia (placeholder)

CEPI

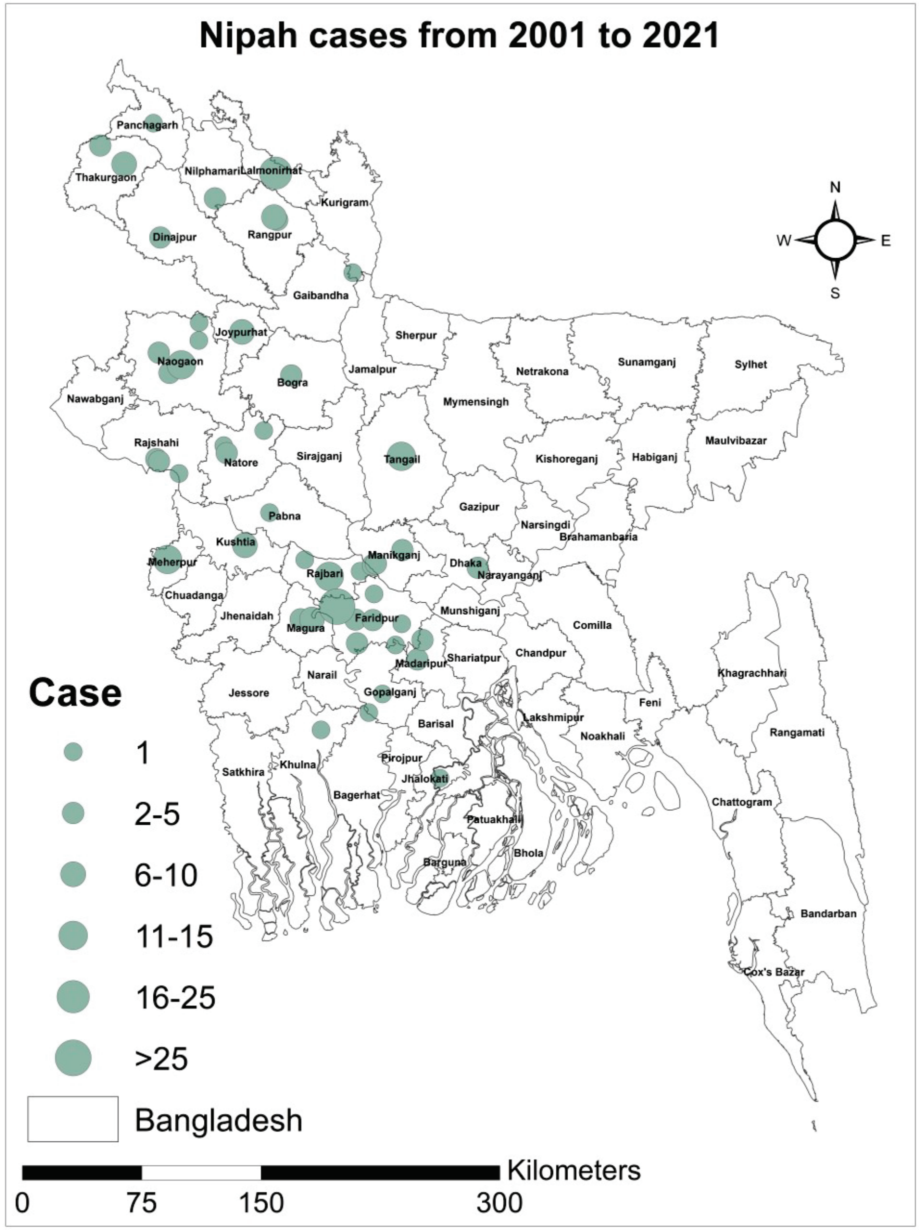
Nipah virus in Bangladesh: 2001 to 2021

Dr. Syed Moinuddin Satter
Assistant Scientist and Deputy Project Coordinator
Principal Investigator, National Nipah Surveillance
Programme for Emerging Infections, icddr,b

Professor Dr. Mahmudur Rahman
MBBS(Bangladesh), MPHM (Thailand), PhD (Cambridge, UK)
Senior Technical GHSA Consultant
Global Health Development/EMPHNET
(Support to the US CDC, Bangladesh Country Team)
Former Director, IEDCR, Bangladesh

Nipah virus transmission in Bangladesh

- First identified as the cause of an outbreak of encephalitis in 2001 in Meherpur district
- Nipah Surveillance and laboratory established in 2006
- Till July, 2021, a total of 322 cases of Nipah infection were recognized; 228 (71 %) died, indicating a very high mortality
- Spillover identified 135 times
- Secondary cases identified 46%
- Current Nipah survivor 79
- Till date, 38 outbreaks have been identified, involving 32 districts out of 64 districts


Nipah cases from 2001 to 2021

Nipah surveillance activity in Bangladesh

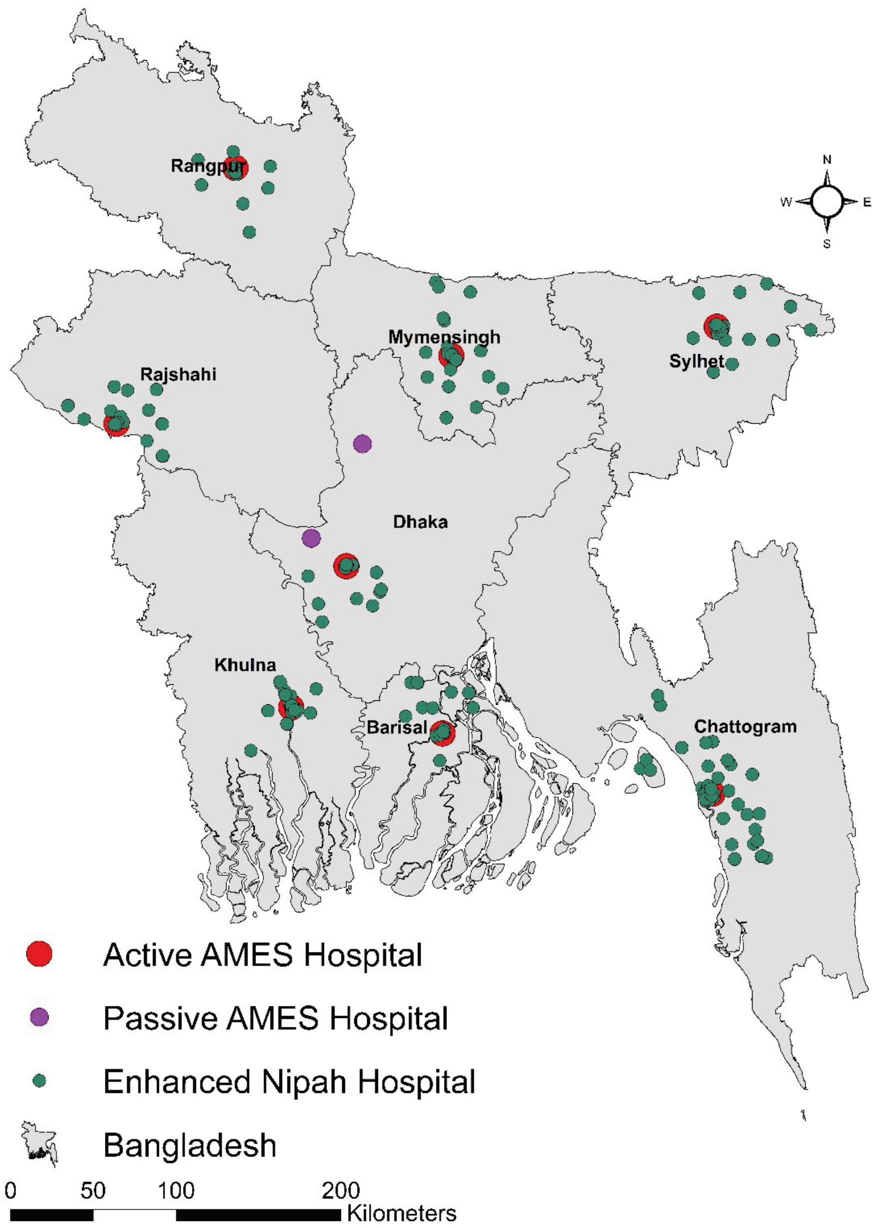
1. Hospital based sentinel surveillance (icddr,b)

- Active surveillance at eight medical collage hospitals (Three additional sites in non-Nipah belt)
- Passive surveillance at two district health care facilities
- Enhanced surveillance activity during Nipah season (Dec’2020-Apl’2021) at 355 health care facilities (HCF)
  - District and sub district public HCF: 99
  - Public HCF: 256
- Year-round Nipah testing

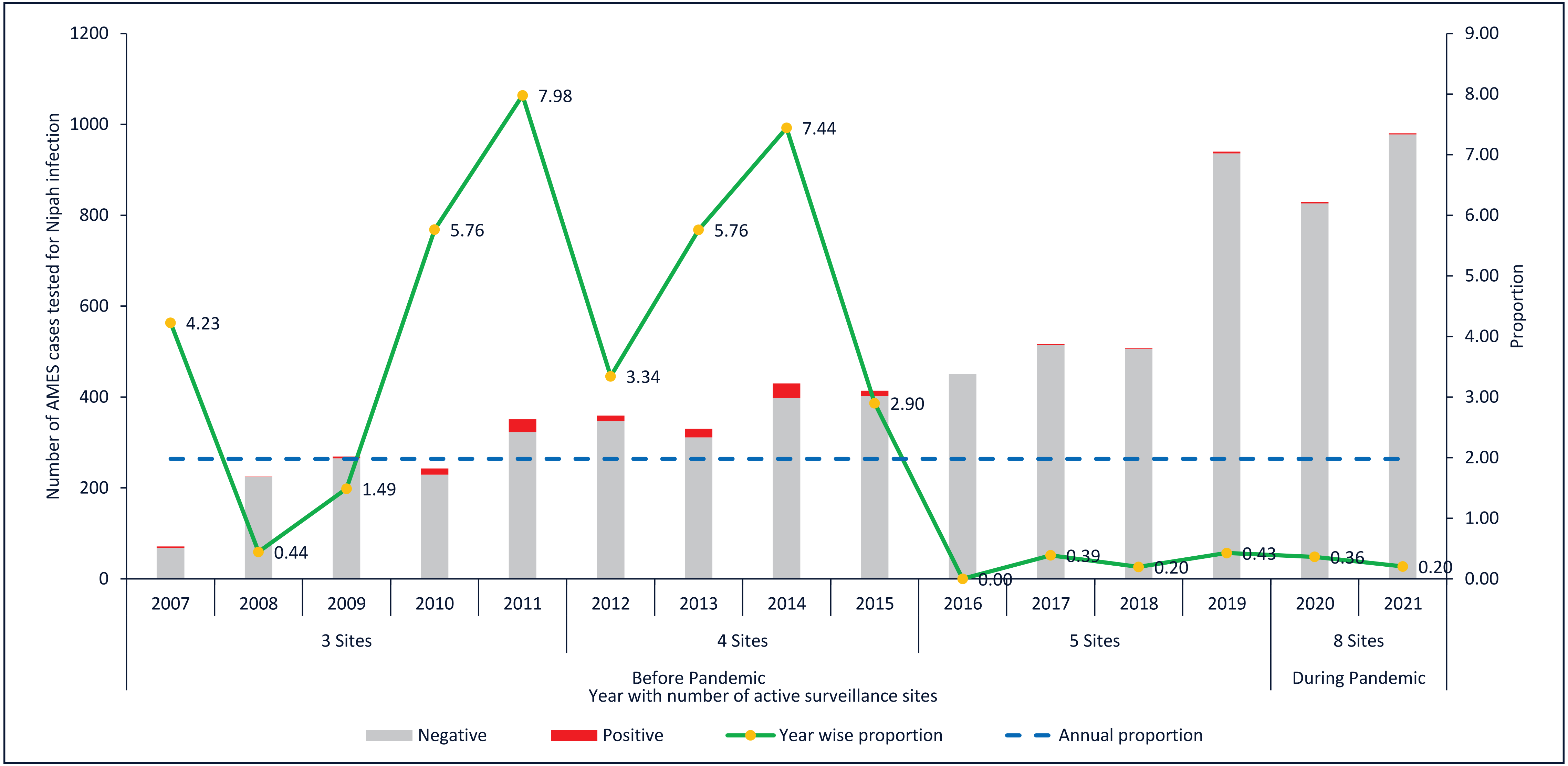
2. Event Based Surveillance at IEDCR

- 24/7 hotline (2)
- Media Monitoring
  - –All national daily newspapers
  - –All TV channels
- Any informal reporting

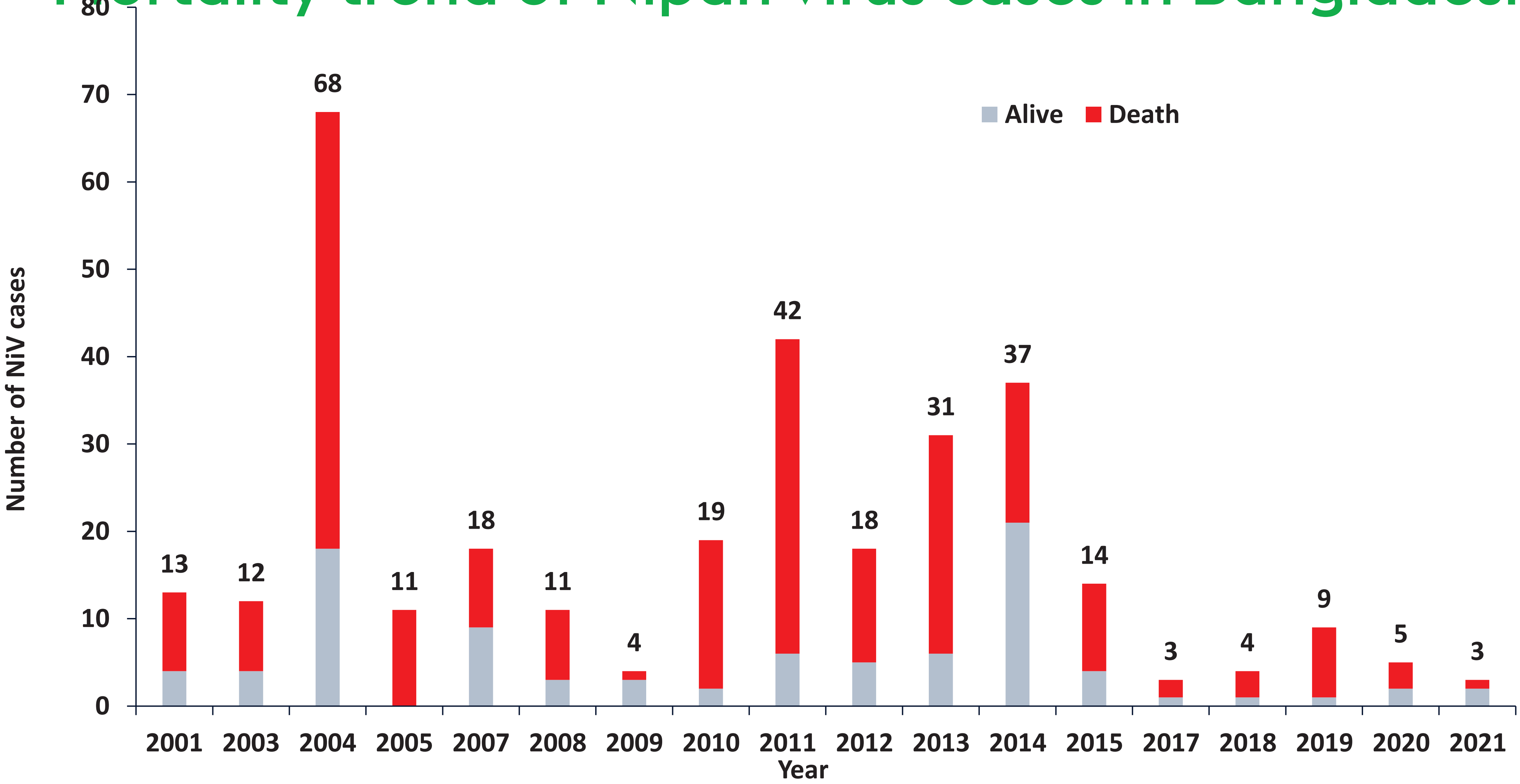
3. Other encephalitis surveillance (JE, WHO and IEDCR)



Trend of Nipah virus cases in Bangladesh



Mortality trend of Nipah virus cases in Bangladesh



Gap in Nipah surveillance

- Population based active surveillance system
- Sequelae of Nipah survivor
- Immunology status of date palm sap harvester (gachi)

CEPI

CEPI Task Force on Nipah Standards, Assays & Animal Models

Dr. Pragya D Yadav,
Scientist E & In-charge, Maximum Containment Facility,
ICMR-NIV, Pune, Department of Health Research, Ministry of Health & Family Welfare, Government of India

1. Nipah Virus: Indian Scenario (Epidemiology)

Siliguri

Nadia

Kozhikode

Ernakulum

Ox 1: 1998
Malaysia
Source: Infected pigs

Ox 4: 2001
India (Ox 1)
Source: Uk

India
(Ox 2): 2007
Source: Uk

India
(Ox 3): 2018
Source: *Pteropus* Bats

India
(Ox 4): 2019
Source: *Pteropus* Bats

Ox 2: 1999; Singapore
Source:
Imported Malay pigs

Ox 3: 2001
Bangladesh
Source: Uk

Yearly Ox documented till date

Ox = Outbreaks
Uk = Unknown

Evolution of detection and diagnosis capacity in India

2001

Siliguri Ox: 30 days; 66 cases; CFR = 74%

- Diagnosis not established.
- No BSL4/BSL3 labs.
- Misdiagnosed: Measles.
- CDC, US confirmed as NiV.

2007

Nadia Ox: 19 days; 6 cases; CFR = 100%

- Diagnosis established.
- Diagnostics provided by CDC (serology & molecular).
- BSL3 labs +.
- Early detection failed.

2015:

- BSL4 Lab operational &
- Capacity for bat surveillance established.
- Bat samples from WB & Assam screened for NiV.
- 8% positivity

2018

Kozhikode Ox: 27 days; 23 cases; CFR = 89%

- Diagnosis in index case not established.
- Early diagnosis & Ox containment.
- Bat survey positivity ~23%.
- Virus isolated
- m102.4 monoclonal imported from UQ for treating Nipah infected patients

2019

Ernakulum Ox: 1 case; No fatality.

- Quick diagnosis.
- Early Ox containment.
- Onsite diagnosis: PoC; Serology & molecular.
- Bat positivity ~12.7%.
- Validated in house diagnosis
- Clinical trial protocol developed (m102.4)

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Core capacity required for surveillance and research for Nipah virus disease

1. Surveillance for early detection of circulation of the virus
    - Human surveillance
    - Bat surveillance
2. Laboratory detection capacity
3. Capacity to conduct systematic epidemiological studies.
4. Clinical trial capacity for newer drugs, monoclonal and vaccines.
5. Clinical Research for appropriate treatment and management of patients
6. Risk communication and community mobilization strategies.

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Human & Bat surveillance

- India has an Integrated Disease Surveillance Program (IDSP) under aegis of Ministry of Health & Family Welfare.
- IDSP picks up clusters of cases and collects outbreak samples.
- Samples are referred to Deptt. of Health Research (DHR) / Indian Council of Medical Research (ICMR) network of Virus Research & Diagnostic Laboratories (VRDLs).
- VRDLs are equipped to detect viruses of public health importance.
- Selected VRDLs with BSL-3 labs are trained to detect high risk pathogens.
- ICMR-NIV, Pune has specialized teams for bat surveillance
- Relevant recent publications:
  - *Emerg Infect Dis. 2019 May;25(5):1003-1006.*
  - *Clin Infect Dis. 2019 Jul 2;69(2):378-379.*
  - J Infect Dis. 2019 May 24;219(12):1867-1878.
  - *BMJ Glob Health. 2018 Nov 9;3(6):e001086.*
  - *Emerg Infect Dis. 2019 May;25(5):1007-1010*

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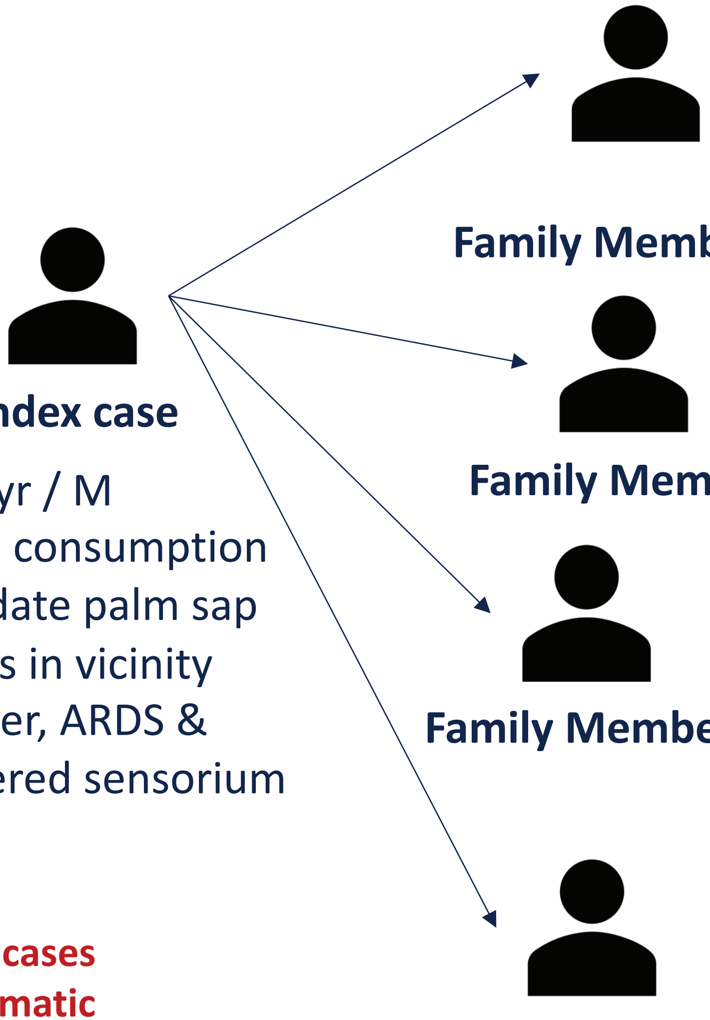
Nipah Virus Outbreaks in India

Month/ Year	Location	No. of cases	No. of deaths	Case Fatality Rate
Feb. 2001	Siliguri	66	45	68%
April 2007	Nadia	05	05	100%
May 2018	Kozhikode	19	17	89.5%
June 2019	Ernakulum	01	01	0%

Siliguri Outbreak: April 9-28, 2007



Index case

- 35 yr / M
- h/o consumption of date palm sap
- Bats in vicinity
- Fever, ARDS & altered sensorium

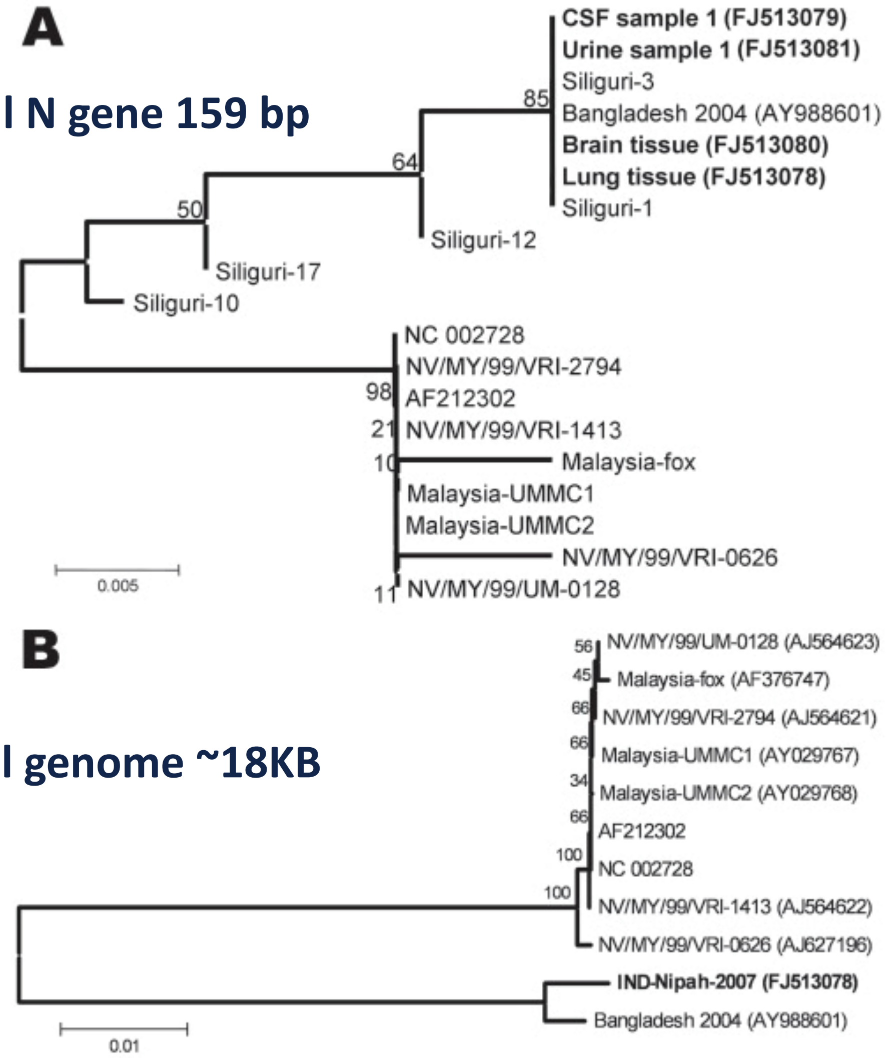
Family Member

Family Member

Family Member

Paramedical Staff

All secondary cases became symptomatic within 12-14 days of contact with index case



Partial N gene 159 bp

Full genome ~18KB

Full-genome NiV sequence from lung tissue: 99.2% nt and 99.8% aa identity with the Bangladesh-2004 isolate.

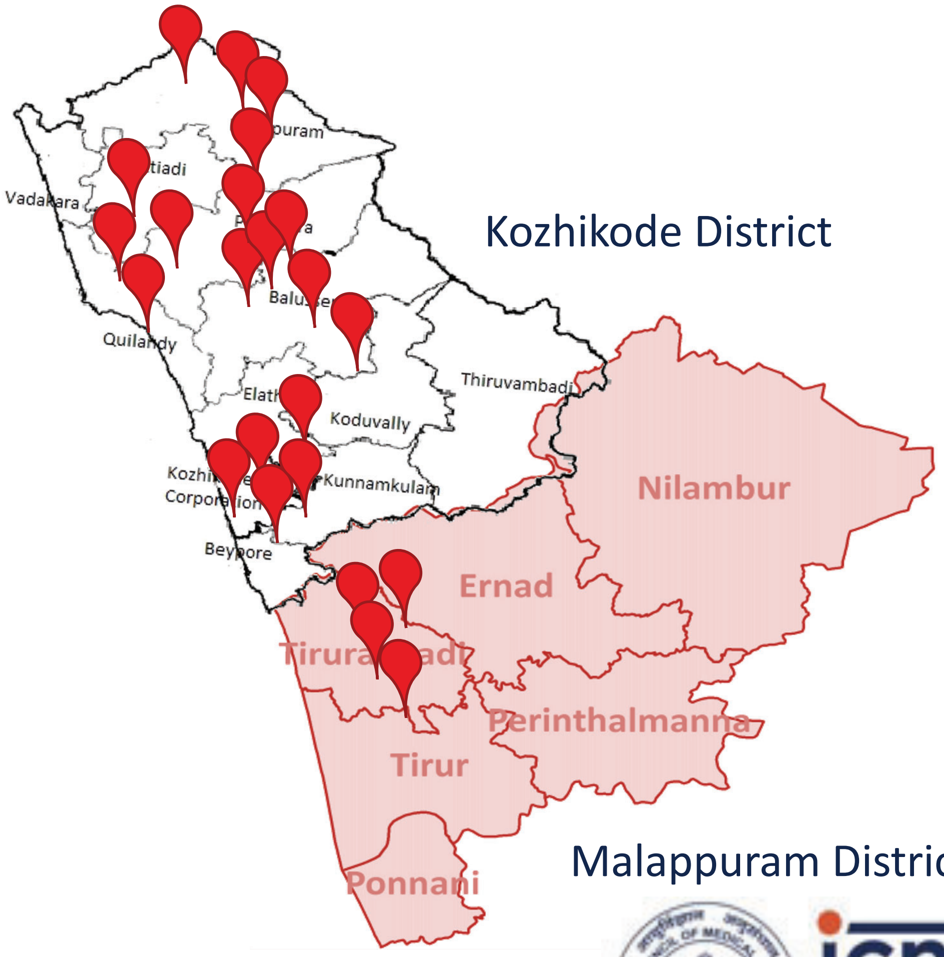
Nipah outbreak in Kozhikode and Malappuram districts (2-29 May 2018), Kerala 2018

23 cases reported

- 18 lab confirmed (NiV RNA +ve); 5 probable (including index case)
- 22 had acute neurological and/or respiratory symptoms
- 16 of the 18 lab confirmed cases died (CFR=89%)

Source of infection of primary case remained unknown

- 21 had history of contact with confirmed/probable cases



Distribution of Nipah cases (n=23) by residence

icmr
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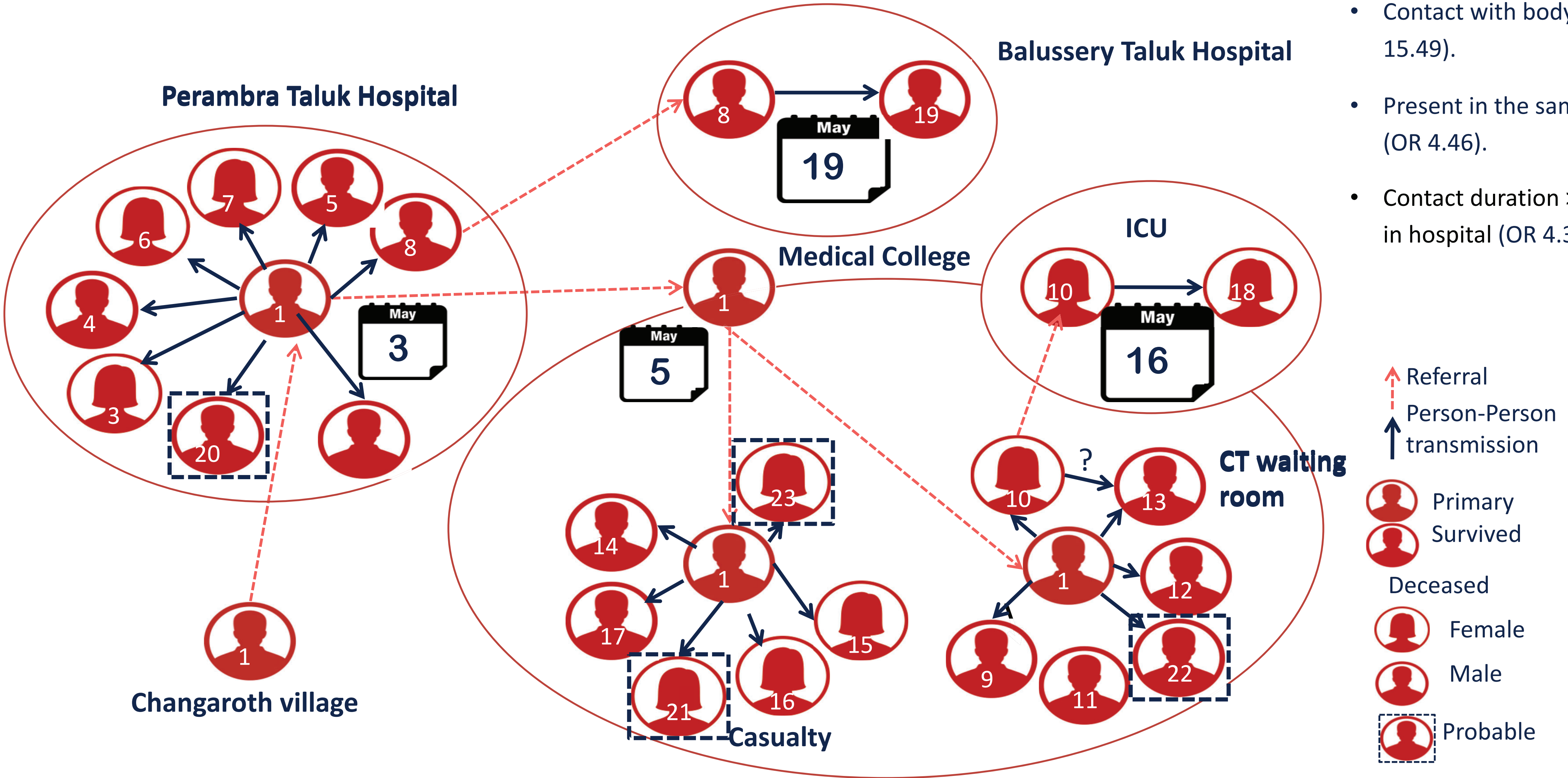
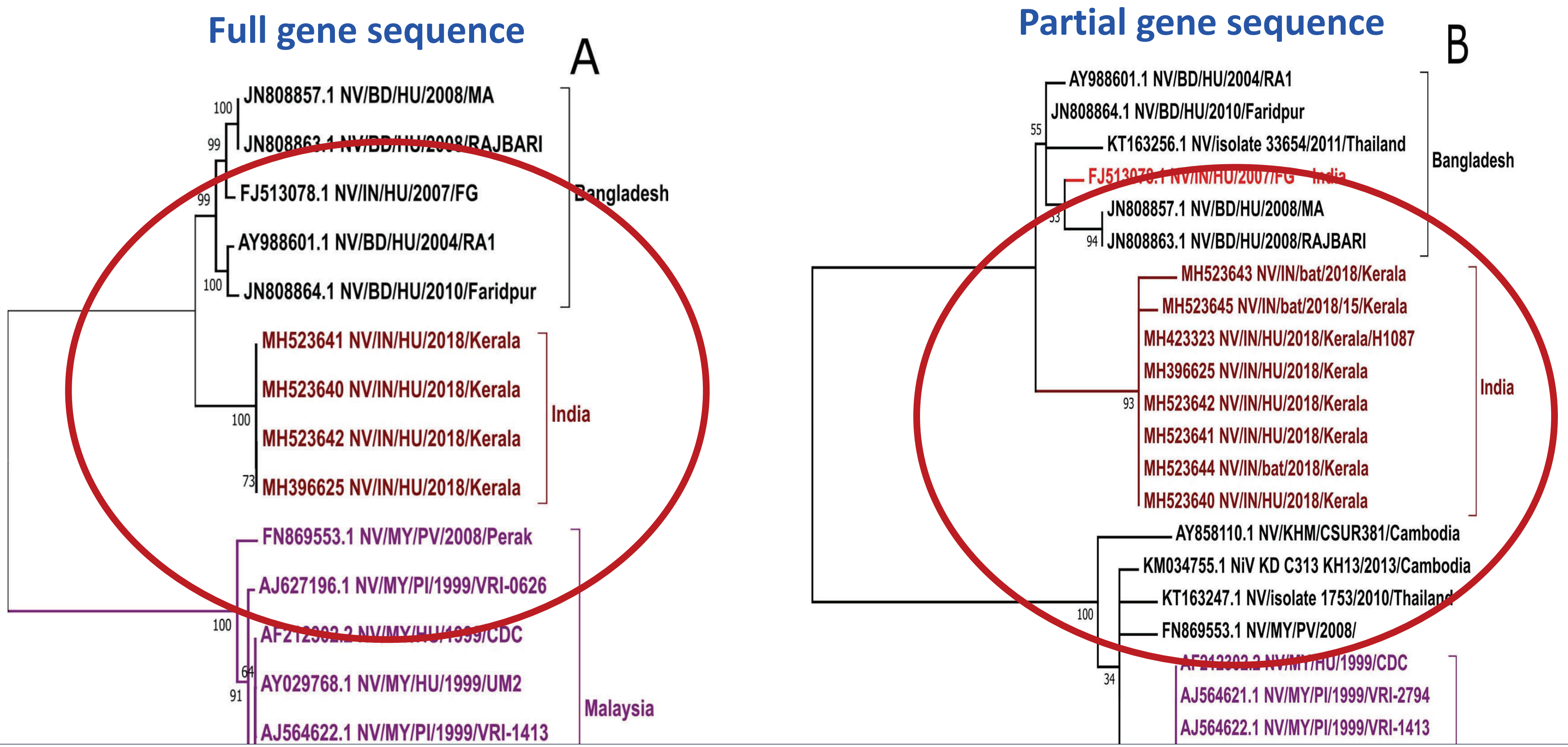
Serving the nation since 1911

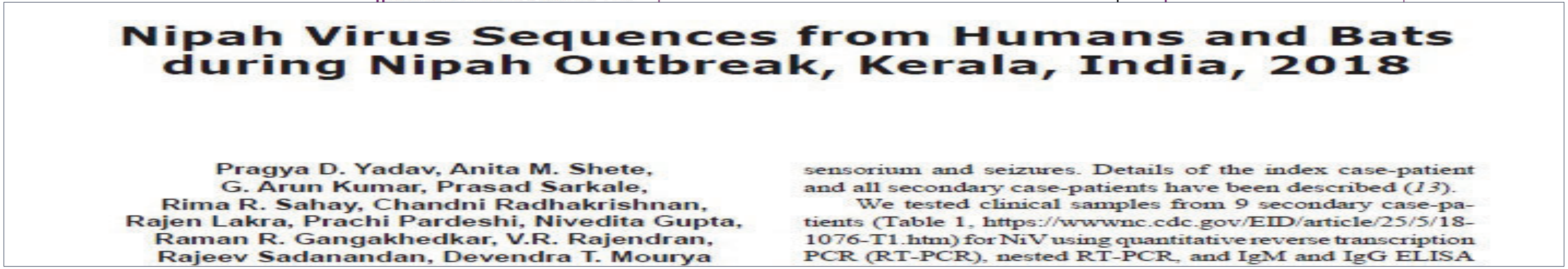
Transmission of Nipah from primary case by date of exposure and location



- **Significant risk factors**
- Contact with body fluids (OR 15.49).
- Present in the same room (OR 4.46).
- Contact duration >24 hours in hospital (OR 4.39).

Bats: Source of Infection in Kozhikode 2018 outbreak





Nipah Virus Sequences from Humans and Bats during Nipah Outbreak, Kerala, India, 2018

Pragya D. Yadav, Anita M. Shete,
G. Arun Kumar, Prasad Sarkale,
Rima R. Sahay, Chandni Radhakrishnan,
Rajen Lakra, Prachi Pardeshi, Nivedita Gupta,
Raman R. Gangakhedkar, V.R. Rajendran,
Rajeev Sadanandan, Devendra T. Mourya

sensorium and seizures. Details of the index case-patient and all secondary case-patients have been described (*13*).

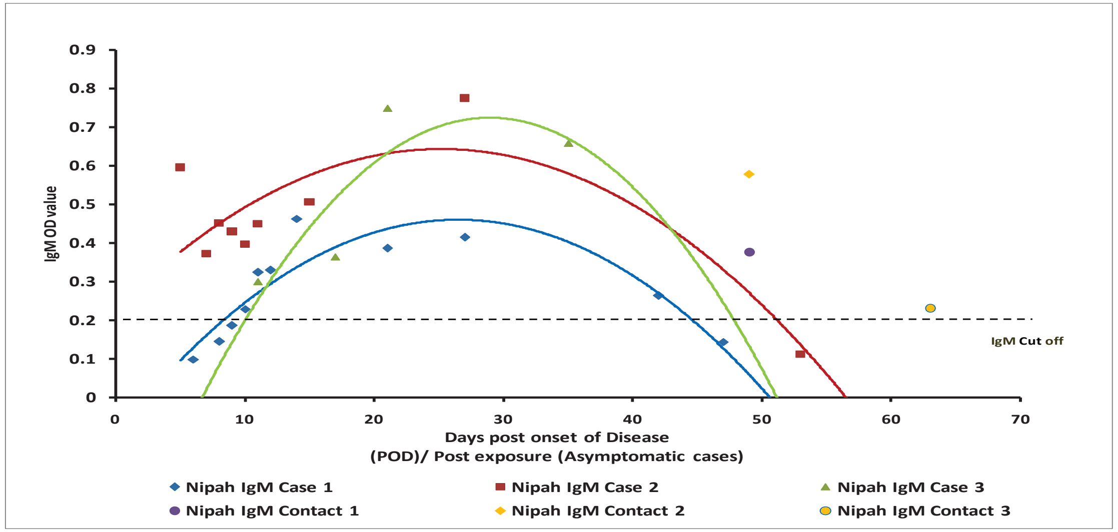
We tested clinical samples from 9 secondary case-patients (Table 1, https://wwwnc.cdc.gov/EID/article/25/5/18-1076-T1.htm) for NiV using quantitative reverse transcription PCR (RT-PCR), nested RT-PCR, and IgM and IgG ELISA

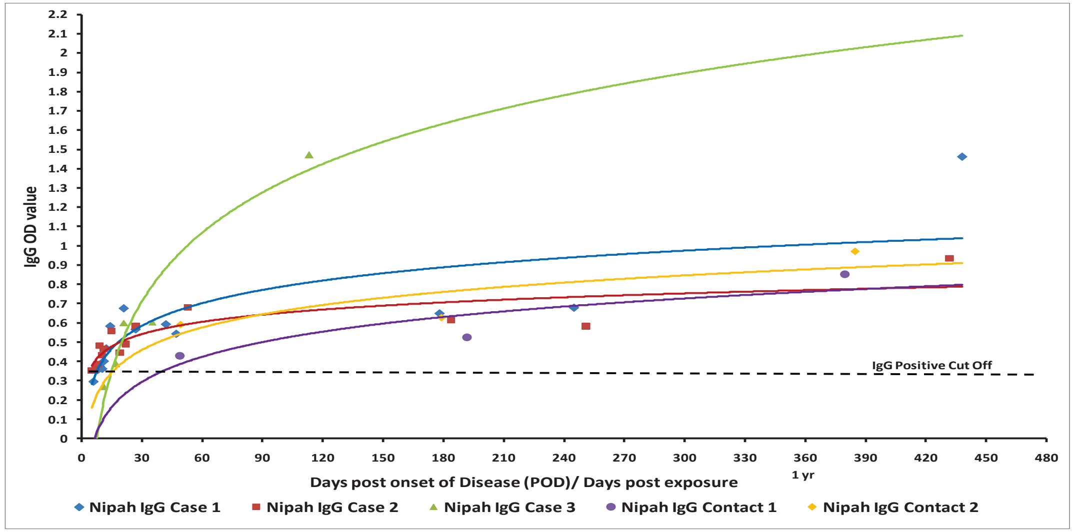
Specialized capacity for bat surveillance & sample processing

Bat Survey Team of ICMR-NIV, Pune
Trapping *pteropus* bat from Kerala

BSL-3 & BSL-4 Laboratory Complex
ICMR-National Institute of Virology, Pune

Persistence of anti-Nipah IgM and IgG antibody
(3 survivors, 3 close contacts)





Total positivity	No. positive samples/total no. of samples tested (%)	
	Anti Nipah human IgG	Anti Nipah human IgM
3/279	2/279(0.716%)	3/279(1.075%)

☐ Evidence for human to human transmission through body fluids of NiV case-patients

☐ • Low prevalence of sub-clinical NiV infection among contacts

Anti-Nipah Human IgM antibodies were detected from POD 5 to POD 42 (O.D ≥0.2)

Anti-Nipah Human IgG antibodies were detected from POD 5 to until 438 POD in survivors and 385 Days Post Exposure in close

Nipah Outbreak of 2019: Ernakulum, Kerala

Epidemiological Assessments

- ICMR team designed a risk categorization algorithm, implemented during contact tracing activities by the district teams
- 330 contacts of the index case were traced/ risk categorized
  - High-risk category included either history of direct contact with body fluids of the index case or having spent >12 hours in close proximity of index case
  - **52 contacts (35 hospital staff, 11 community members, 6 family members) were categorized into high-risk category**
  - None of these were found to be positive for Nipah virus infection
- ICMR team also aided the state to revise the Nipah surveillance and contact tracing guidelines that were finalized

Laboratory capacity for detection of Nipah virus

- ICMR-NIV, Pune team had set up the testing facilities at GMC Ernakulam within 24 hours:

- Light weight
- Portable
- Rugged (No A/C)
- Battery powered
- Ready to use, stabilized consumables

Components:

1. Sample Prep kit
2. Autoprep RNA extraction kit
3. POC Nipah Real Time machine

ICMR’s contribution in understanding NiV prevalence & further support to public health system

- Conducted surveys for Nipah virus and anti-NiV IgG antibodies in bats, pigs and human population from North-East region.
- Deputed experts to support the state government towards investigation, prevention, and control of Nipah virus outbreak in Kerala during 2018-2019
- Training on PPE donning/doffing, sample collection & triple packaging for Medical officers, HCWs ASHA and ANMs.
- Training for safe burial of human remains in cemetery.
- Un-folding the mystery- Source of outbreak in Kerala with positivity in *Pteropus* bats.
- Laboratory capacity building by training 12 VRDL laboratory for Nipah and other high risk pathogens.
- Capacity building for NiV testing at ICMR-NIV, Kerala unit
- Nationwide surveillance of for NiV in bat population revealed presence of virus in Kerala, West Bengal and Maharashtra.

Key partners

- Indian Council of Medical Research (ICMR) Headquarter (Implementing agency)
- ICMR Institutes:
  - National Institute of Virology, Pune (virus detection, in-house diagnostics, bat survey)
  - National Institute of Epidemiology, Chennai (epidemiological studies)
  - National AIDS Research Institute, Pune (clinical trial and clinical pharmacy)
- Department of Health Research (DHR) /ICMR Virus Research & Diagnostic Laboratory (VRDL) network (virus detection & surveillance)
- National Institute of Health/National Institute of Allergy & Infectious Diseases, USA (clinical trials with newer MCMs)
- Coalition for Epidemic Preparedness & Innovation, Norway (vaccine trials)
- World Health Organization –SEARO & Hq (sharing WHO blueprint implementation plan and connecting with other affected countries)
- IEDCR, Bangladesh (intercountry Nipah clinical trial)

Containment of NiV in Kerala

OVER 1,000 PEOPLE IN CONTACT LIST

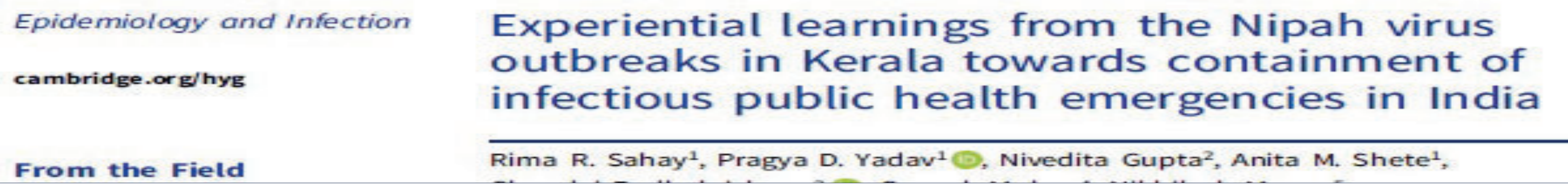
- As of now, there 1,407 people in the list of people who could have been in contact with Nipah-infected persons
- The health department has also issued guidelines regarding how to deal with a person who shows Nipah symptoms
- Human Monoclonal 102.4, the medicine for Nipah, is likely to arrive from Australia on Friday
- Certain protocol needed to be followed before administering the medicine
- It is a phase-two trial drug that was found effective in patients who were infected with similar types of virus

Medical team visiting the house where the mother of Muhammad Sabith and V K Salih, victims of Nipah infection, resides at Panthirikkara on Thursday

Training for safe burial of dead body in cemetery

Training on sample collection & triple packaging for Medical officers, ASHA and ANMs

Intensive field work for case contact tracing



Epidemiology and Infection

cambridge.org/hyg

From the Field

Experiential learnings from the Nipah virus outbreaks in Kerala towards containment of infectious public health emergencies in India

Rima R. Sahay[1], Pragya D. Yadav[1], Nivedita Gupta[2], Anita M. Shete[1],

2. Nipah Virus: Research and Development

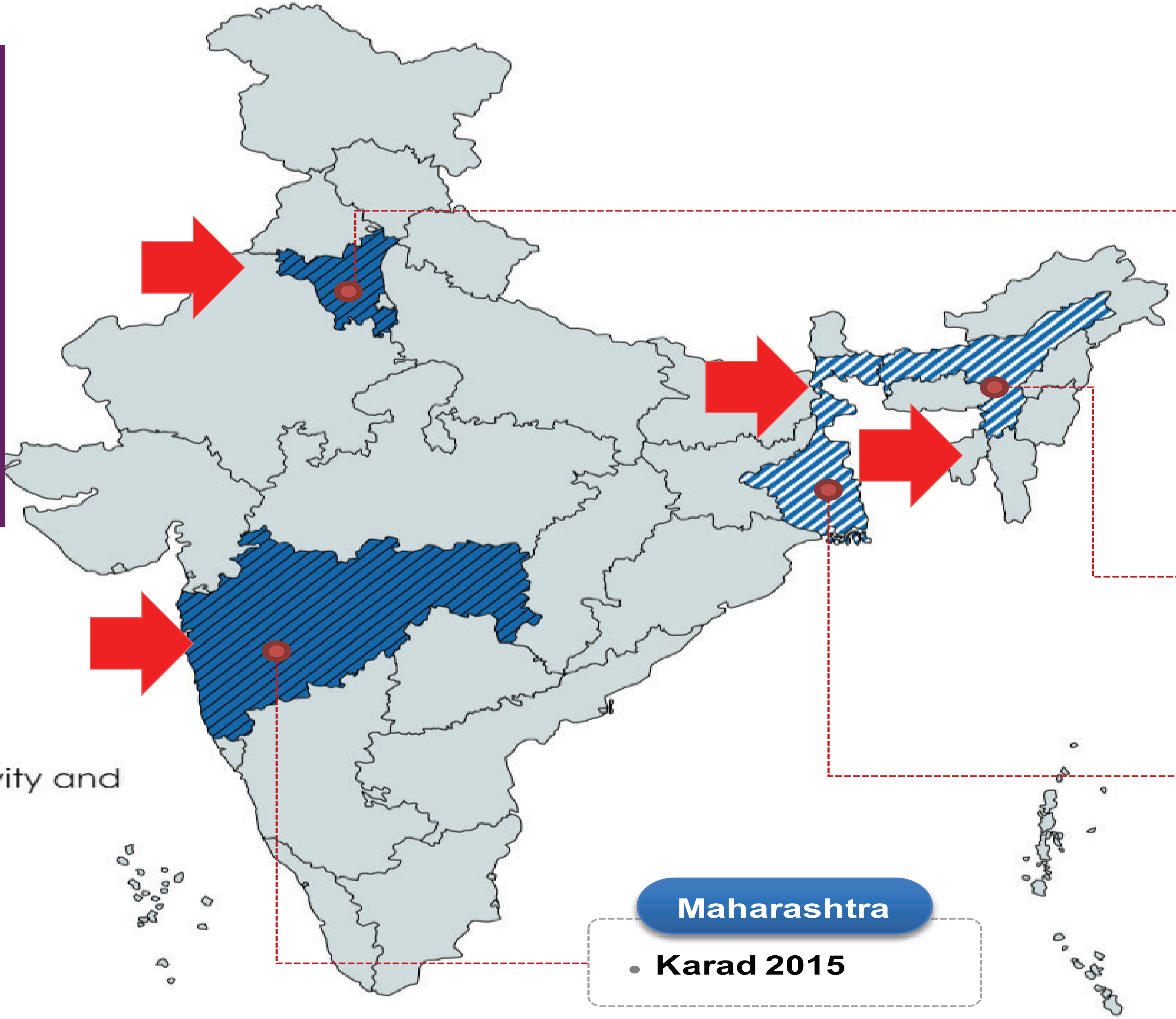
- ❑ Development of Point of care Diagnostic kit in public private partnership [Molbio Diag. TM]
- ❑ NiV isolate characterized
- ❑ Collaboration with Bangladesh
- ❑ Countrywide bat studies for NiV initiated
- ❑ Capacity building for NiV testing at ICMR-NIV, Kerala
- ❑ Un-folding the mystery- Source of outbreak in Kerala with positivity in *Pteropus bats*
- ❑ Sero-prevalence of NiV specific antibodies among close contacts Kerala, 2018
- ❑ On 3rd June 2019 - a college student resident of Ernakulam was confirmed to be positive for NiV

- ❑NiV positivity in Pteropus bats 12/52=23%
- ❑NiV sequence from *Pteropus* bats and human homology 99.7-100%

Nipah virus positivity recorded in Pteropus bat in India from 2011-18

Species screened

- *Rousettus leschenaulti*
- *Pteropus medius*
- *Cynopterus sphinx*
- *Pipistrellus tenuis mimus*
- *Megaderma lyra*



▨ NiV Seropositivity

▨ NiV Viral RNA posistivity and seropositivity

Harayana

- 2008

Assam

- Dhubri, 2014-15

West Bengal

- Cooch behar, 2015
- Myanaguri, 2009

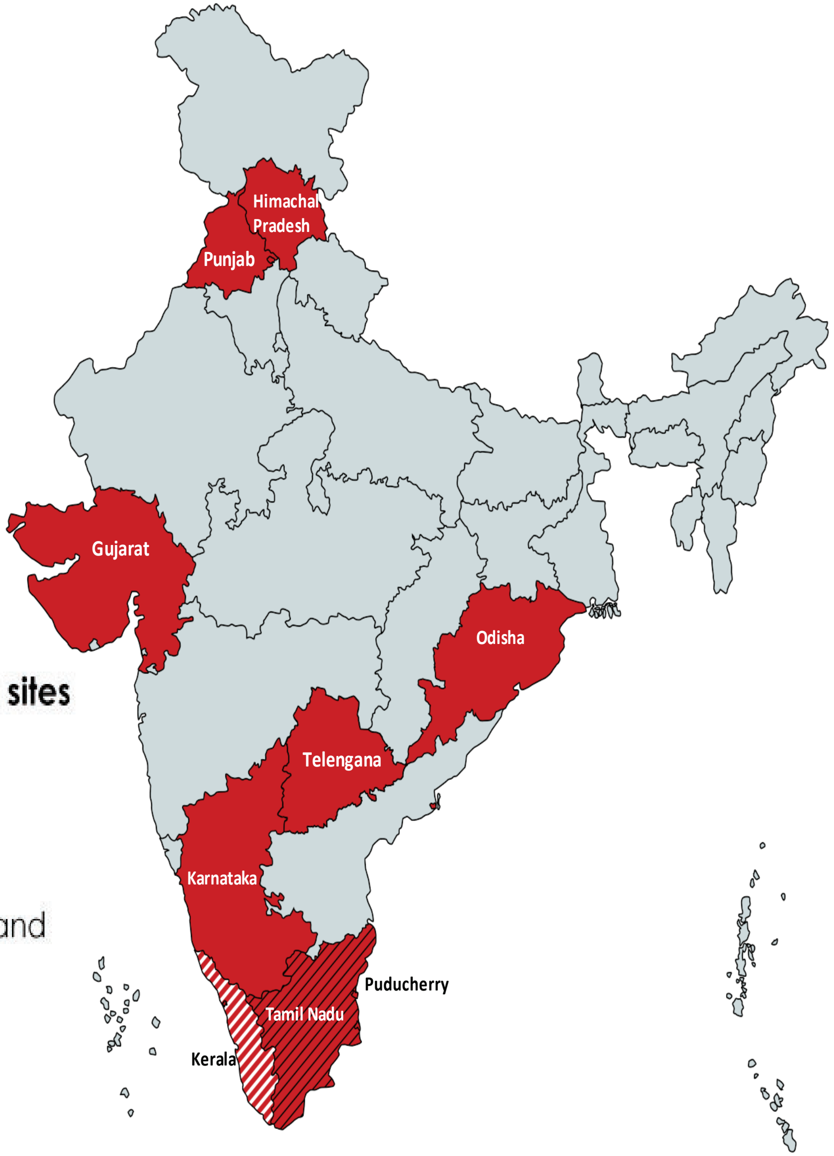
Maharashtra

- Karad 2015

CEPI

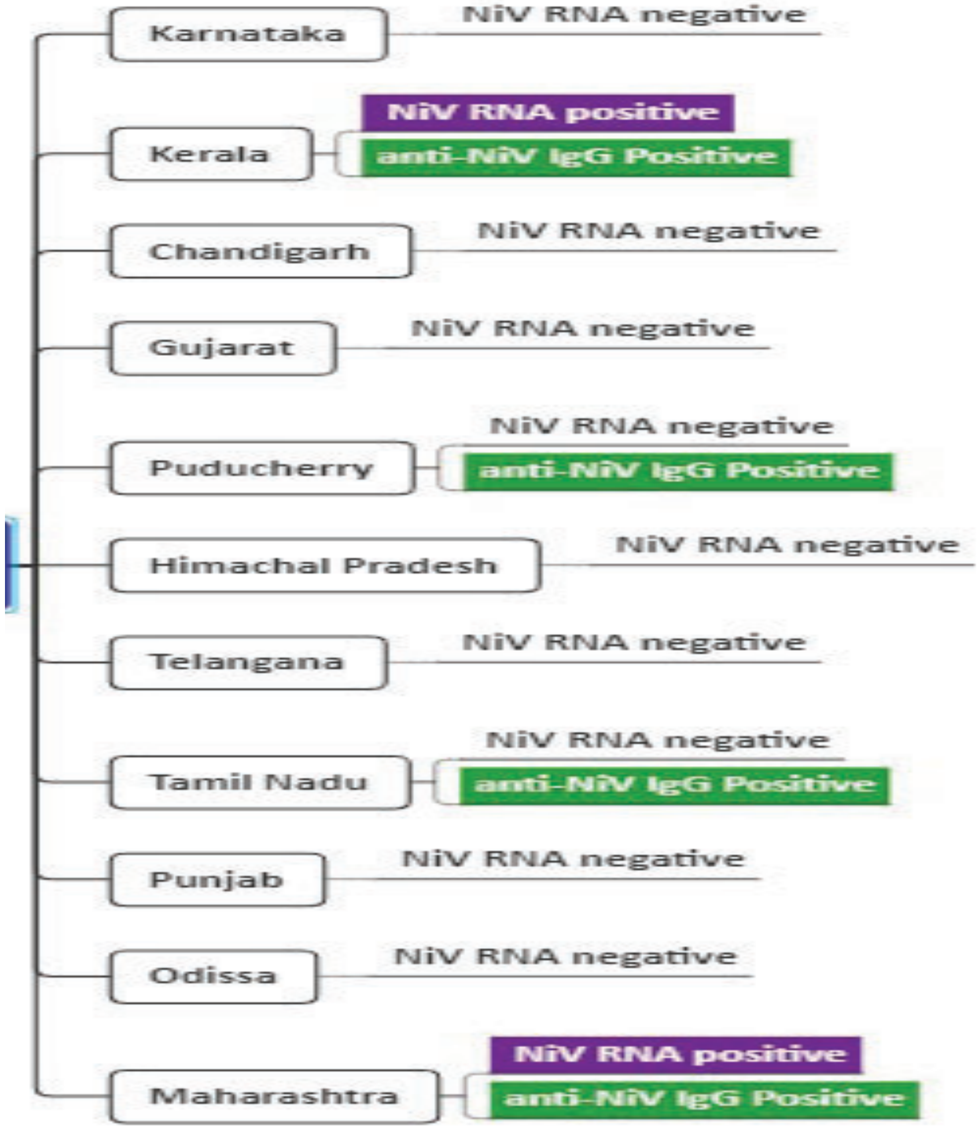
Country wide survey of Nipah virus in bats from 2019

Survey of 11 states completed



Bat specimen collection sites 2018-19

- 🟥 collection sites
- ▨ NiV seropositivity
- ▧ NiV viral RNA positivity and seropositivity



Karnataka	NiV RNA negative
Kerala	NiV RNA positive anti-NiV IgG Positive
Chandigarh	NiV RNA negative
Gujarat	NiV RNA negative
Puducherry	NiV RNA negative anti-NiV IgG Positive
Himachal Pradesh	NiV RNA negative
Telangana	NiV RNA negative
Tamil Nadu	NiV RNA negative anti-NiV IgG Positive
Punjab	NiV RNA negative
Odissa	NiV RNA negative
Maharashtra	NiV RNA positive anti-NiV IgG Positive

Development of the Diagnostic assays

1. Development of Anti-Nipah human IgM antibody detection
2. Development of Anti-Nipah human IgG antibody detection
3. Development of Anti-Nipah bat IgG antibody detection
4. Clinical trial for NiV monoclonal documents prepared
5. Study on pathogenicity of Nipah virus (Kerala isolate) in Syrian hamster model
6. Standardization of plaque assay for Nipah Virus in Vero CCL81

- ☐ Evidence for human to human transmission through body fluids of NiV case-patients
- ☐ • Low prevalence of sub-clinical NiV infection among contacts

External Validation of Nipah virus Point of Care assay and ELISA

Assays developed for Nipah detection

1. TruenatTM Nipah Point of Care Test (POCT), developed by Molbio Diagnostics and optimized by ICMR-NIV, Pune.
2. Nipah Human IgG ELISA
3. Nipah Human IgM ELISA

❑ Indigenous assays developed by ICMR NIV, Pune

❑ CDCSO (DCGI) approved the clinical protocol of PoC for NiV detection

❑Nipah Human IgM and IgG ELISA reagents from CDC, USA were used as a gold standard.

❑99.26% specificity and 100% sensitivity was found for both the assays.

Nipah POCT Validation in collaboration with Institute of epidemiology disease control and research, Dhaka

- MoU was signed between ICMR-NIV , Pune and IEDCR, Dhaka , November 2020
- NIV team visited IEDCR during period of 3-7 November and performed the validation
- Used the clinical samples of IEDCR for validation of Nipah assays .

Anti-Nipah Human IgM and IgG ELISA onsite validation in Kerala and Bangladesh
IEDCR, Dhaka and Kerala indicated 100% concordant results in comparison of CDC reagents

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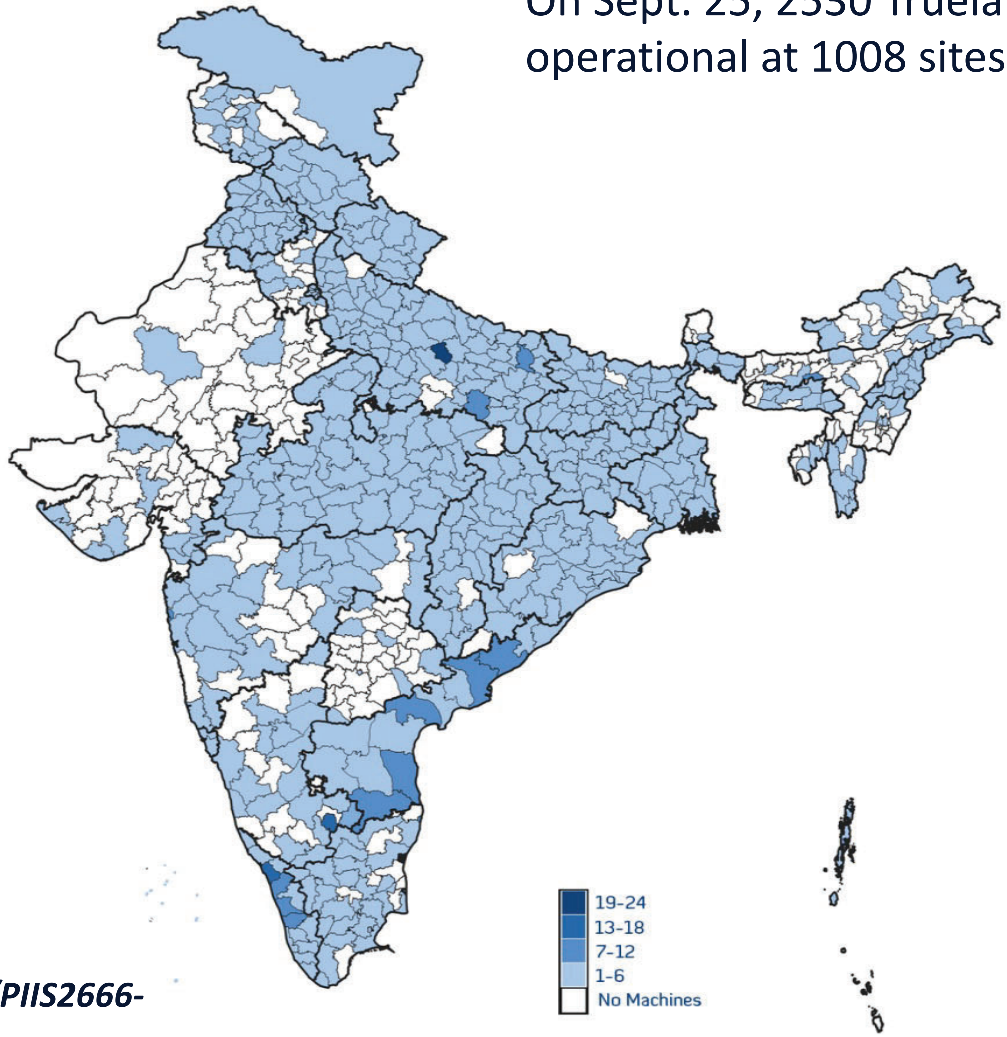
Serving the nation since 1911

Repurposing TrueLabTM (TrueNat) Diagnostic Platform during Pandemic

On Sept. 25, 2530 Truelab^{TM} workstations were operational at 1008 sites in 530 districts of India.

COVID-19:

1. Beta CoV E gene screening assay
2. RdRp gene confirmatory assay
3. Multiplex E gene & Orf1a gene assay

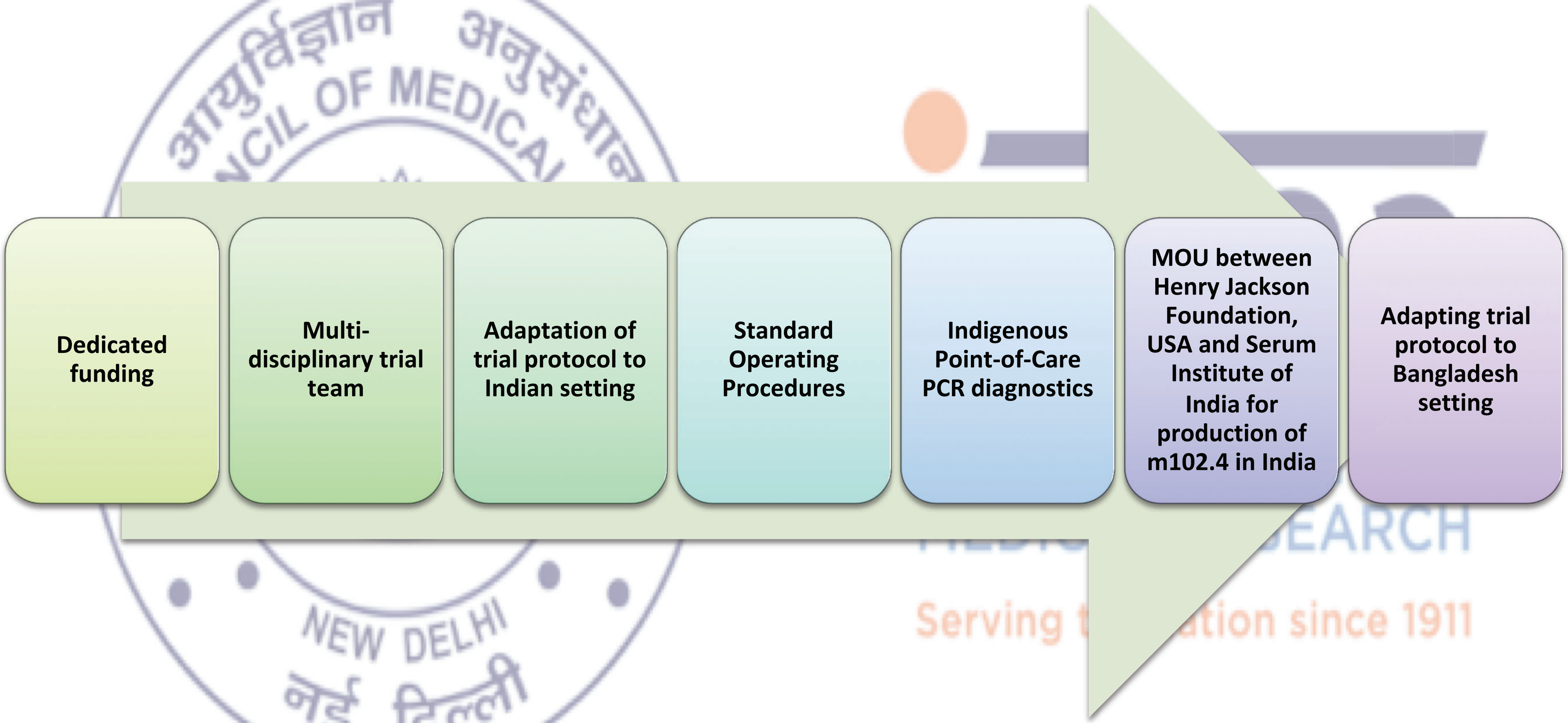


Reference:

Basawarajappa et al; IJMR; epub ahead of print

N Gupta et al; https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247(20)30164-6/fulltext

ICMR’s commitment to Nipah virus infection therapeutic clinical trial



- Dedicated funding
- Multi-disciplinary trial team
- Adaptation of trial protocol to Indian setting
- Standard Operating Procedures
- Indigenous Point-of-Care PCR diagnostics
- MOU between Henry Jackson Foundation, USA and Serum Institute of India for production of m102.4 in India
- Adapting trial protocol to Bangladesh setting

ICMR-WHO collaborative International Consultation for R&D on Nipah Virus and Nipah road map [2018-19]

- ☐ Monoclonal antibody trials
- ☐ Vaccine possibilities
- ☐ Diagnostic issues
- ☐ Preparedness for next outbreak
- ☐ R& D for Nipah

Nipah task force Expert meeting for Team members from NIH, Bangladesh , Malaysia , WHO and ICMR and Non-ICMR other institutes of India

Followed by consultation meeting for the implementation of Nipah Clinical Trial in Dhaka Bangladesh from 24th to 26th September 2019

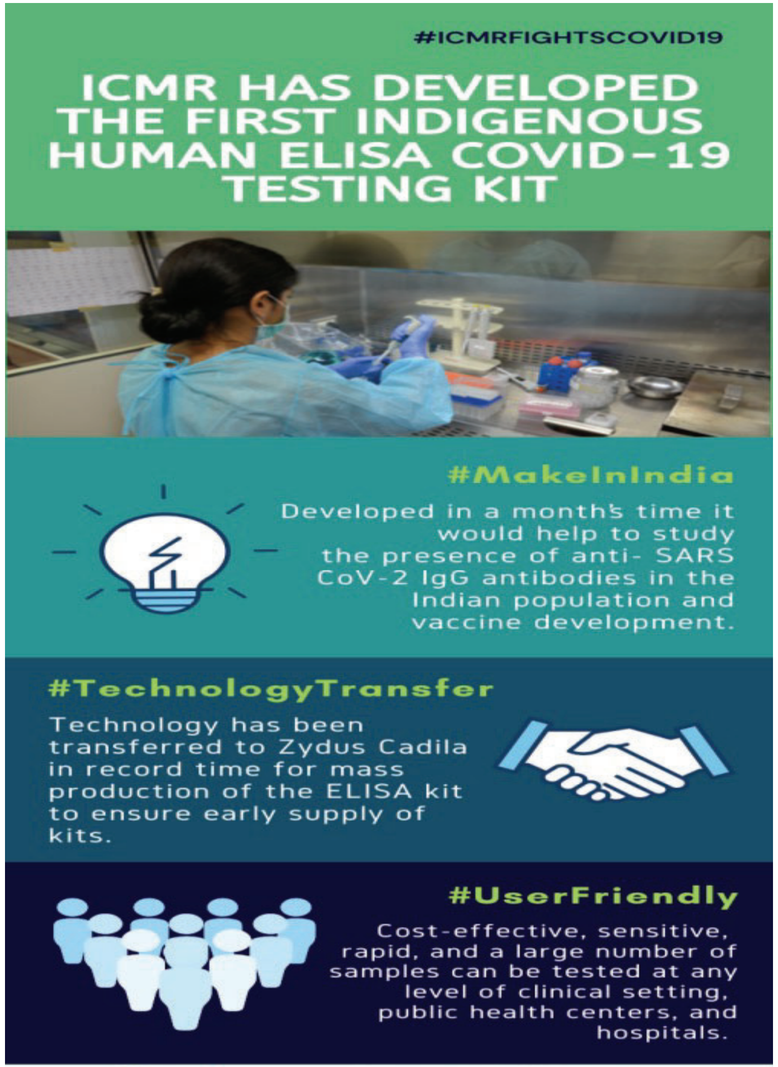
3. Impact of COVID-19 on henipavirus projects

- *Please describe the impact COVID-19 has had on henipavirus-related work. This could include impact on staff, resources, laboratories, etc.*
- *Please include projections of how you anticipate projects will be delayed.*
- *In very few cases, COVID-19 research may accelerate R&D for other viruses. Please describe if this is the case for your working group(s).*

Contribution in Pandemic

- First Genome sequencing and virus isolation
- Variant of concern and its studies
- Validation of Real time PCR, RNA extraction, ELISA , Antigen kits
- Development of assays eg. ELISA, IFA, PRNT, MNT etc
- Development of animals model for SARS CoV-2
- Screening of Anti-viral protocol and compounds
- Development of Monoclonal and Polyclonal Antibody
- Indigenous Vaccine development with Bharat Biotech International Limited
- Preparing antigen bulk for raising equine sera for therapy
- Preclinical study for vaccine in collaboration with Serum Institute and Zydus Cadilla

Indigenous Development COVID 'KAWACH' ELISA



#ICMRFIGHTSCOVID19

ICMR HAS DEVELOPED THE FIRST INDIGENOUS HUMAN ELISA COVID-19 TESTING KIT

#MakeInIndia

Developed in a month's time it would help to study the presence of anti- SARS CoV-2 IgG antibodies in the Indian population and vaccine development.

#TechnologyTransfer

Technology has been transferred to Zydus Cadila in record time for mass production of the ELISA kit to ensure early supply of kits.

#UserFriendly

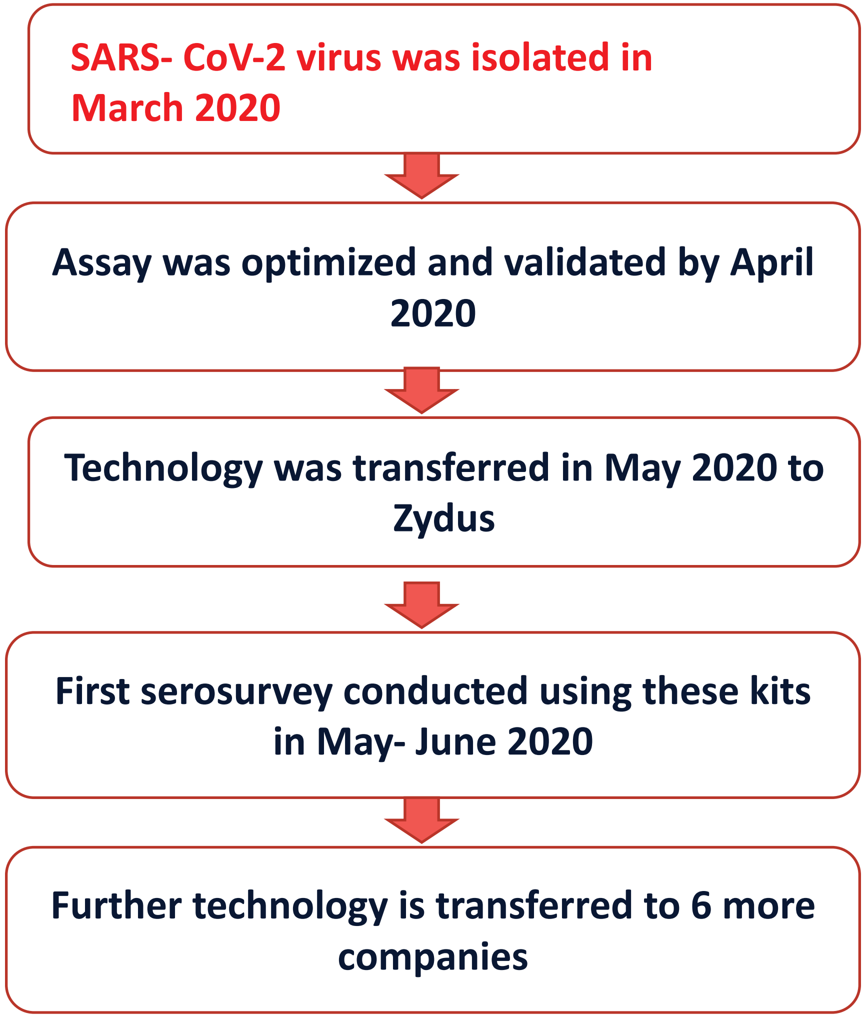
Cost-effective, sensitive, rapid, and a large number of samples can be tested at any level of clinical setting, public health centers, and hospitals.

Technology transferred to Zydus Cadila & seven other companies

To check presence of IgG (determine exposure COVID-19 in past)

#ICMRFightsCovid19

ICMR's technology has been transferred to Zydus Cadila in record time for mass production of the ELISA kit to ensure early supply of COVID-19 testing kits



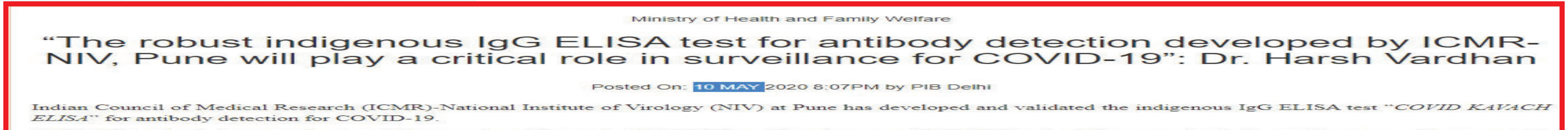
SARS- CoV-2 virus was isolated in March 2020

Assay was optimized and validated by April 2020

Technology was transferred in May 2020 to Zydus

First serosurvey conducted using these kits in May- June 2020

Further technology is transferred to 6 more companies



Ministry of Health and Family Welfare

"The robust indigenous IgG ELISA test for antibody detection developed by ICMR-NIV, Pune will play a critical role in surveillance for COVID-19": Dr. Harsh Vardhan

Posted On: 10 MAY 2020 8:07PM by PIB Delhi

Indian Council of Medical Research (ICMR)-National Institute of Virology (NIV) at Pune has developed and validated the indigenous IgG ELISA test "*COVID KAVACH ELISA*" for antibody detection for COVID-19.

Therapeutics and Prophylactics for COVID-19

Plasma Therapy

Equine antisera

Purified $F(ab)_2$ fragments of antisera against SARS CoV-2 in Syrian hamster model

Monoclonal antibody against SARS-CoV-2 in Syrian hamster model

Zydus Cadilla

Prophylactics

Inactivated whole virion vaccine (COVAXIN)

Bharat Biotech

DNA vaccine

Zydus Cadilla

- ICMR-NIV has collaborated with multiple institutes and Pharma companies for preclinical research and clinical trials for therapeutic and prophylactic products against SARS-CoV-2

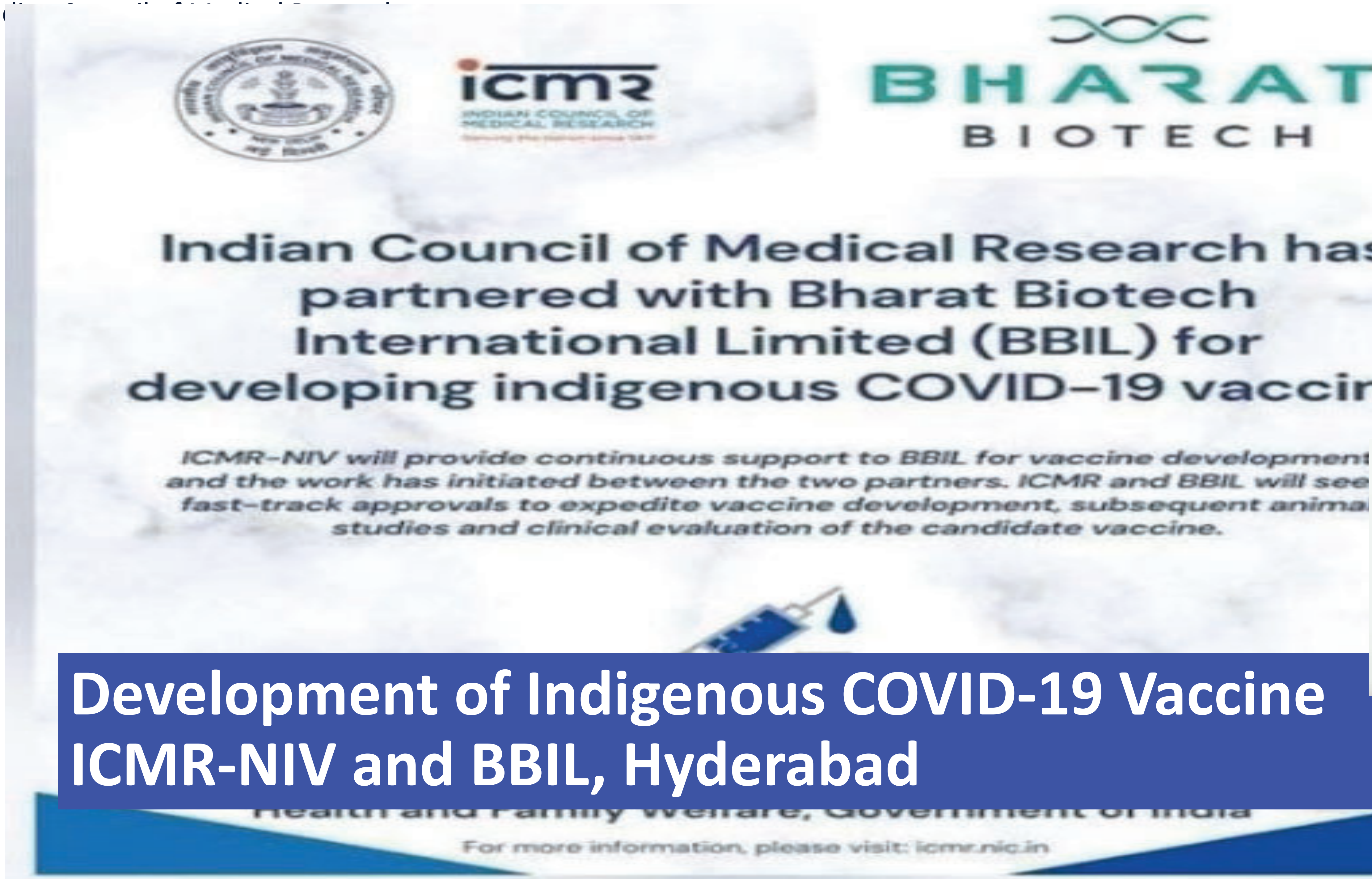
Clinical Trial > BMJ. 2020 Oct 22;371:m3939. doi: 10.1136/bmj.m3939.

Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID Trial)

Anup Agarwal [1], Aparna Mukherjee [2], Gunjan Kumar [1], Pranab Chatterjee [1], Tarun Bhatnagar [3], Pankaj Malhotra [4], PLACID Trial Collaborators

Collaborators, Affiliations + expand

In



BHARAT
BIOTECH

Indian Council of Medical Research has partnered with Bharat Biotech International Limited (BBIL) for developing indigenous COVID–19 vaccine

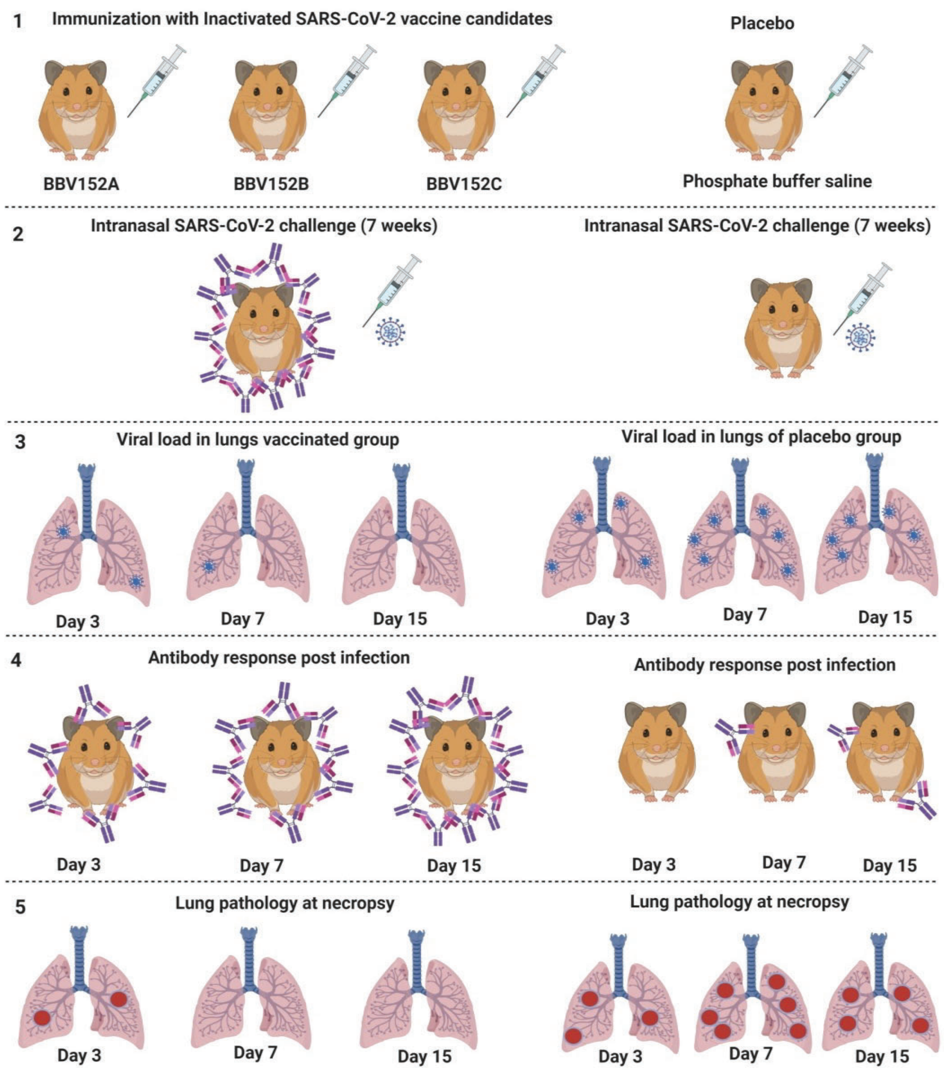
ICMR–NIV will provide continuous support to BBIL for vaccine developmen and the work has initiated between the two partners. ICMR and BBIL will see fast–track approvals to expedite vaccine development, subsequent anima studies and clinical evaluation of the candidate vaccine.

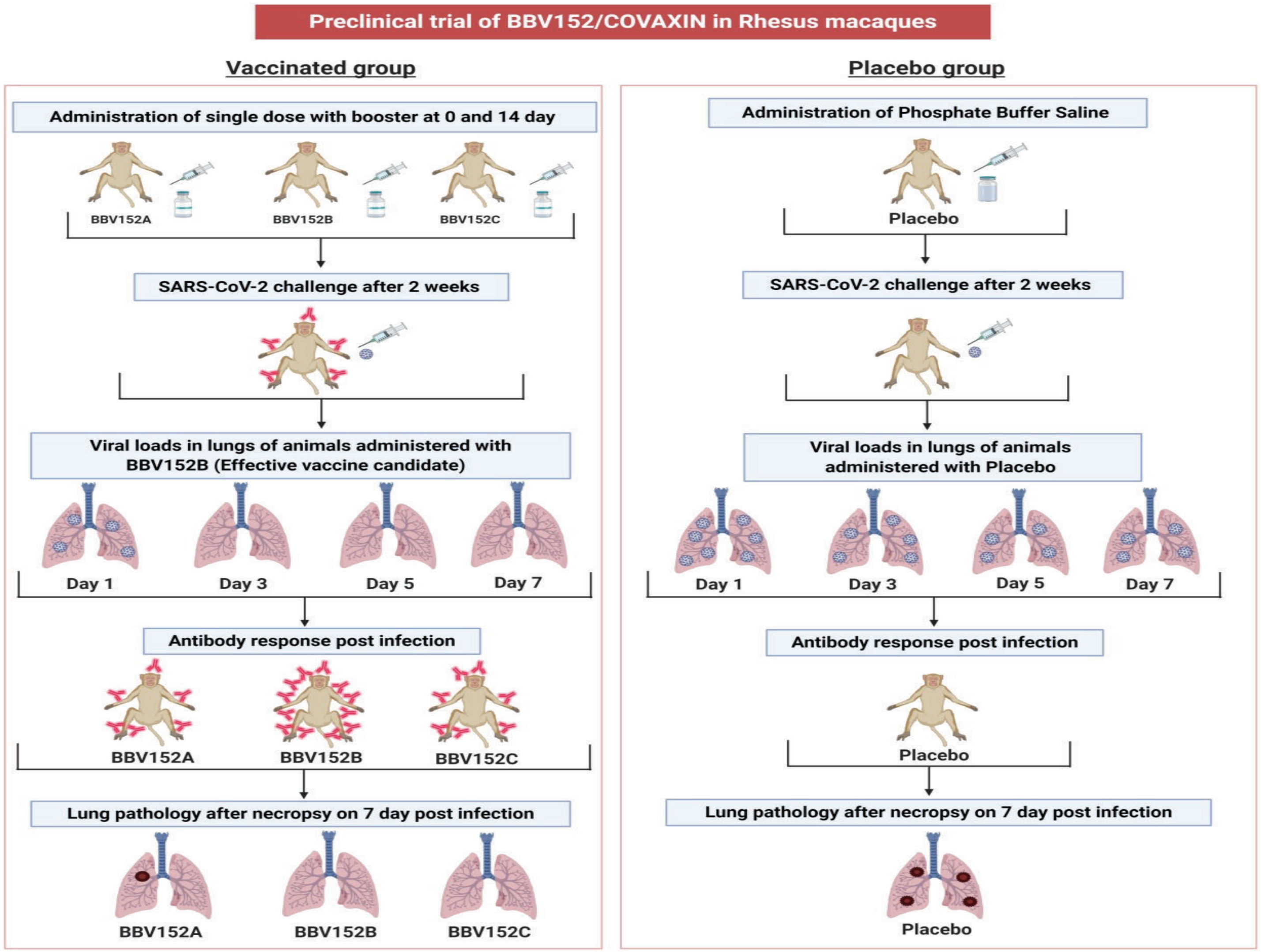
Health and Family Welfare, Government of India

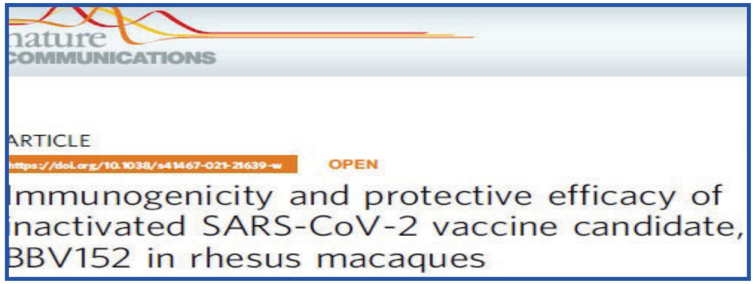
For more information, please visit: icmr.nic.in

Development of Indigenous COVID-19 Vaccine ICMR-NIV and BBIL, Hyderabad

Preclinical study of COVAXIN™






nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-021-21639-w OPEN

Immunogenicity and protective efficacy of inactivated SARS-CoV-2 vaccine candidate, BBV152 in rhesus macaques


iScience

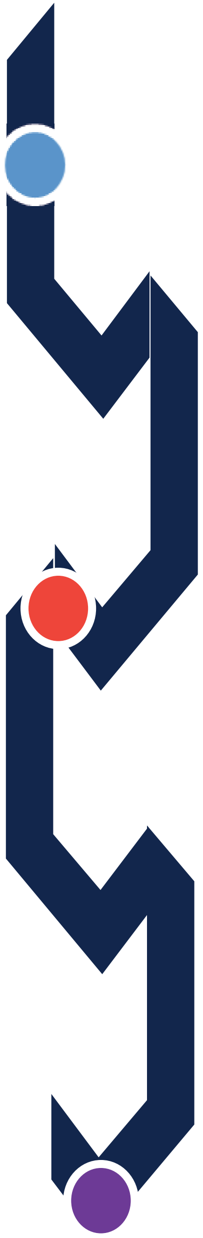
Volume 24, Issue 2, 19 February 2021, 102054

CellPress

Article

Immunogenicity and protective efficacy of BBV152, whole virion inactivated SARS-CoV-2 vaccine candidates in the Syrian hamster model

Sreelekshmy Mohandas [1], Pragya D. Yadav [1, 5], Anita Shete-Aich [1], Priya Abraham [1], Krishna Mohan Vadrevu [2], Gajanan Sapkal [1], Chandrashekhar Mote [3], Dimpal Nyayanit [1], Nivedita Gupta [4], Vellimedu Kannappa Srinivas [2], Manoj Kadam [1], Abhimanyu Kumar [1], Triparna Majumdar [1], Rajlaxmi Jain [1], Gururaj Deshpande [1], Savita Patil [1], Prasad Sarkale [1], Deepak Patil [1] … Balram Bhargava [4]



Phase-I (13-30 July 2020)

- **375** healthy participants (18-55 age group enrolled)
- 100 each - randomly assigned to 3 vaccine groups
- 75 were randomly assigned to the control group
- The most common solicited **adverse events were mild**
- **82.3-91.9% seroconversion**

THE LANCET
Infectious Diseases

Access provided by National Institute of Virology

ARTICLES | VOLUME 21, ISSUE 5, P637-646, MAY 01, 2021

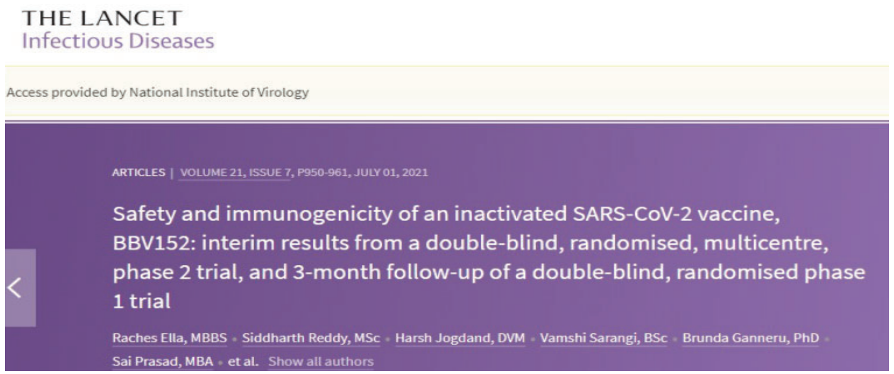
Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial

Raches Ella, MBBS • Krishna Mohan Vadrevu, PhD • Harsh Jogdand, DVM • Sai Prasad, MSc • Siddharth Reddy, MSc • Vamshi Sarangi, BSc • et al. Show all authors

Published: January 21, 2021 • DOI: https://doi.org/10.1016/S1473-3099(20)30942-7 • Check for updates

Phase-II (5-12 Sep 2020)

- **380** healthy participants (12-65 age group enrolled)
- GMT titres ranged from 100·9-197·0
- **Seroconversion at Day 56 is 88.0%-96·6%**
- 3-month post-second-dose GMTs ranged from 39·9-69·5
- 6 µg with Algel-IMDG formulation selected for the phase-III



THE LANCET
Infectious Diseases

Access provided by National Institute of Virology

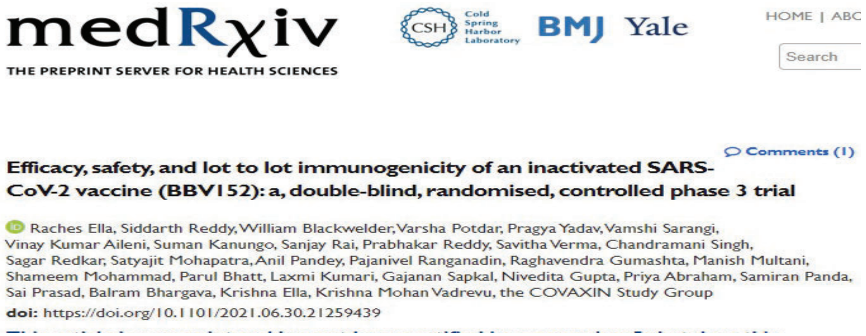
ARTICLES | VOLUME 21, ISSUE 7, P950-961, JULY 01, 2021

Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial

Raches Ella, MBBS • Siddharth Reddy, MSc • Harsh Jogdand, DVM • Vamshi Sarangi, BSc • Brunda Ganneru, PhD • Sai Prasad, MBA • et al. Show all authors

Phase-III (16 Nov 2020-7 Jan 2021)

- **25,798 participants** (18-98 age group enrolled)
- **78% efficacy-** against Mild, moderate and severe COVID-19
- **93% efficacy-** against severe COVID-19 reducing hospitalization
- **63% efficacy-** against asymptomatic COVID-19 transmission



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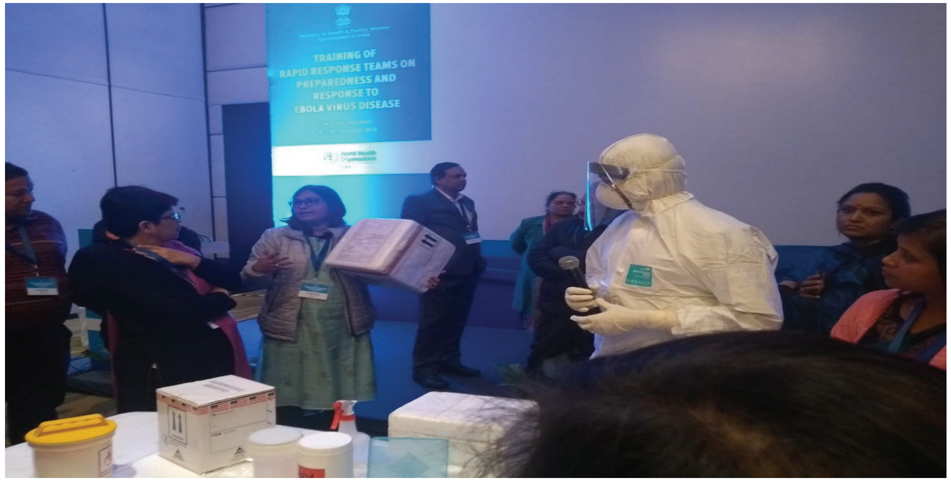
Comments (1)

Efficacy, safety, and lot to lot immunogenicity of an inactivated SARS-CoV-2 vaccine (BBV152): a, double-blind, randomised, controlled phase 3 trial

Raches Ella, Siddarth Reddy, William Blackwelder, Varsha Potdar, Pragya Yadav, Vamshi Sarangi, Vinay Kumar Aileni, Suman Kanungo, Sanjay Rai, Prabhakar Reddy, Savitha Verma, Chandramani Singh, Sagar Redkar, Satyajit Mohapatra, Anil Pandey, Pajanivel Ranganadin, Raghavendra Gumashta, Manish Multani, Shameem Mohammad, Parul Bhatt, Laxmi Kumari, Gajanan Sapkal, Nivedita Gupta, Priya Abraham, Samiran Panda, Sai Prasad, Balram Bhargava, Krishna Ella, Krishna Mohan Vadrevu, the COVAXIN Study Group

doi: https://doi.org/10.1101/2021.06.30.21259439

Training of Rapid Response Teams Public Health Preparedness and Response to Ebola and Nipah Virus Disease (Oct-Dec 2019)
Bangalore, Guwahati, Pune, New Delhi , Bhubaneswar



Impact of COVID-19 on Nipah virus R&D in India

Impact	Lesson learned and advantages gained
With all the challenges of natural disaster happening and two severe waves of pandemic in 2020 and 2021 and emerge of VOC has impacted the Nipah virus bat survey in different states due to intermittent lockdown in different states .	➢ Enhanced strengthening, testing of the laboratories in the country including mobile laboratory and containment facility ➢ Laboratory capacity for preclinical studies of vaccine and clinical trial sites improved and enhanced in country ➢ Ready for Nipah vaccine and drugs trials and preclinical study
Both the waves of the pandemic had led the public health system to completely driven for the SARS-CoV-2 surveillance and control. This had led to reduce surveillance of other diseases including Nipah and other high-risk pathogens .	➢ Self reliance for the development of PPEs, Vaccine, along with conducting pre-clinical study in animal models and multi-centric trials on different products. ➢ Excellent private and Government collaborations
This has also impacted the further strengthening of the RESEARCH platform and Nipah clinical trial work	➢ Bio-risk mitigation practices improved, creation of isolation facility and PPE donning and doffing training all over the country and its importance is now inculcated amongst the health care and frontline workers
All efforts and research are diverted to SARS-CoV-2 pandemic.	➢ Working in extreme diversity and showing the humungous efforts at each level
No cases referred so we don't know the immediate status	➢ Establishment of robust validation of kits including PCR, RNA, Serological assay
R& D research focused complete as per need of country for COVID 19	➢ Fast track approval from different sectors to enhance studies

4. Major obstacles and how Task Force can facilitate moving forward

Major obstacles for Nipah	Way out
➢ Diagnosis and scaling up ➢ [Molecular and serological assays and its quality] ➢ Containment facility ➢ Augment clinical research capacity.	✓ Strengthen AES/SARI network including Nipah as one of screening agent ✓ Validation at different facility to make robust testing including standard diagnostic is needed ✓ External validation of the diagnostic assays
➢ Risk communication and community mobilization strategies during Nipah virus outbreak as well as during implementation of clinical trials during outbreak.	✓ Enhancing number of such facilities and work culture
➢ **Cutting edge technology for development of vaccine candidates**	✓ Development of the vaccine and therapeutic products and its clinical trial at fast speed ✓ Need help of CEPI and other organization
➢ Augment clinical trial capacity with newer medical countermeasures (drugs, vaccines and monoclonals)	✓ Safety and efficacy studies and Immunogenicity studies in multiple-partners network
➢ Epidemiological studies to enable early detection of Nipah virus (including modelling).	✓ Need multicentre approach and smooth process
➢ As the cases of Nipah outbreaks are mostly sporadic, its difficult to initiate any such study. ➢ Dedicated team should plan and ready for such outbreaks to act immediately in clinical trail mode. ➢ Preparedness	✓ Need for the trained manpower for field, laboratory and clinical trail related work

“COVID-19 has taught that diseases have no borders, pathogens treat humans equally. Remember the strength which we have gained , lessons which we have learnt to fight unending war of infectious disease but lets do it together”

Pragya Yadav

CEPI

Nipah virus – Updates from Malaysia

Li-Yen Chang
Associate Professor
Department of Medical Microbiology
Faculty of Medicine
Universiti Malaya, MALAYSIA

Nipah virus work in Malaysia – to date

No NiV outbreaks since first outbreak in 1998

1997 – 1st death recorded in Kinta District, Perak

Clinical, pathological, epidemiological studies and reports

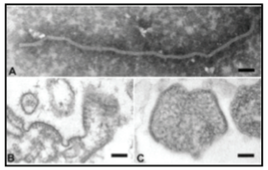
Culling of pigs to end the outbreak

Basic virological investigations (antigenicity, protein-protein interactions, virus replication and assembly, transmission dynamics, detection assays, vaccine candidates)

2010

1998 – NiV outbreak started in Malaysia

1999 – NiV was isolated



Chua KB et al, Science, 2000

1998-2010 – Several NiV strains from humans, pigs and bats were isolated, sequenced and characterized

2015

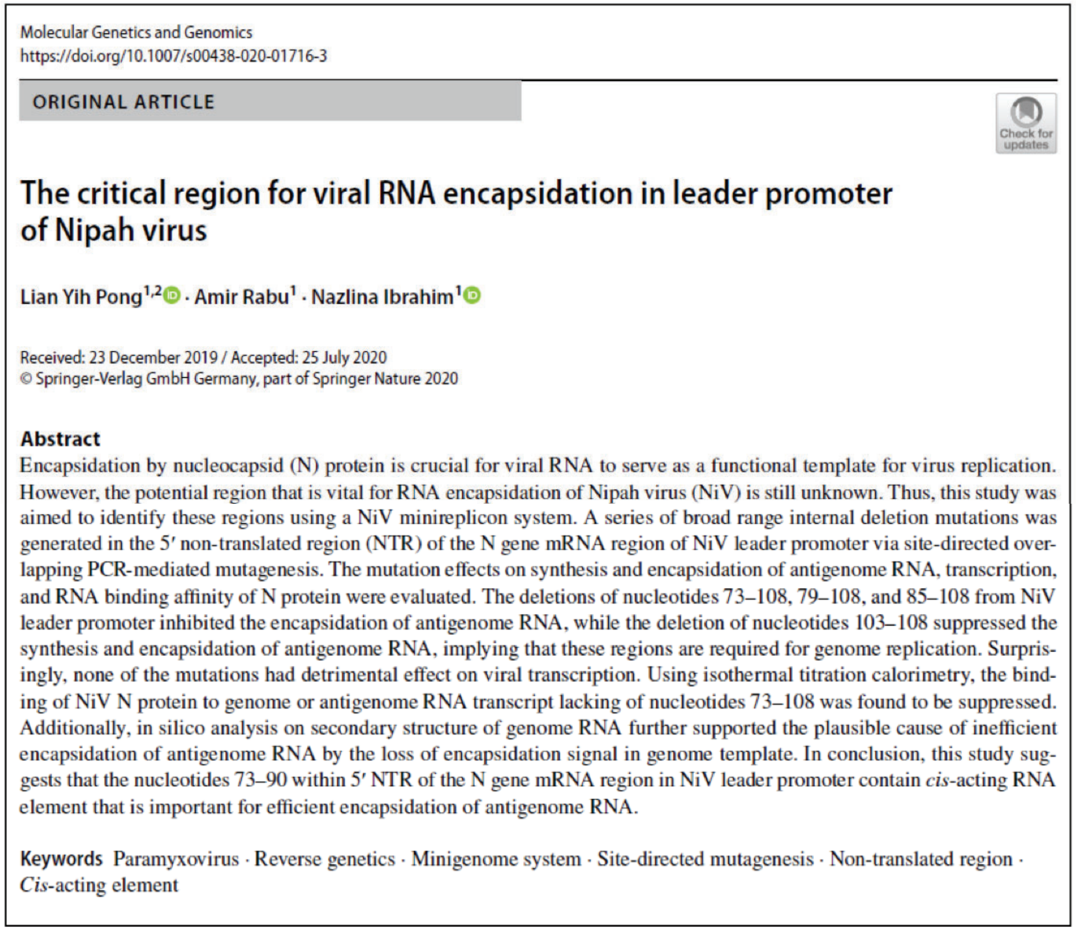
2021

Seroprevalence, viral RNA encapsidation

Related to bats >>
Bat species diversity,
Knowledge, attitude and practice – bat-borne diseases

UNIVERSITI MALAYA

Recent NiV research work in Malaysia



Molecular Genetics and Genomics
https://doi.org/10.1007/s00438-020-01716-3

ORIGINAL ARTICLE

The critical region for viral RNA encapsidation in leader promoter of Nipah virus

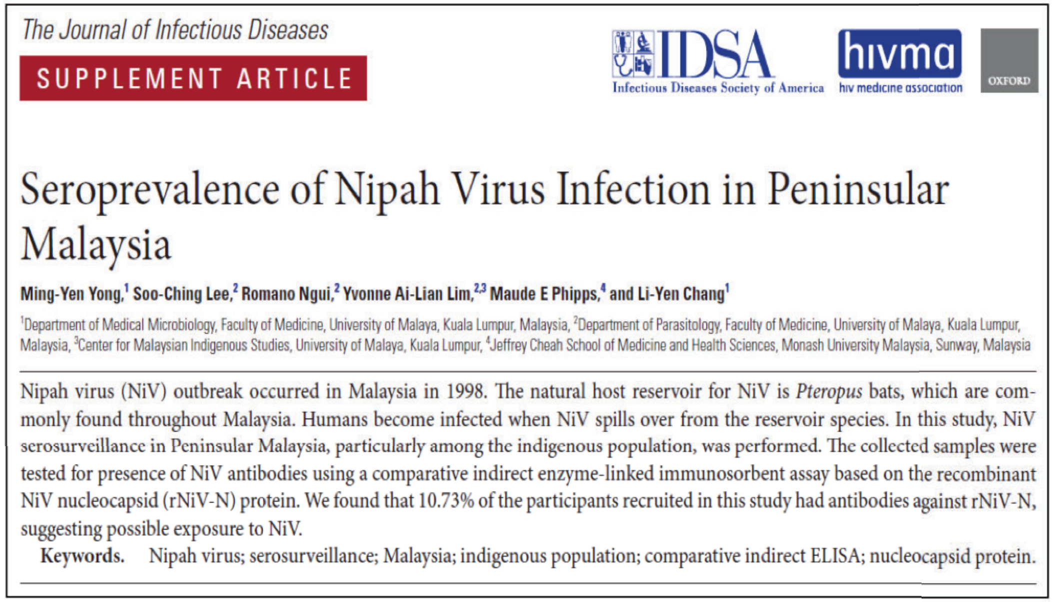
Lian Yih Pong[1,2] · **Amir Rabu**[1] · **Nazlina Ibrahim**[1]

Received: 23 December 2019 / Accepted: 25 July 2020

Abstract

Encapsidation by nucleocapsid (N) protein is crucial for viral RNA to serve as a functional template for virus replication. However, the potential region that is vital for RNA encapsidation of Nipah virus (NiV) is still unknown. Thus, this study was aimed to identify these regions using a NiV minireplicon system. A series of broad range internal deletion mutations was generated in the 5′ non-translated region (NTR) of the N gene mRNA region of NiV leader promoter via site-directed overlapping PCR-mediated mutagenesis. The mutation effects on synthesis and encapsidation of antigenome RNA, transcription, and RNA binding affinity of N protein were evaluated. The deletions of nucleotides 73–108, 79–108, and 85–108 from NiV leader promoter inhibited the encapsidation of antigenome RNA, while the deletion of nucleotides 103–108 suppressed the synthesis and encapsidation of antigenome RNA, implying that these regions are required for genome replication. Surprisingly, none of the mutations had detrimental effect on viral transcription. Using isothermal titration calorimetry, the binding of NiV N protein to genome or antigenome RNA transcript lacking of nucleotides 73–108 was found to be suppressed. Additionally, in silico analysis on secondary structure of genome RNA further supported the plausible cause of inefficient encapsidation of antigenome RNA by the loss of encapsidation signal in genome template. In conclusion, this study suggests that the nucleotides 73–90 within 5′ NTR of the N gene mRNA region in NiV leader promoter contain *cis*-acting RNA element that is important for efficient encapsidation of antigenome RNA.

Keywords Paramyxovirus · Reverse genetics · Minigenome system · Site-directed mutagenesis · Non-translated region · *Cis*-acting element



The Journal of Infectious Diseases
SUPPLEMENT ARTICLE
IDSA Infectious Diseases Society of America · hivma hiv medicine association · OXFORD

Seroprevalence of Nipah Virus Infection in Peninsular Malaysia

Ming-Yen Yong,[1] Soo-Ching Lee,[2] Romano Ngui,[2] Yvonne Ai-Lian Lim,[2,3] Maude E Phipps,[4] and Li-Yen Chang[1]

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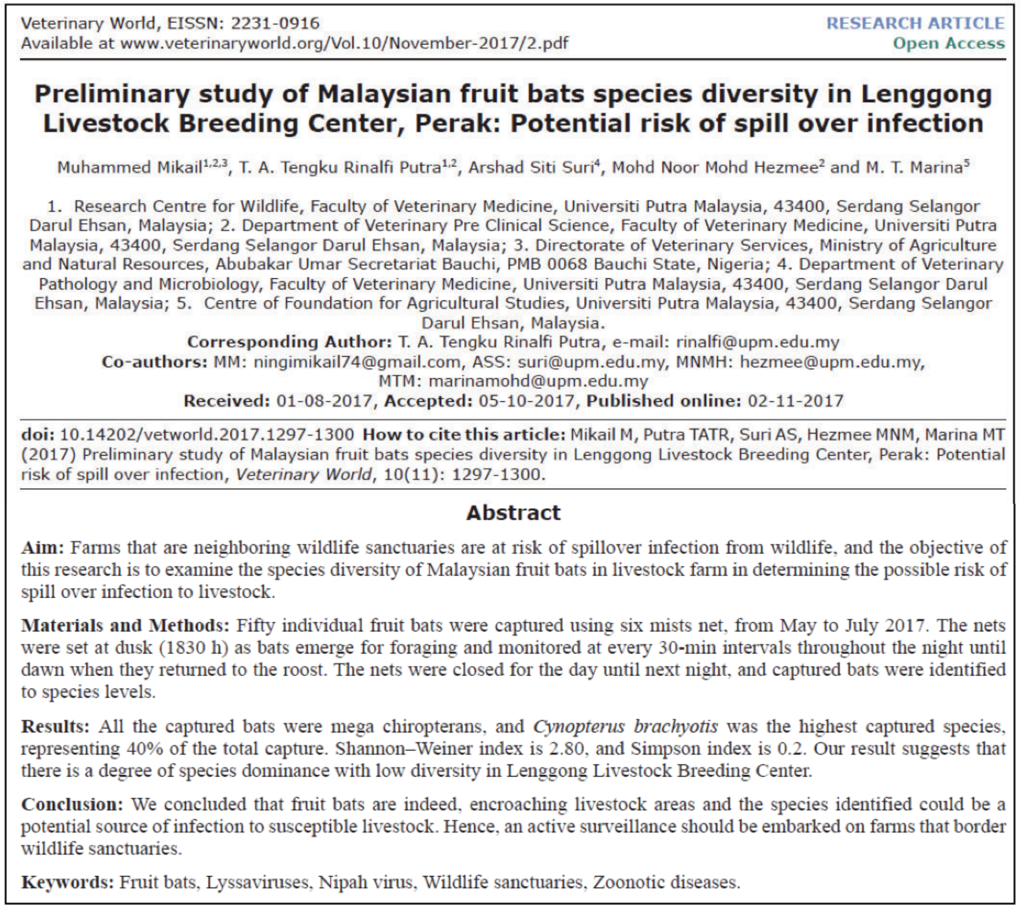
Nipah virus (NiV) outbreak occurred in Malaysia in 1998. The natural host reservoir for NiV is *Pteropus* bats, which are commonly found throughout Malaysia. Humans become infected when NiV spills over from the reservoir species. In this study, NiV serosurveillance in Peninsular Malaysia, particularly among the indigenous population, was performed. The collected samples were tested for presence of NiV antibodies using a comparative indirect enzyme-linked immunosorbent assay based on the recombinant NiV nucleocapsid (rNiV-N) protein. We found that 10.73% of the participants recruited in this study had antibodies against rNiV-N, suggesting possible exposure to NiV.

Keywords. Nipah virus; serosurveillance; Malaysia; indigenous population; comparative indirect ELISA; nucleocapsid protein.

Recent NiV-related research work in Malaysia



Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.10/November-2017/2.pdf

RESEARCH ARTICLE
Open Access

Preliminary study of Malaysian fruit bats species diversity in Lenggong Livestock Breeding Center, Perak: Potential risk of spill over infection

Muhammed Mikail[1,2,3], T. A. Tengku Rinalfi Putra[1,2], Arshad Siti Suri[4], Mohd Noor Mohd Hezmee[2] and M. T. Marina[5]

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Received: 01-08-2017, **Accepted:** 05-10-2017, **Published online:** 02-11-2017

doi: 10.14202/vetworld.2017.1297-1300 **How to cite this article:** Mikail M, Putra TATR, Suri AS, Hezmee MNM, Marina MT (2017) Preliminary study of Malaysian fruit bats species diversity in Lenggong Livestock Breeding Center, Perak: Potential risk of spill over infection, *Veterinary World*, 10(11): 1297-1300.

Abstract

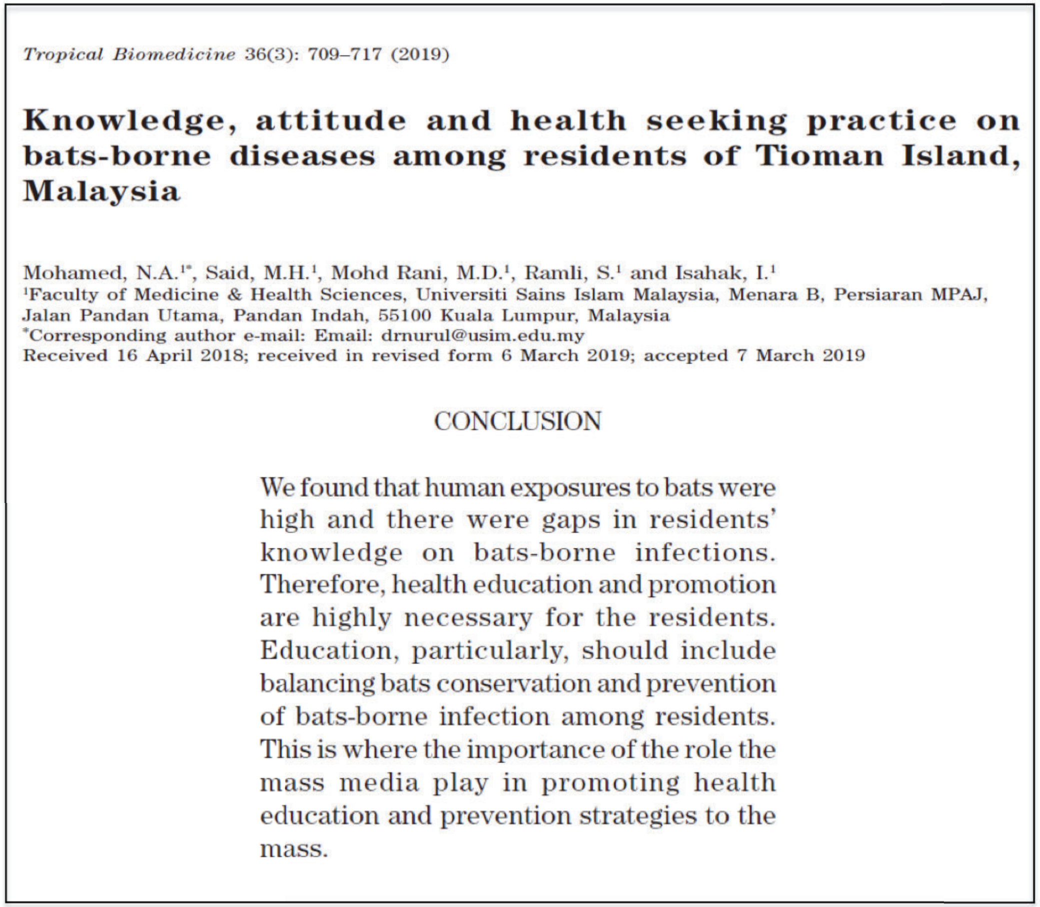
Aim: Farms that are neighboring wildlife sanctuaries are at risk of spillover infection from wildlife, and the objective of this research is to examine the species diversity of Malaysian fruit bats in livestock farm in determining the possible risk of spill over infection to livestock.

Materials and Methods: Fifty individual fruit bats were captured using six mists net, from May to July 2017. The nets were set at dusk (1830 h) as bats emerge for foraging and monitored at every 30-min intervals throughout the night until dawn when they returned to the roost. The nets were closed for the day until next night, and captured bats were identified to species levels.

Results: All the captured bats were mega chiropterans, and *Cynopterus brachyotis* was the highest captured species, representing 40% of the total capture. Shannon–Weiner index is 2.80, and Simpson index is 0.2. Our result suggests that there is a degree of species dominance with low diversity in Lenggong Livestock Breeding Center.

Conclusion: We concluded that fruit bats are indeed, encroaching livestock areas and the species identified could be a potential source of infection to susceptible livestock. Hence, an active surveillance should be embarked on farms that border wildlife sanctuaries.

Keywords: Fruit bats, Lyssaviruses, Nipah virus, Wildlife sanctuaries, Zoonotic diseases.



Tropical Biomedicine 36(3): 709–717 (2019)

Knowledge, attitude and health seeking practice on bats-borne diseases among residents of Tioman Island, Malaysia

Mohamed, N.A.[1*], Said, M.H.[1], Mohd Rani, M.D.[1], Ramli, S.[1] and Isahak, I.[1]
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Received 16 April 2018; received in revised form 6 March 2019; accepted 7 March 2019

CONCLUSION

We found that human exposures to bats were high and there were gaps in residents' knowledge on bats-borne infections. Therefore, health education and promotion are highly necessary for the residents. Education, particularly, should include balancing bats conservation and prevention of bats-borne infection among residents. This is where the importance of the role the mass media play in promoting health education and prevention strategies to the mass.

Impact of Covid-19 on henipavirus projects

Item	Impact
Proposal submission and new project funding	No impact in general, but less prioritized now
Staffing, resources, laboratories	Not applicable, except for project requiring resources and infrastructure from hospitals
Progress of funded projects	Delayed due to WFH, no inter-state and inter-district travel
Acceleration of research due to COVID-19	Not applicable

Major obstacles and how Task Force can facilitate

1. Project funding and support
2. Link and connect with potential partners

Thank you

Affected country updates
Q&A

Antibody standard

NiV antibody standard development I

Li-Yen Chang
Associate Professor
Department of Medical Microbiology
Faculty of Medicine
Universiti Malaya, MALAYSIA

UNIVERSITI
MALAYA

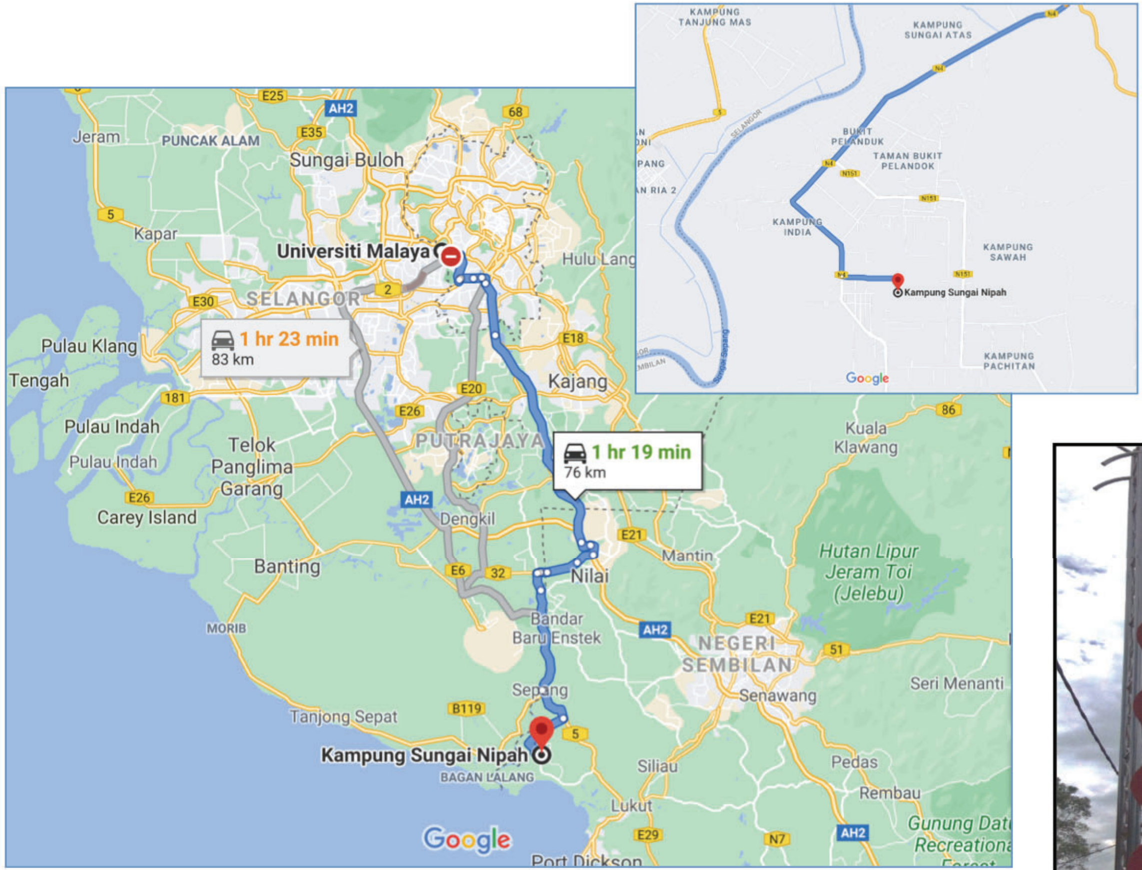
NiV encephalitis in Malaysia 1998/1999

Total: 265 cases, 105 deaths

At least 94 NiV patients were treated at Universiti Malaya Medical Centre (UMMC)

Neurology Unit, Dept. Medicine UMMC followed-up with some surviving NiV patients (~68%)

Post-NiV outbreak visit in 2015



No NiV surveilance program

Follow-up visit to Kampung Sungai Nipah

Blood samples were collected from 24 individuals, all except one was detected positive by NiV pseudovirus VNT

UM-CEPI-NIBSC NiV Convalescent Sera Project

Scope of Work:

1. Quantify and characterize NiV specific antibody in collected sera/plasma from NiV survivors
2. Transfer sera/plasma to NIBSC to develop working standard and WHO-endorsed antibody standard
3. Characterize the memory B- and T-cells in the PBLs of NiV survivors
4. Sharing of assay methodology for technology transfer

Thank you

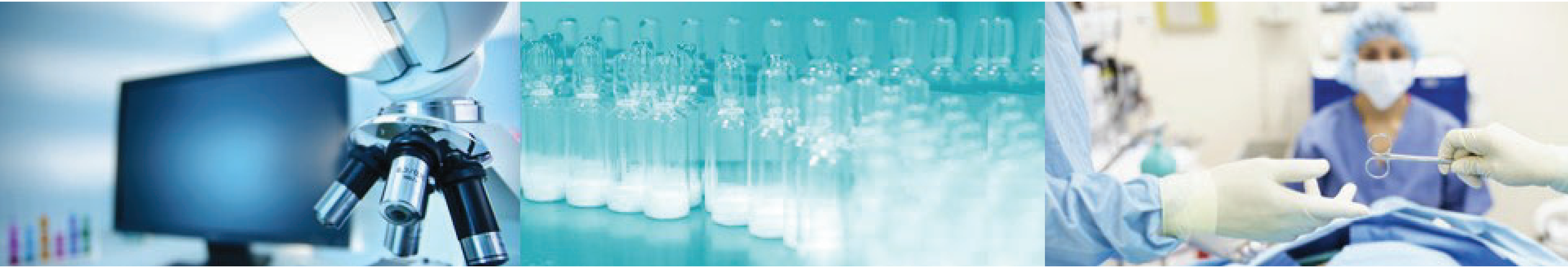
Medicines & Healthcare products
Regulatory Agency

NIBSC

Nipah antibody standard development - NIBSC

Mark Hassall

7th September 2021



Why do we need biological standards in assays

The potency of biological products can differ between different tests and laboratories and this needs to be controlled for

Without any physical measure of activity they can be measured by referencing against a standard

Serology testing in bioassays can be harmonised by comparison to the performance of a reference standard

For clinical testing this allows comparison of vaccine responses from different assays or labs

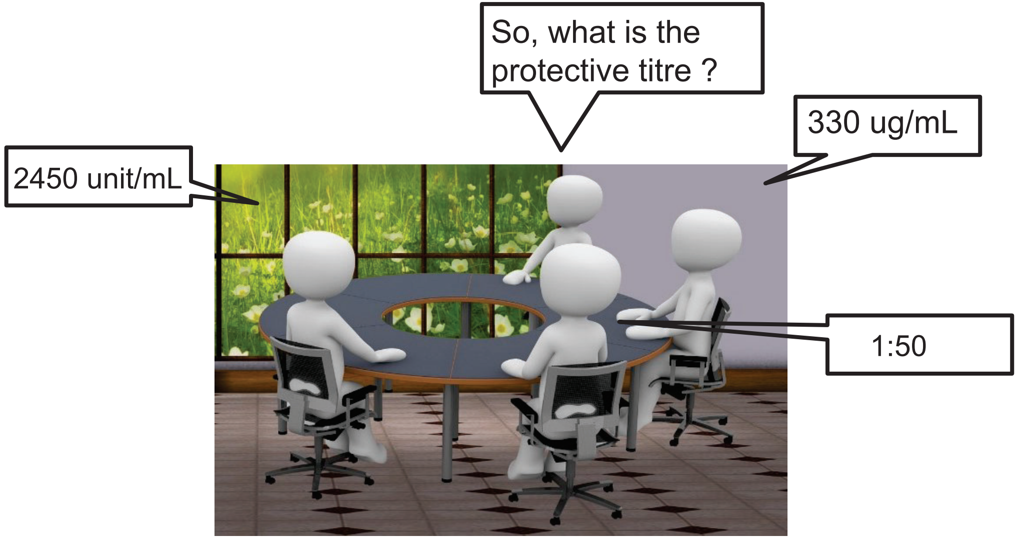
Hierarchy of standards:

- Primary calibrant – WHO IS
- External control/secondary standards
- Assay standards
  - Internal calibrator/standard
  - run control
  - working reagent
  - Secondary or tertiary reagent

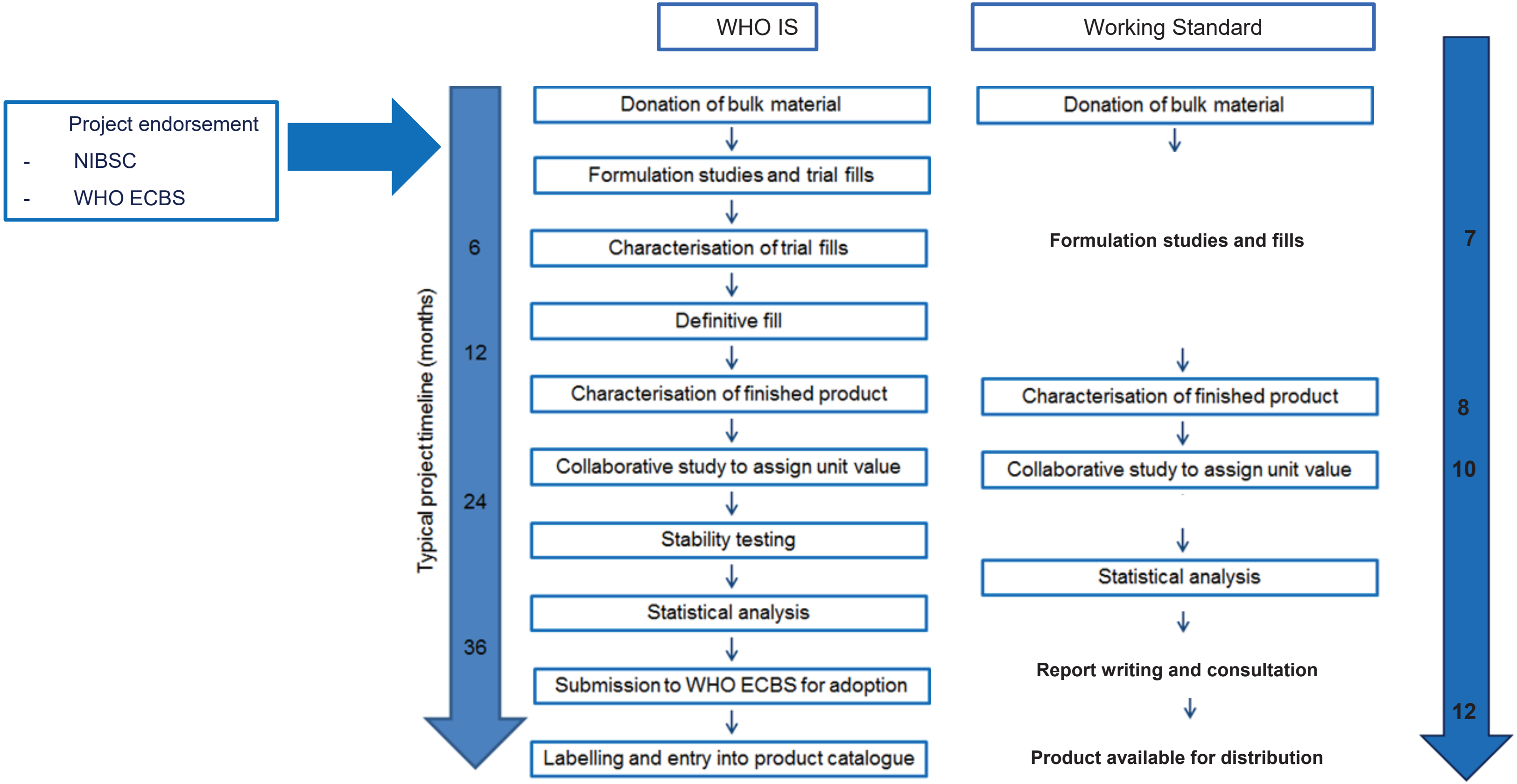
Primary calibrant

WHO International Standard – established by the WHO ECBS

Define the common language to express potency of the samples



Standards projects



Project endorsement
- NIBSC
- WHO ECBS

WHO IS

Typical project timeline (months)

Donation of bulk material
↓
Formulation studies and trial fills
↓
Characterisation of trial fills
↓
Definitive fill
↓
Characterisation of finished product
↓
Collaborative study to assign unit value
↓
Stability testing
↓
Statistical analysis
↓
Submission to WHO ECBS for adoption
↓
Labelling and entry into product catalogue

Working Standard

Donation of bulk material
↓
Formulation studies and fills
↓
Characterisation of finished product
↓
Collaborative study to assign unit value
↓
Statistical analysis
↓
Report writing and consultation
↓
Product available for distribution

Sample collection and preparation

- UM will collect samples from convalescent patient and ship to PHE
- NIBSC will provide PHE with any blood virology report & previous PCR test results associated with samples
- Samples tested for Nipah virus and If sample is negative ship to NIBSC
- Samples received at NIBSC CL3 lab
- Samples treated by solvent detergent
- Samples tested by ELISA and PV assay
- Samples prepared for final formulation

Nipah antibody standard timelines

Cepi Working standard

- Endorsement by the WHO Expert committee on Biological standardization (Jan 2019)
- Sample collection from source country/partner – MTA signed with UM (Aug-Sept 2021)
- Sample testing by PHE then transfer to NIBSC (Sept 2021)
- Characterisation of antibody at NIBSC by ELISA, neutralisation assay (Sept-Oct 2021)
- Data reporting to partner and recommendation for larger donation (Oct 2021)
- Sample testing by PHE then transfer to NIBSC (Dec 2021)
- Formulation of working standard candidate and reference panel (Jan 2022)
- Feasibility study (Feb-Mar 2022)
- Analysis and reporting of study (Mar-Apr 2022)
- Establishment of working standard and reference panel (Apr 2022)

Nipah antibody standard timelines

International standard

- Stability study for candidate International Standard (Feb 2022-Feb 2023)
- Organisation of the WHO Collaborative study (Apr-May 2022)
- WHO Collaborative study (Jun-Sep 2022)
- Statistical analysis (Sep-Oct 2022)
- Collaborative study report and submission to WHO (Oct-Nov 2022)
- Establishment of International Standard by WHO ECBS (Mar or Oct 2023)
- Deliverable: WHO International Standard made available for distribution (May or Dec 2023)

What is the purpose of a collaborative study?

- It is to gather a set of data to confirm that an antibody reference candidate is fit for purpose
    - It should work in a range of assays (ELISA, neutralisation)
    - It allows harmonisation of results from different labs/assays
    - It works across a titration range (limit of detection)
- To assign an (international)unitage to the standard and a consensus on the panel levels (high, medium, low)
- It is <u>not</u> for validating or for testing assays

Collaborative study

Ahead of receiving the study samples you will be sent

- A study protocol
  - Introduction to the study - aims
  - Description of the study samples (numbers, volume, origin, treatments, etc.)
  - How many independent assays and possible dilutions suggestion
  - How to report the results - timelines
  - Contact details
- A result reporting Excel file
  - Assay reporting tables already setup
  - Data reporting tabs for each assay method
  - Comments box

When the samples are shipped participants will be notified of shipment and arrival dates, and

- Information for use – including MSDS

After the collaborative study is completed the data will be analysed at NIBSC and a report with the results issued (labs are anonymised). All participants will have the opportunity to comment

NIBSC

Mark Page
Emma Bentley
Giada Mattiuzzo
Mark Hassall

PHE

Marion Killip

Antibody standard
Q&A

CEPI

Natural History Studies

Sept 1, 2021

Concept and Assumptions

- CEPI and NIAID will design an animal modeling approach that will serve CEPI and NIAID (maybe also other USG entities) with robust Nipah animal models that will suit regulatory applications for product approval using the Animal Rule approval pathway
    - Studies will be executed to high data quality standards (GLP or well-documented)
    - Assumption: 2 animal species will be used, Syrian golden hamsters and African Green monkeys (SGH and AGM)
- Studies will be protocol driven, reviewed by stakeholders
- Using a pre-IND submission package for formal FDA interaction through CEPI regulatory support, Study Protocols will be presented to FDA for review and approval, feedback to be shared with stakeholders
- Work to be performed by CEPI Animal Model Network Partner lab

Progress on design of natural history studies

- July 20, CEPI circulated design plans for hamster and AGM studies to experts and stakeholders:
  - CEPI internal, NIAID team (NI-V PO, CREID, Preclinical services)
  - RML, IRF, UTMB
  - CEPI statistical support
  - CEPI laboratory network partner engaged to carry out this work

- Ongoing work, planned through Q3 2021:
  - Designing studies (complete with statistical rationale), endpoints and scoresheet design to support euthanasia criteria
  - Compiling, reviewing study protocols, obtaining regulatory support from CEPI and other stakeholders
  - Submission of pre-IND package for model building
  - NIAID/BEI production of NIV-B stock (characterized, with CofA) expected in Fall 2021

Summary of proposed activities, circulated for feedback 7/20

1. Virus stock

Strain at hand: NiV Bangladesh

BEI Inventory: 35 vials, supported with CofA

NIAID stock generation effort

2. Confirmation of virulence studies

Use new stock

Hamster first

Then AGM

3. Dose titration studies

Bracket confirmation of virulence doses

Determine adequate dose for reproducible modelling

Hamster and AGM

4. Natural History studies

Detailed study at chosen dose level

Serial sac design was favored for certain aspects

Hamster and AGM

Results will provide a complete package describing model

Comments and issues raised by reviewers

“Design as written is repetitive, and experiments may be combined for collection of enough data and statistical power.”

“Desirable to define an LD50, LD99 if possible.” Plan is to actually use the LD99 dose or something close to it in our models studies testing the NiV-B stock.

CEPI statistical consultant proposes: **Adaptive dose finding study** can be informed by using a starting dose of virus expected to cause severe disease/lethality in hamster (e.g. 10^7 pfu/mL or TCID50 for hamsters); a similar design can be evaluated for AGM (different dose)

Choose 3 doses, ideally equally spaced and logarithmic (eg. **10^6**, **10^7**, and **10^8**), n=5 or 6 animals per group

Collect data, refine curve with second block design, calculate LD50 and LD99

Benefit: spares use of many animals in large groups to define LD50 in a large blind study; two experiments’ data can be compiled if data are independent blocks

“Should we consider a study that is designed to rule out any role that sedation/manipulation might play in NiV disease progression?”

Suggestion that NIAID’s PCS could do this in parallel. Maybe also evaluate alternative routes of inoculation (IP vs IN)

“Are we using more than one site for data collection/model establishment?”

Probably, and especially if NIAID chips in for parallel studies. Raises questions of how endpoints collected at various sites will be standardized to allow comparison. E.g. Need consistent euthanasia criteria and health scoring sheets to perform consistent work, as well as consistent animal species ages, weights, feeding and handling methods, etc.

“Use TCID50 or PFU/mL, and be consistent.” Perhaps early studies could present data in both formats, enabling comparisons.

If we are evaluating hematology and clinical chemistry parameters, we should make sure normal values for these in the animals under test are established at the site.

Model characterization studies, serial-sac design

- Well-designed serial sac studies could enable collection of model characterization over infection time period, yet also collect timepoints
- Animals would be randomized to sac days (eg. 2, 4, 6, 8, 10 etc, out as far as day 28 or 35 (if any survivors in those groups).
- The collection of longitudinal data from this study will be useful in designing challenge and other studies:
    - The serial sacrifice and natural mortality will produce a survival curve that can be estimated using the Kaplan Meier method. Parameters such as average and median survival time can be estimated.
    - The logitudinal nature of the data allows for a more sophisticated analyses of data using mixed models to estimate characteristics of the infected animals. This may allow us to define additional target parameters for assessing vaccines or other treatments.

Study Elements

- Telemetry for temperature, activity levels, RR, BP, SPO_2, etc
  - Also EEG?, CVC was not favored.
- Frequency of clinical monitoring (at least 2x per day, 3 or 4 times during disease timeframe)
- Clinical signs expected: depression, lethargy, fever, loss of appetite – Draft clinical score sheet TBD
- Observations on labored breathing, nasal (bloody) discharge
- Observations for neurological signs, seizures, muscle twitch tremors, paralysis (may not be consistent)
- Blood sampling to evaluate: viral load (infectious virus and RT-PCR), IgM and IgG, wbc and serum clinical chemistry (what analytes? CRP, etc)
- Euthanasia criteria plan should be based on clinical scoring (what are driving attributes of clinical scoring?)
  - Febrile response (loss thereof?)
  - Observed changes in posture and activity levels
  - Changes in breathing patterns, labored breathing
- Necropsy and tissue histology. Can discuss full tissue list.

CREID

Centers for Research in Emerging Infectious Diseases

Sara E. Woodson, PhD
Program Officer
September 1, 2021

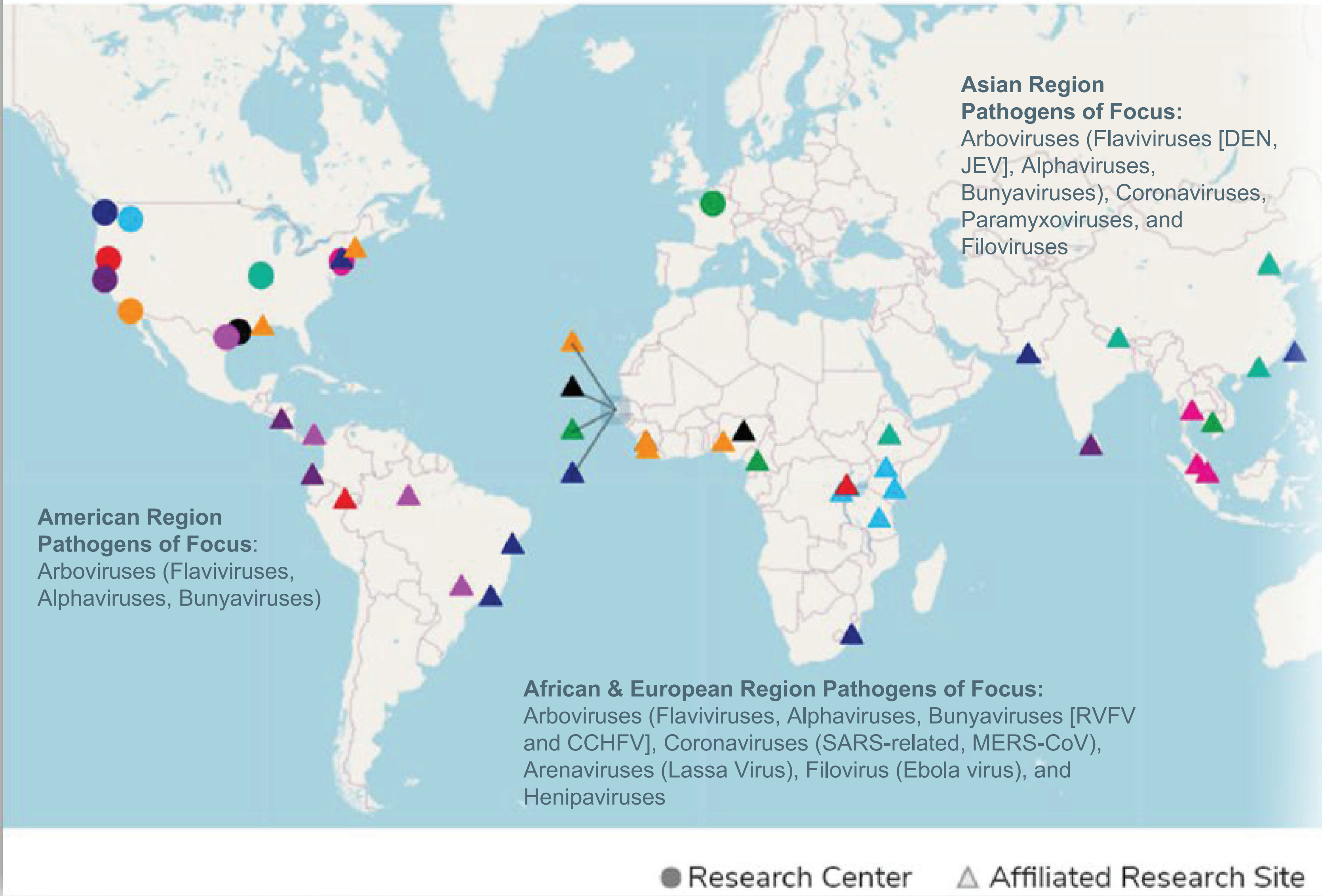
Website: https://creid-network.org/

Email: NIAIDCREIDNetwork@niaid.nih.gov

CREID Program Goals & Objectives

- Improve knowledge of emerging and re-emerging infectious diseases
    - Conducting pathogen/host surveillance and clinical research studies
    - Studying pathogen transmission
    - Developing reagents and diagnostic tests for detection of emerging pathogens and vectors
- Increase outbreak research response flexibility and capacity
- Contribute to training the next generation of EID investigators & leaders

Where is CREID?



Asian Region Pathogens of Focus:
Arboviruses (Flaviviruses [DEN, JEV], Alphaviruses, Bunyaviruses), Coronaviruses, Paramyxoviruses, and Filoviruses

American Region Pathogens of Focus:
Arboviruses (Flaviviruses, Alphaviruses, Bunyaviruses)

African & European Region Pathogens of Focus:
Arboviruses (Flaviviruses, Alphaviruses, Bunyaviruses [RVFV and CCHFV], Coronaviruses (SARS-related, MERS-CoV), Arenaviruses (Lassa Virus), Filovirus (Ebola virus), and Henipaviruses

● Research Center △ Affiliated Research Site

Research Centers:

- ● American and Asian Centers for Arboviral Research and Enhanced Surveillance (A2CARES)
- ● Center for Research in Emerging Infectious Diseases-East and Central Africa (CREID-ECA)
- ● Center for Research in Emerging Infectious Disease -Epidemiology, surveillance, pathogenesis (CREID-ESP)
- ● Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics (CREATE-NEO)
- ● Emerging Infectious Diseases: South East Asia Research Collaboration Hub (EID-SEARCH)
- ● EpiCenter for Emerging Infectious Disease Intelligence (EEIDI)
- ● Pasteur International Center for Research on Emerging Infectious Diseases (PICREID)
- ● United World Antivirus Research Network (UWARN)
- ● West African Center for Emerging Infectious Diseases (WAC-EID)
- ● West African Research Network for Infectious Diseases (WARN-ID)

Where is CREID?

Asian Region Pathogens of Focus:
Arboviruses (Flaviviruses [DEN, JEV], Alphaviruses, Bunyaviruses), Coronaviruses, Paramyxoviruses, and Filoviruses

American Region Pathogens of Focus:
Arboviruses (Flaviviruses, Alphaviruses, Bunyaviruses)

African & European Region Pathogens of Focus:
Arboviruses (Flaviviruses, Alphaviruses, Bunyaviruses [RVFV and CCHFV], Coronaviruses (SARS-related, MERS-CoV), Arenaviruses (Lassa Virus), Filovirus (Ebola virus), and Henipaviruses

● Research Center △ Affiliated Research Site

Research Centers:

- ● American and Asian Centers for Arboviral Research and Enhanced Surveillance (A2CARES)
- ● Center for Research in Emerging Infectious Diseases-East and Central Africa (CREID-ECA)
- ● Center for Research in Emerging Infectious Disease -Epidemiology, surveillance, pathogenesis (CREID-ESP)
- ● Coordinating Research on Emerging Arboviral Threats Encompassing the Neotropics (CREATE-NEO)
- ● Emerging Infectious Diseases: South East Asia Research Collaboration Hub (EID-SEARCH)
- ● EpiCenter for Emerging Infectious Disease Intelligence (EEIDI)
- ● Pasteur International Center for Research on Emerging Infectious Diseases (PICREID)
- ● United World Antivirus Research Network (UWARN)
- ● West African Center for Emerging Infectious Diseases (WAC-EID)
- ● West African Research Network for Infectious Diseases (WARN-ID)

CREID Network *Henipavirus* Activities
-In Progress

- Surveillance & Seroprevalence for *Henipavirus* infections
  - Wildlife (e.g. bats) & Humans
    - >1,600 bat samples (17 Asian species, 11 West African)
      - Swine, NHPs, and other species sampling under way.
    - >2,000 human samples AFI
- Characterization of *Henipaviruses* for Spillover Potential
  - Phylogenetic analysis
  - Receptor utilization
  - Human cell type susceptibility

Image from:
https://hub.jhu.edu/2020/01/03/nipah-virus-tracking-2499-em1-art1-rea-health/

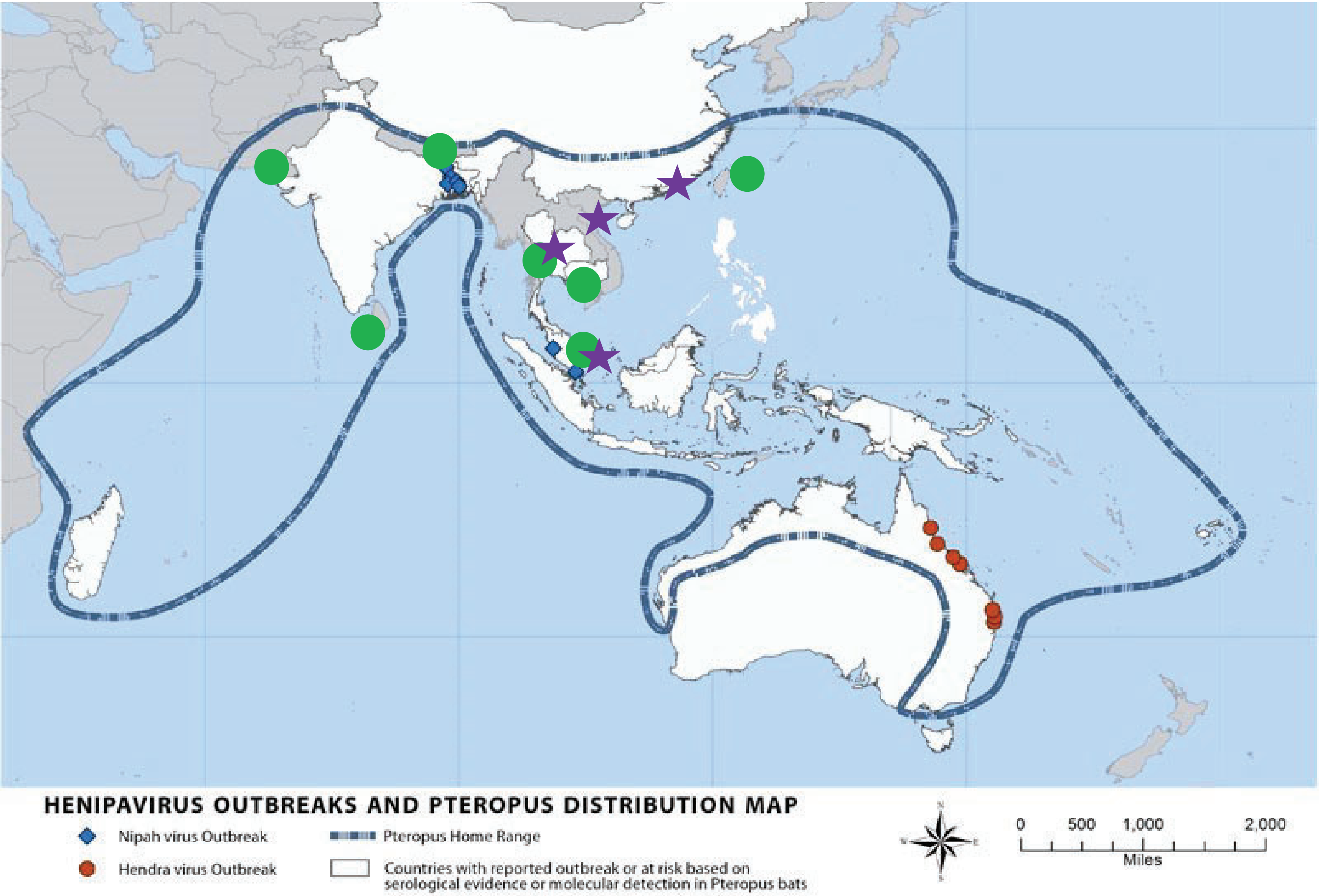
CREID
Centers for Research in Emerging Infectious Diseases

Nipah Virus Relevant CREID Field Sites

● Human Sampling
- Pakistan
- Nepal
- Sri Lanka
- Thailand
- Cambodia
- Malaysia
- Taiwan

★ Animal Sampling
- Thailand
- Malaysia
- Hong Kong
- Vietnam*

(*Pilot Program Award)



HENIPAVIRUS OUTBREAKS AND PTEROPUS DISTRIBUTION MAP

Image from: https://www.cdc.gov/vhf/nipah/outbreaks/distribution-map.html

Henipavirus or Nipah Assays in CREID Network

- Sequencing
  - RT-qPCR & RT-PCR
  - Next Gen
- Serological Assays
  - ELISA
  - Multiplex Arrays
  - Neutralization (FRNTs)
- T-cell Activation

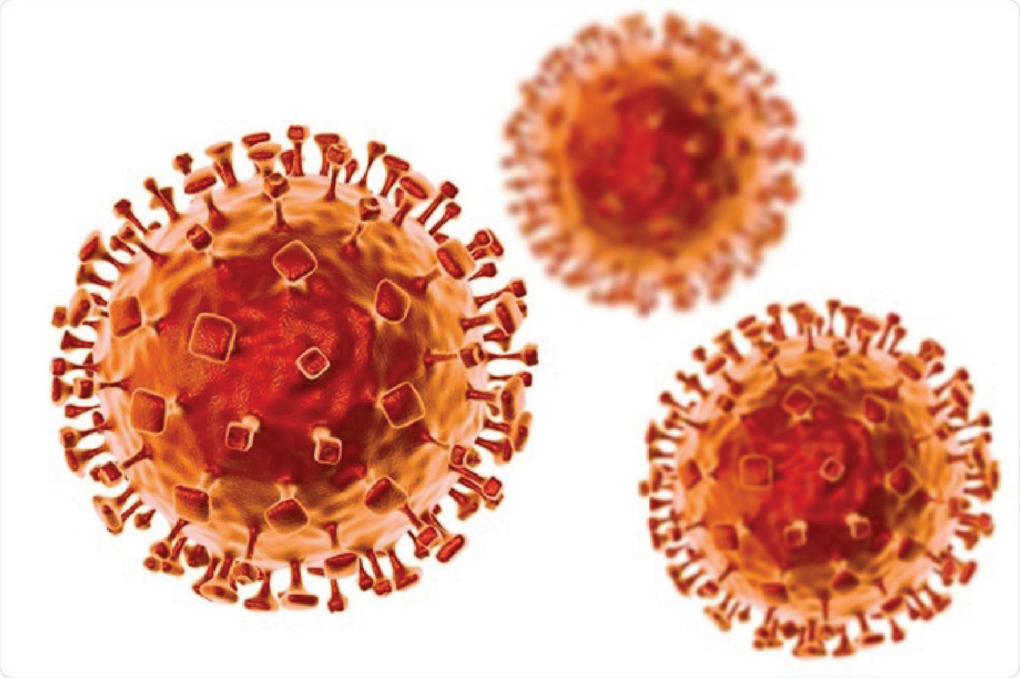


Image from:
https://www.news-medical.net/health/Nipah-Virus-Diagnosis-and-Treatment.aspx

Centers for Research in Emerging Infectious Diseases

CREID Research Center Details

- Emerging Infectious Diseases: South-East Asia Research Collaboration Hub *aka* EID-SEARCH
- EcoHealth Alliance, PI Peter Daszak
- Primary Collaborating Institutions:
  - Chulalongkorn University Hospital
  - Conservation Medicine Ltd.
  - Uniformed Services University
  - University of North Carolina-Chapel Hill
  - Duke-NUS

- West African Center for Emerging Infectious Diseases *aka* WAC-EID
- University of Texas Medical Branch, PI Scott Weaver
- Primary Collaborating Institutions:
  - University of Jos
  - Jos University Teaching Hospital
  - Kenema Government Hospital
  - National Veterinary Research Institute, Vom
  - Njala University
  - Institut Pasteur de Dakar
  - Duke-NUS

RfP: Characterizing the epidemiological diversity of Nipah strains

An epidemiological investigation

PL: Maïna L'Azou Jackson

PM: Roice Fulton

RfP available at:

https://cepi.net/get_involved/cfps/?learn-more-7099=5

https://cepi.net/wp-content/uploads/2021/08/CEPI-Nipah-RfP-Call-Text.pdf

Background

- The vast majority of experimental Nipah virus research in the past 20 years has been performed with a single isolate obtained from a patient during the Nipah virus outbreak in Malaysia (Nipah Virus, 1999 Malaysia).
- More recent work has expanded to include an isolate from a patient in Bangladesh (Nipah virus, 2004 Bangladesh). However, this still means that all experimental knowledge on Nipah virus has been derived from work with only 2 virus strains, limiting inferences and interpretations about the impact of strain variation on pathogenicity, virus shedding, transmission, and efficacy of countermeasures.
  - With a regulatory approach based on animal experiments, there will be a need to characterize the challenge strains and compare them with the circulating strains.
- Difficult-to-obtain samples
  - Disease progression is rapid, and delays in seeking care coupled with lack of rapid diagnostics means that only rarely are patients diagnosed with Nipah virus infection before death, severely limiting opportunities to collect biological samples.
  - Furthermore, outbreaks have occurred in areas where diagnostic autopsy is not the standard of care, due to religious and cultural concerns or to limited capacity, further limiting samples available for analysis.
- The true geographical spread of Nipah is unknown and cases likely to pass undetected in certain countries (communications with US CDC)

Emily S. Gurley Christina F. Spiropoulou and Emmie de Wit. Twenty Years of Nipah Virus Research: Where Do We Go From Here? The Journal of Infectious Diseases® 2020;221(S4):S359–62

Goal and needs

- **Goal**: To fundamentally expand our phylogenetic understanding of Nipah virus in all affected countries and any associated epidemiology in humans to ensure vaccine development efforts *are suitably addressing any strain variation.*
- 607 reported cases of Nipah
  - of which 110 patients have been sampled
  - A recent paper (Whitmer et al., 2021) reported 55 full length genomes. There were initially only 20 full length genomes but this paper has identified and sequenced an additional 35 full length genomes (22 directly from Bangladeshi clinical specimens and 13 viral isolates - 6 Malaysian and 7 Bangladeshi)
    - Research concluded that additional genotyping is needed in and beyond the Nipah 'belt'
- There is a need to revisit the meta and epi data - as new evidence is emerging from sequencing activities - to characterize the diversity of NIV and understand if for example the different NiV B clades 1 and 2 genotypes translate into different phenotypes, such as differences in human-to-human transmission rates, pathology, morbidity, and mortality.

Deliverables

- This RfP is open to any organization or consortium of organizations with the requisite expertise, experience and resources to complete the following tasks, grouped into workstreams (proposed outputs are included per workstream below, but may be adapted by applicants to the specifics of their proposed implementation):
- **Workstream 1**: Retrospectively explore whether there are additional "historical" human NIV samples that could be sequenced from known affected geographical areas
  - Output 1: Map and describe extant samples and isolates from human Nipah cases

    Explore the possibilities for additional analyses on these samples to produce new biologic data and, with assistance from qualified biocontainment labs made available through CEPI or via applicant partnerships, investigate whether virus can be isolated and cultured for use as a future research strain
  - Output 2: Complement the existing genome sequences by sequencing any extant NIV samples which have not yet been sequenced
  - Output 3: Ensure sequences are made available in public genome database(s)
- **Workstream 2**: Retrospectively analyze the metadata associated with extant human NIV sequences
  - Output 1: Build out database on existing genomes with associated metadata
  - Output 2: Retrospective analysis of the existing metadata associated with NIV sequences, including assessment of differences between countries and strains over time
  - Output 3: Peer-reviewed publication(s) of results of the above analyses (WS1 & WS2)
- **Workstream 3:** Prospectively provide additional support to existing surveillance systems to: - sequence Nipah samples from human clinical cases, - report them in public genomic databases in a timely manner, and collect epi data and prospectively analyse metadata associated with these sequences in known affected geographical areas

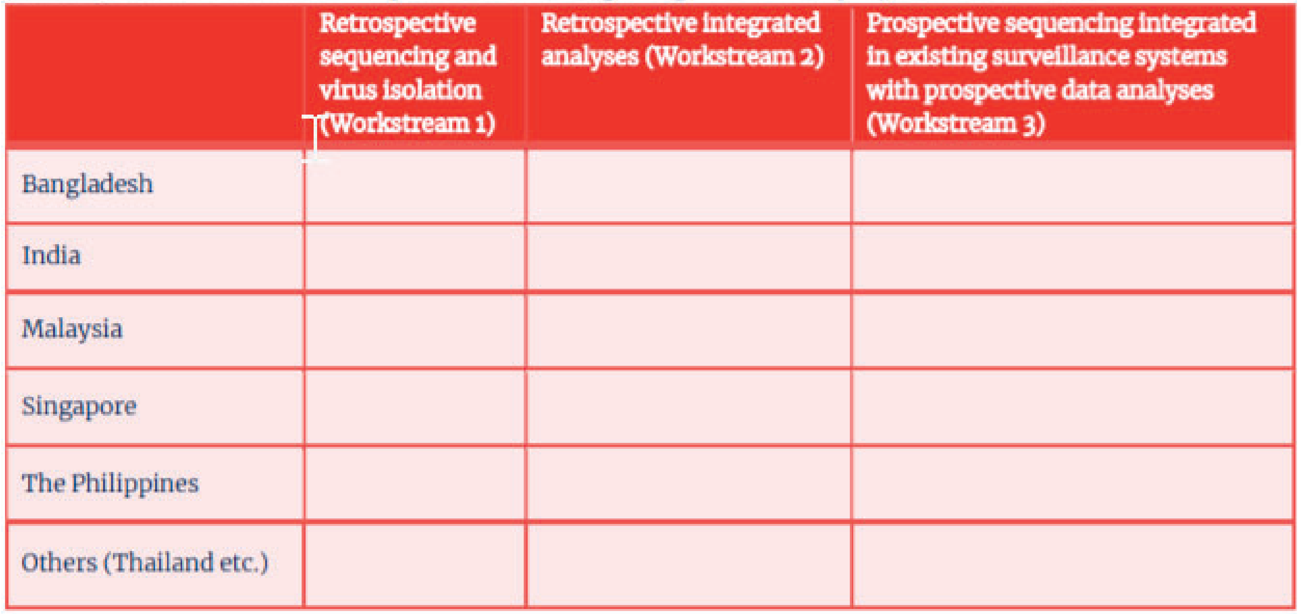
CEPI

WS I, 2 and 3 are independent from each other but are complementary, can be managed separately or not

Matrix of candidate Nipah-affected target regions versus project workstreams

Potential scenarios for allocating workstreams

Table 1. Matrix of candidate Nipah-affected target regions versus project workstreams



	Retrospective sequencing and virus isolation (Workstream 1)	Retrospective integrated analyses (Workstream 2)	Prospective sequencing integrated in existing surveillance systems with prospective data analyses (Workstream 3)
Bangladesh			
India			
Malaysia			
Singapore			
The Philippines			
Others (Thailand etc.)			

All the activities in both the columns and rows are independent from each other but are complementary, can be managed separately or not

Timelines

Activity	Date
Request for proposals advertised	Thursday, 5 August 2021
Notification of intent to participate sent to epidemiology@cepi.net	Wednesday, 1 September 2021
Deadline for applicants questions regarding proposal details or process	Wednesday, 1 September 2021
FAQ published to CEPI website at https://cepi.net/get_involved/cfps/	Wednesday, 8 September 2021
Deadline for submission of proposal packages	**Sunday, 3 October 2021 (23:59:59 Central European Time)**
Selection process completed	End October 2021
Contract initiation and agreement	Through November 2021

CEPI & NIAID initiatives
Q&A

CEPI

From: Raúl Gómez Román

To: Mark Hassall; Spiropoulou, Christina (CDC/DDID/NCEZID/DHCPP); 王佑春; mark.page@nibsc.org; Dr Syed M Satter; Mahmudur Rahman; LiYen Chang; Pragya yadav; Levis, Robin; Broder, Christopher; Park, Eun-Chung (NIH/NIAID) [E]; Woodson, Sara (NIH/NIAID) [E]; Geisbert, Thomas W.; De wit, Emmie (NIH/NIAID) [E]; Steve Luby; John James Openshaw; Ksiazek, Thomas G.; Glenn.Marsh@csiro.au; Yoshihiro KAKU; Simon Graham; Dalan Bailey; Keith Chappell; Paul Young; Christopher da Costa; Michael Egan; Hilde Depraetere; Chieko KAI; Katie Ewer; Teresa Lambe; KNEZEVIC, Ivana; YOO, Si Hyung; Elwyn Griffiths; Giada Mattiuzzo; Emma Bentley; Rick Nichols; Paul Kristiansen; Carolyn Clark; Amy C. Shurtleff; Eric Mungai; Sophie Houard; Mahmudur Rahman; Maïna L"Azou Jackson; Mariann Vaule Bringsværd; Katie Smith; In-Kyu Yoon; Mark Polhemus

Cc: Valentina Bernasconi; Neil George Cherian; William Dowling; Debra Yeskey; dr.rima.sahay@gmail.com; Patterson, Jean (NIH/NIAID) [E]; Ana Paula De Almeida Aranha; honko@bu.edu; ahgriff@bu.edu; Sullivan, Nancy (NIH/VRC) [E]; Rick Nichols; Heidi Sneddon; Victor Leyva-Grado; Tracy Chen; Roice Fulton; rebecca.loomis@nih.gov; Nairuti Patel

Subject: Nipah Task Force (confirmed)

Attachments: CEPI Nipah Task Force September 2021 final.pdf

Dear Members of CEPI's Task Force on Nipah Standards, Assays & Animal Models,

Please find the attached pre-reads ahead of our call next week. We look forward to our discussions.

Best wishes,
Raúl

Topic Presenter Allocated time Objective
Introduction & welcome Raúl Gómez Román, CEPI 5 min
Updates from henipavirus-affected countries Glenn Marsh, Australia
Syed Satter, Bangladesh
Pragya Yadav, India
LiYen Chang, Malaysia
10 min each
\+ 5 min Q&A To gain visibility on recent henipavirus work and on how COVID-19 has affected R&D in country
NiV antibody standard development I LiYen Chang, University of Malaya 10 min To share information on objectives, including NiV assays in the pipeline
NiV antibody standard development II Mark Hassall, NIBSC 10 min To gain visibility on the plans and timelines for the feasibility study leading to the development of an international NiV antibody standard
10 min Q&A on antibody standard development
Natural history studies Carolyn Clark/Amy Shurtleff , CEPI 10 min To share information/updates on objectives, status and expected timelines.

Updates from CREID network Sara Woodson, NIAID 10 min
Updates from CEPI Epi RfP Maïna L'Azou Jackson, CEPI 10 min
5 min Q&A on CEPI & NIAID initiatives
Action items & closing Raúl Gómez Román, CEPI 5 min

Microsoft Teams meeting
Join on your computer or mobile app
Meeting options

Sensitivity: CEPI Internal

Solving Opportunities for Spillover (SOS): Frequency and Mechanisms of Cross-species Transmission of Nipah Virus in Bangladesh

SPECIFIC AIMS

Nipah virus has characteristics that suggest it could pose a meaningful pandemic threat: human infections are highly fatal [1], there are currently no therapies or vaccines, and it can spread person-to-person through the respiratory route [1]. Nipah virus is not currently transmitted efficiently between people [1], but this characteristic is mutable and could change in the future – other viruses have accomplished this through infections in intermediate hosts (e.g., SARS coronavirus). Due to the risk of pandemic emergence of Nipah virus, it is prudent to learn more about the frequency, geographic distribution, and nature of spillover events so that we can prevent them. Since the first recognized outbreak in Bangladesh in 2001 [2], human Nipah infections have been detected nearly every year [1]. Human spillovers primarily occur through consumption of date palm sap contaminated with bat saliva or urine [3]. Human spillovers through date palm sap occur primarily during winter, when the sap is consumed fresh, but more spillovers occur in colder winters [4,5]. **Temperature variations explain well the inter-annual variation in human spillover events, but the mechanisms driving these differences remain unexplored** [5]. Moreover, Nipah outbreaks in humans are uncommon events and may not represent the true number of spillovers occurring from bats. Antibodies against Nipah virus have been found in several domesticated animal species in Bangladesh [6], indicating additional spillover events from bats. However, little is known about the role of these animals in Nipah virus ecology. In particular, **the timing, frequency, and transmission routes of spillovers into other animals in Bangladesh have not been investigated**. If we are to meaningfully mitigate our risk for Nipah virus pandemics, we must solve the remaining questions about spillovers. Specifically, **we must understand how many spillovers are occurring, which animal species are involved, and what ecological forces drive variation in spillovers**.

This multidisciplinary study, integrating epidemiology, ecology, and anthropology, will solve these most pressing questions related to Nipah virus spillover. This work ~~is centered on the~~ establishes~~ment of~~ longitudinal animal cohorts for measuring Nipah seroincidence and household surveys to capture variation in human date palm sap consumption at two sites in Bangladesh.

Specific Aim 1: Characterize the frequency, nature, and timing of contact between bats, humans, and potential intermediate hosts. We hypothesize that humans and domesticated animals have numerous interactions with each other and bats that could lead to Nipah virus spillover. In areas near bat roosts in Faridpur and Chittagong, we will use surveys, social mapping, and camera traps to describe the ecological interactions between human and domesticated animal populations at a fine geographic scale.

Specific Aim 2: Measure seroincidence of Nipah virus spillovers in mammals. We hypothesize that several domesticated animal species have non-zero incidence of Nipah virus exposure due to contact with bats. We will sample cohorts of cattle, pigs, goats, dogs, and cats living near bat roosts to measure seroprevalence at baseline and seroconversion over time.

Specific Aim 3: Identify drivers of variation in human sap consumption behavior. We hypothesize that date palm physiology and human date palm sap consumption vary with weather patterns. Household cohorts will report their sap consumption weekly during winter to track intra-annual and inter-annual patterns of sap consumption while we monitor weather patterns, volume of sap production, and sap quality to identify drivers of human consumption.

The **expected products** of this study include: **1)** descriptions of interactions between bats, humans, and domesticated animals that could facilitate Nipah spillover; **2)** quantification of spillover events occurring from bats into domesticated animals; and **3)** identification of sources of variation in date palm sap production and human consumption.

Commented [spl1]: Clearly, this is a proposal about Nipah virus, but it is also a remarkable opportunity to study the little understood phenomena of spillover. You may consider embedding this as part of the rationale.

Commented [spl2]: Likely to change during the time the grant is funded. Would be careful not to buttress the rationale too centrally on this point.

Commented [3]: Make sure that we are emphasizing:
- why this matters and the context it is in
- the primary objective
- the primary hypothesis/question

Commented [spl4]: We do not solve questions. We answer them.

Commented [spl5]: There are two different notions of spillover here. First, is spillover from bats into domestic animals. The second notion of spillover is how this bat virus gets into people. The phrasing of the sentence is ambiguous regarding which notion of spillover is being referred to. I was assuming the first notion, and from that perspective the phrasing is not technically correct. I recommend defining terms so that these two different notions are designated separately so that your meaning is clearly communicated.

Commented [spl6]: Since domestic animals are fairly short-lived and prior studies demonstrate antibodies against Nipah, don't we already have sufficient evidence to embrace this hypothesis?

Commented [spl7]: Useful to know, but not as closely tied to the animal spillover ideas that I see is core to this proposal.

Commented [spl8]: Couldn't we already do this without even implementing the study? What does the study add?

Commented [spl9]: How does this help us?

Commented [10]: Add one more sentence that expresses the main outcome and why it matters.

I was thinking our outcome was new, highly impactful knowledge about Nipah ecology in Bangladesh, but will also provide an important model for studying Nipah in other locations/cultural contexts.

But I'm not sure what will be the best way to frame it...

RESEARCH STRATEGY – SIGNIFICANCE

Nipah virus is an emerging zoonotic pathogen that has caused outbreaks of human illness with high case fatality in Malaysia, Bangladesh, India, and likely the Philippines (Arunkumar et al., 2019; Chadha et al., 2006; Ching et al., 2015; Kaw Bing Chua, 2003; Nikolay et al., 2019). Systematic surveillance of human Nipah cases only occurs in a limited region within Bangladesh (Naser et al., 2015), so the known cases outside of Bangladesh have exclusively been detected as part of outbreaks. Since Bangladesh represents a small portion of the geographic range that *Pteropus* fruit bat hosts of Nipah virus occupy in South and Southeast Asia (**Figure**), many human Nipah infections in this region are not captured with current surveillance efforts. Furthermore, Nipah virus poses some risk for pandemic spread (Luby, 2013). Observed outbreaks with person-to-person transmission have not been sustained for more than five generations, but its elevated mutation rate as an RNA virus, and its geographic niche in a densely populated and interconnected region of Asia highlight the need for continued study of this virus. Each new spillover event from bats represents an opportunity for a new Nipah virus strain to emerge with greater transmissibility in humans and thus greater pandemic potential.

Commented [11]: Roughly 1-2 pages, with maybe one figure (map of Pteropus range and surveillance hospital locations)

Commented [12]: Some draft text from our previous document.

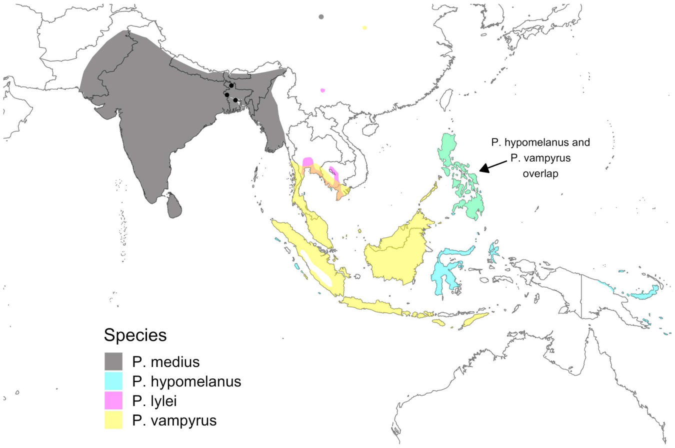


Figure. The overlapping geographic ranges of *Pteropus* fruit bat species with evidence of Nipah virus infection via sequencing or virus isolation. Black points show the location of hospitals with systematic surveillance of human Nipah cases in Bangladesh.

Beyond the aims outlined above for understanding the ecology of Nipah virus at the human-animal interface in Bangladesh, there are opportunities to expand the impact of this work. For example, the study framework and samples collected from bats and other animals could be used to study infections with other viruses shed by *Pteropus* bats, including novel paramyxoviruses recently identified from these roosts. Multiple studies have found evidence of human and animal exposure to other bat paramyxoviruses (Albariño et al., 2014; Baker et al., 2013; Philbey et al., 1998; Yaiw et al., 2007). Since paramyxoviruses unrelated to Nipah virus have been detected in *P. medius* in Bangladesh (Anthony et al., 2013) and other *Pteropus* species in Asia and Australia (K B Chua et al., 2001; Marsh et al., 2012; Sasaki et al., 2012), it is possible that these viruses may share some pathways to animal and human spillover with Nipah virus. Furthermore, the study framework devised for this project could easily be scaled and adapted for use in understanding Nipah virus ecology in other regions within the range of *Pteropus* spp. reservoir hosts (**Figure 1**). Projects such as these could explore some of the ecological and cultural dimensions that may explain why some regions of Asia with documented Nipah virus circulation in bat populations have yet to experience documented outbreaks of human disease.

Commented [spl13]: This seems too early to present these ideas. I recommend focusing on the exciting learning/understanding the can come from this work. You can include them later, in terms of closing comments, other work that this might lead to. Stated differently, I anticipate the review committee will be much more interested in what can be learned during this five years, then in setting up a productive infrastructure.

- However, during two outbreaks in 2001 and 2003, epidemiologic studies showed that cases were more likely to have had contact with livestock than controls (Hsu et al. 2004).
- In addition, one child with NiV reported exposure to goats who had died from apparent neurological illness, but these animals were not available for exam during the investigation (Luby et al. 2009).

RESEARCH STRATEGY – INNOVATION

The primary aim of surveillance for spillovers has focused on human infections, yet many of these likely go undetected nonetheless. In Bangladesh, where active surveillance for acute cases has been ongoing at hospitals since 2007, an estimated half of all human cases in the catchment area of hospitals still go undetected (Hegde et al. 2019). When surveillance for acute cases of emerging pathogens is limited, serological surveys are frequently used to detect pathogen spillover into human populations outside of documented outbreaks (Cappelle et al., 2020; Yong et al., 2020). However, this approach would be inefficient for Nipah virus due to the rarity of spillover in a given year and the high case fatality of Nipah virus infection in humans, often exceeding 70% for primary cases in Bangladesh (Nikolay et al., 2019).

Looking for infections exclusively in humans is also an overly narrow approach for detecting Nipah virus spillover events, especially given the importance of intermediate hosts in past outbreaks. The human outbreak of Nipah virus in Malaysia in 1998-1999 was driven by transmission from pigs following spillover from bats (Kaw Bing Chua et al., 2002; Kaw Bing Chua, 2003). A similar outbreak occurred in the Philippines in 2014 with horses as the likely intermediate hosts (Ching et al., 2015). Although there has been no clear involvement of intermediate hosts in human outbreaks in Bangladesh, there is evidence of Nipah virus exposure in domestic animals. Studies of animals living around *Pteropus* bat roosts in areas of Bangladesh with past Nipah virus outbreaks have found antibodies against Nipah virus in cattle, goats, and pigs (Chowdhury et al., 2014) as well as in dogs and cats (Islam et al., unpublished data). These data suggest that transmission of Nipah virus to humans through domestic animals is possible and may contribute to spillover risk in regions where outbreaks involving intermediate hosts have not yet been observed.

The advantage of animal serological surveys is that they are easier and less costly to perform than human surveys, so greater sample sizes can be obtained. Domesticated animals may also have more frequent interactions with bats or bat excreta than humans and lower case fatality following exposure, thereby increasing the probability of spillover events. Thus, serological surveys of animals are likely to yield better information on the zoonotic force of infection of Nipah virus than records of human outbreaks or human serological surveys alone. Animal serosurveys could also be expanded to regions outside of the small catchment area for human Nipah surveillance in Bangladesh and into other countries with *Pteropus* bat populations (**Figure**). Data from such animal serosurveys would increase our understanding of the frequency of Nipah virus spillover events occurring from bats. Knowledge of these animal spillover events would aid in evaluating risks for subsequent human spillover and the opportunities for viral adaptation in different hosts.

Commented [14]: Roughly 1-2 pages, with the conceptual figure that Raina suggested

Commented [15]: Some draft text from our previous document.

Commented [spl16]: This would seem a good location to include the two ideas in the bullet points above the research strategy.

Commented [spl17]: I recommend leading with scientific rather than logistical advantages.

Commented [spl18]: Yes, but this is not the strategy that is proposed for this study.

In addition to more accurate estimates of Nipah virus spillover, there is a need to understand the ecological drivers of spillover in the cultural context of Bangladesh. Consumption of date palm sap contaminated with Nipah virus from fruit bat excreta or saliva is the primary spillover route in this country and variation in sap consumption rates partially explain the geographic localization of documented human cases (Gurley et al., 2017). Nevertheless, there is substantial annual variation in the number of Nipah virus spillover events that is not matched with longitudinal data on date palm sap production and consumption in Bangladesh. Moreover, more spillover events appear to happen in years with cooler winters (McKee et al., 2021), suggesting that climate may affect bat behavior, Nipah virus shedding from bats or survival in sap, date palm biology, human consumption behavior, or a combination of these factors. Thus, there is a need to collect concurrent longitudinal data on Nipah virus shedding in bat populations, date palm sap physiology, and date palm sap production and consumption to understand how these factors interact to explain yearly variation in spillover events.

RESEARCH STRATEGY – APPROACH

Preliminary data

Ecological interactions with bats: Previous studies have identified ecological interactions between humans, bats, and domesticated animals, although the role these interactions play in Nipah virus ecology are unclear. As mentioned above, the primary route for human exposure to Nipah virus is through consumption of raw or fermented date palm sap [3,7]. *Pteropus medius* fruit bats frequently visit date palm trees to feed on sap during collection and can contaminate sap with their saliva, urine, or feces [8,9]. Some households also report feeding raw date palm sap to their livestock [7]. *P. medius* bats also feed frequently on cultivated fruit trees in populated areas [7], dropping partially eaten fruits that are sometimes consumed by humans or fed to domesticated animals [6,7]. While consumption of bat-bitten fruit is not a significant risk factor for human infection [3], it may contribute to exposure in domesticated animals, as was hypothesized to occur~~red~~ with pigs in the 1998-1999 Nipah outbreak in Malaysia [10]. A study of Nipah seroprevalence in domesticated animals found that seropositive cattle and goats were more likely to have been fed fruits partially eaten by bird and/or bats and palmyra palm fruit juice than seronegative animals [6]. Bat hunting is a direct interaction with bats practiced in Bangladesh for consumption or medicinal use [11], although no outbreaks have been associated with hunters [7]. Finally, dogs, cats, and pigs may be exposed when hunting or scavenging [12–14], including possible consumption of dead bats or bat placenta.

Commented [spl19]: A2.

Commented [spl20]: E2. I would make clear that this is our group's work.

Nipah virus exposure in domesticated animals: There is existing evidence that domesticated animals are exposed to Nipah virus in Bangladesh outside of the context of human outbreaks, but systematic surveillance is missing. A serological survey of cattle, goats, and pigs sampled between 2009 and 2011 from seven districts of central and western Bangladesh (the region where most human outbreaks have been reported) found 6.5% of cattle, 4.3% of goats, and 44.2% of pigs were positive for antibodies against Nipah virus glycoprotein using a Luminex-based multiplexed microsphere assay [6]. A second study of animals sampled between 2013 and 2015 from five districts in the same region of Bangladesh using an enzyme-linked immunosorbent assay for Nipah immunoglobulin G found 1.1% of cattle, 1.2% of goats, 3.2% of dogs, and 4.7% of cats were seropositive (Islam et al., unpublished data). Pigs and cats are highly susceptible to Nipah virus infection in experimental settings and shed virus from the nasopharynx [15,16]. Other studies outside of Bangladesh have reported exposure to Nipah virus or other related henipaviruses. Infected or seropositive goats, dogs, and cats were detected during the 1998-1999 outbreak among pigs and humans in Malaysia [17–19]. During a henipavirus outbreak among humans and horses in the Philippines in 2014, four cats and one dog died after consuming horse meat, and four dogs were found with neutralizing antibodies against Nipah virus [13]. Sheep and goats in Ghana [20] and pigs and horses in Nigeria [21] have also been reported seropositive for henipavirus exposure.

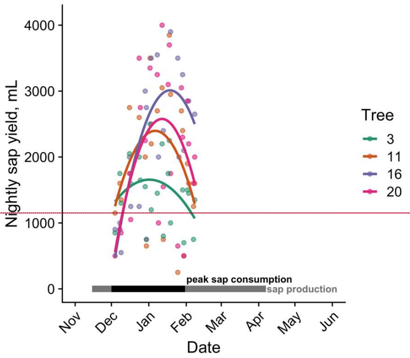
Commented [spl21]: E2.

Commented [spl22]: I recommend noting that Nipah virus is a generalist, that is it can in fact nearly any animal because the cellular receptor it leverages for infection is the highly filer genetically conserved ephrin B2/B3 receptor. Indeed, this is part of what makes mammal surveillance so efficient.

Date palm physiology and sap consumption patterns: The limited data available suggest that sap production and consumption vary seasonally (**Figure 1**) and that annual temperature fluctuations in winter may explain changes in sap consumption and the resulting risk of human spillovers. Fresh date

Figure 1.

Seasonal date palm sap production and consumption.



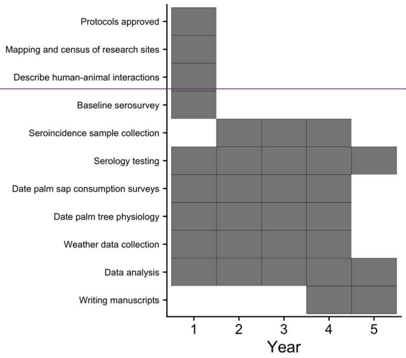
palm sap is usually harvested fresh in winter between mid-November and early March [22]; peak months for household consumption of fresh sap are in January and February (Shanta et al., unpublished data). In some areas of Bangladesh where fermented date palm sap (*tari*) is consumed, *tari* production occurs year-round, although production peaks during winter to meet higher customer demand for *tari* [23]. Sap yield also appears to peak during January during the coolest weather, although these data come from only one study of four trees in the winter of 1911-1912 [22] (**Figure 1**). Information from sap collectors also indicates that sap flows more freely and is higher quality during clear, cool nights while warm or cloudy weather diminish sap yield and quality [9,22]. However, no longitudinal data on date palm sap yield and quality over multiple years are available to determine the effects of weather. Nearly all human Nipah outbreaks have occurred in winter, with the highest proportion of spillover events occurring in January [4]. Additionally, more spillovers occur in winter weeks following a previous week with cooler temperatures [4] and more spillover events have been observed in years with colder winters [5]. Human sap consumption also varies annually. The proportion of households consuming date palm sap declined across Bangladesh during the winters of 2014 through 2016 (Shanta et al., unpublished data). Coincidentally, the winters became progressively warmer during those years and the number of reported human Nipah spillover events declined [5]. It is possible that warmer temperatures during those winters decreased the availability or quality of date palm sap, resulting in reduced consumption and the associated risk of Nipah virus exposure.

Commented [spl23]: Can you strengthen the case on why this knowledge is scientifically important? If

Overall strategy

Figure 2.

Project timeline.



The three specific aims of this study will be pursued in stages over five years (**Figure 2**). In Year 1, we will map the study sites, census animal populations, and interview households about their interactions with animals and typical date palm sap consumption behavior (Aim 1). Cohorts of censused domesticated animals and human households will be enrolled in Year 1 and an initial serological survey will establish baseline levels of Nipah virus exposure. Collection of sera from domesticated animal cohorts will follow every six months in Years 2–4 to measure Nipah seroincidence (Aim 2). A cohort of date palm trees will also be identified at each site in Year 1 for weekly measurements of sap yield and quality in winter through Year 4, along with temperature and other weather variables. Records of date palm sap consumption during winters of Year 1–4 will be collected weekly from household cohorts (Aim 3). Data analysis and testing of sera will occur continuously through Year 5.

Commented [24]: Talk about how work will be partitioned among teams and how they will collaborate.

Might consider moving this towards the end of the document.

Commented [25]: Add in preliminary work by USU to design and validate beads.

Study Sites

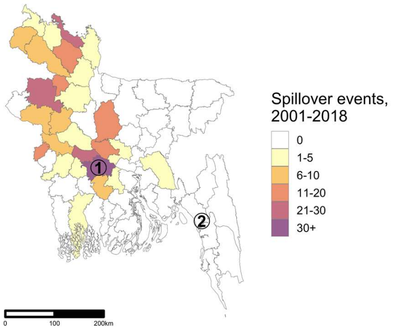
Research will be conducted at two study sites in Bangladesh that differ in the number of reported Nipah spillovers despite similarities in their ecology. A goal of this study is to understand how spillover events in domesticated animals relate to observed human risk at these sites, if at all. *Pteropus medius* bat populations roosting in Faridpur and Chittagong do not have overlapping home ranges and are genetically distinct [24,25]. Nipah virus shedding in urine has been detected repeatedly in longitudinal surveys of bats at both sites (Gurley et al., unpublished data). Humans and animals live in close

Commented [spl26]: I do not see this goal mentioned anywhere in the specific aims. It seems odd that it pops up here in the methods section.

proximity to bats in each area, but Faridpur has reported the highest number of Nipah spillover events into humans in Bangladesh while spillovers have never been detected in Chittagong (**Figure 3**). Communities in Faridpur also report higher rates of drinking fresh date palm sap than those in Chittagong [7]. Furthermore, seropositive cattle and goats [6] and dogs and cats (Islam et al., unpublished data) have also been reported from studies in Faridpur. It is likely that Nipah virus spillover events are occurring undetected in animals and humans in Chittagong ~~due to lack of surveillance effort~~. However, there may be other important biological and cultural factors explaining variation in spillover risk between these two areas that will be explored in this study, including the frequency of interactions between bats, humans, and other animals and different patterns of date palm sap production and consumption.

Figure 3.

Proposed study sites in 1) Faridpur and 2) Chittagong.



Commented [spl27]: Also, D7.

Commented [spl28]: But with an N=2, we are not powered to assess whether any differences we observe are due to unmeasured characteristics that are different between the two sites.

Aim 1 Strategy

Commented [29]: Reiterate the main goal, the hypotheses that will be tested.

Make sure we make super clear everything we are going to measure, how we are going to evaluate it, and what the implications are.

Community enrollment: Previously identified roosts of *Pteropus medius* fruit bats in Faridpur (~2000 bats) and Chittagong (~8000 bats) will serve as central points for enrolling communities into the study. Based on a survey of Bangladeshi communities on household size [7], we conservatively estimate that there are 200 households in a given suburban community. Survey estimates of the average numbers of domesticated animals owned per household indicate that at least three communities will need to be enrolled at each site to capture enough individual cattle, goats, pigs, dogs, and cats for the serological assessments in Aim 2 and for the required number of households for assessing trends in date palm sap consumption in Aim 3. Communities nearest to the focal bat roosts will be enrolled first, with a maximum radius of 15 km for community enrollment based on the observed home range size of *P. medius* [25].

Mapping and census: Teams dispatched to each community within the Faridpur and Chittagong sites will first speak with local leaders to delineate the boundaries of the community. Once boundaries are established, teams will then map the location of households and other structures, populations of livestock and peri-domestic animals (cattle, goats, pigs, dogs, and cats), fruit bat roosts, cultivated fruit trees (e.g, mango, lychee), date palm trees, and any other natural resources that are shared between host groups (**Figure 4**). GPS locations for each point will be recorded and then used to develop preliminary maps of each community and the estimated populations of humans, bats, and domesticated animal species.

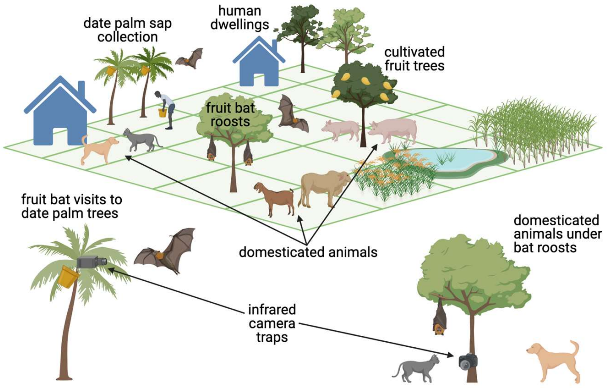


Figure 4. Mapping and recording interactions between bats, humans and domesticated animals.

Describing human-animal and animal-animal interactions: This portion of the work will occur in three parts. First, teams will enroll members of each community for a social mapping exercise. In this exercise, members will collaborate in focus groups to identify where domesticated animal populations or bat roosts are located in the community and the known forms of interactions. Human-domesticated animal interactions may include owning and caring for livestock, dogs, or cats. Human-bat interactions could include bat hunting or observing bats visiting a residential fruit tree or eating bat-bitten fruit. Domesticated animal-bat interactions might involve livestock, cats, or dogs grazing or scavenging underneath bat roosts or popular bat feeding sites; domesticated animals being fed bat-bitten fruits or date palm sap; or observations of carnivores scavenging dead bats or bat placenta.

Second, qualitative information on ecological interactions gathered from the social mapping will be used to design surveys to quantify the types and intensity of human-animal and animal-animal interactions. In addition to the example interactions given above, the survey will ask households about their typical consumption of date palm sap, either fresh or fermented. Questions about the number of household members that drink sap during winter, how frequently household members drink sap, and the typical volume consumed. These data will provide a baseline for the longitudinal assessments of sap consumption in Aim 3. Two hundred randomly selected households across enrolled communities at each site will be administered the survey in Year 1 to evaluate a difference in the proportion of households consuming sap between study sites.

> *Power calculation:* With 200 households interviewed at each site, this sample size will be sufficient to identify a moderate effect size (d = 0.4) for a difference in the proportion of households consuming sap between sites with 0.8 power and a significance level of 0.05. As discussed in the strategy for Aim 3, this number of households will be sufficient for assessing differences in sap consumption between study sites or years.

Commented [spl30]: I concede that you have the power to do this, but I'm less clear on what scientific inference of general interest it allows.

Third, nighttime space use and food resource consumption will be described using camera traps in Year 1. Based on the social mapping exercise and household interviews, teams will identify *Pteropus medius* bat roosts (beyond the focal roost at each study site), fruit trees that are popular feeding sites for bats and other animals, and date palm trees that are harvested for fresh sap. A motion-sensor tripped infrared camera will be mounted at the base of each bat roost tree (Figure 4) for three consecutive nights each month to record any visitations by livestock, dogs, cats, or other wild animals at night and any interactions they may have with bats (e.g., scavenging dead bats). Cameras will be installed at the base of fruit trees that are known popular feeding sites for bats in each community; recordings will be performed over three consecutive nights during the tree species' peak fruiting time. Finally, if date palm trees are found within the community and are harvested for fresh sap in winter, cameras will record visits by bats or other animals (e.g., rodents). Cameras will be mounted at the top of the tree near the collection pot to observe animals touching or licking sap [9] (Figure 4). A target of five date palm sap trees per community will be recorded for three consecutive nights in winter (between December and February). All cameras will be set at dusk and record animal visits until cameras are retrieved at dawn. A hired guard will protect the cameras during each night they are mounted.

Limitations:

Commented [31]: Add in section for anticipated challenges and their solutions.

Aim 2 Strategy

Commented [32]: Reiterate the main goal, the hypotheses that will be tested.

Make sure we make super clear everything we are going to measure, how we are going to evaluate it, and what the implications are.

Need some information about design of serological assay

Baseline seroprevalence survey and force of infection: Between the surveyed communities from Aim 1, teams will aim to capture 150 animals each of cattle, goats, pigs, dogs, and cats in Year 1 from the Faridpur and Chittagong study sites (**Figure 5**). Teams will capture animals and take a sample of whole blood. Blood samples will be placed in a vacutainer, centrifuged to separate the serum, and immediately preserved in liquid nitrogen in the field. Samples will then be stored in a -20 °C freezer until testing. Serum samples will be tested for the presence of Nipah virus antibodies using a bead-based assay.

Commented [33]: Henrik: Any lab challenge studies showing the feasibility for these animals to become infected? Do they die/get sick?

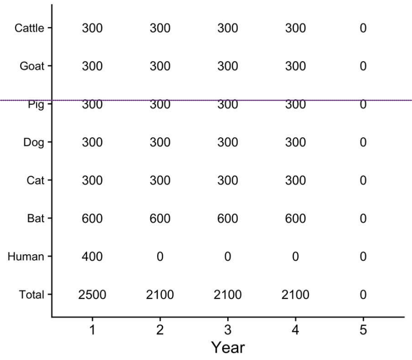
Zia: Can we also consider shrew and rodents?

Commented [34]: Henrik, lab studies were done on cats and pigs, as referenced in the preliminary data section above. Cats develop clinical disease, pigs do not. Its unclear of disease would have been fatal in cats, as they were euthanized prior to that endpoint.

Zia, we could, and I think it would be interesting to see those results. However, given that there is no prior information on NiV infection in these animals and the fact that we have a limited budget, I think we might have to keep our target animal species limited to the five already mentioned. I would be willing to include them as long as the budget allows.

Figure 5.

Animal serum sampling schedule.



Year	1	2	3	4	5
Cattle	300	300	300	300	0
Goat	300	300	300	300	0
Pig	300	300	300	300	0
Dog	300	300	300	300	0
Cat	300	300	300	300	0
Bat	600	600	600	600	0
Human	400	0	0	0	0
Total	2500	2100	2100	2100	0

> *Power calculation:* Based on a survey showing Nipah seroprevalence 1.1% in cattle, 1.2% in goats, 3.2% in dogs, and 4.7% in cats, a sample size of 150 animals

is more than sufficient to measure a seroprevalence greater than zero in the baseline survey, with a power of 0.8 and a significance level of 0.05.

During capture, teams will record the age in months of each animal if known from the owner. Otherwise, teams will estimate the age of animals or classify animals into juvenile or adult age groups. The serology data will then be used to calculate baseline seroprevalence by animal species, and using the age information, seroprevalence by age classes. The latter data will then be used to fit catalytic models to estimate the average annual force of infection from NiV in the region for each animal species [26]. These will be used as a baseline estimate of annual incidence that will be compared to the prospective study.

Commented [spl35]: Consider with some veterinary support if a finer grained estimate could be generated.

Estimating seroincidence prospectively: During animal captures in Year 1, teams will identify the number of juvenile cattle, goats, pigs, dogs, and cats that are Nipah seronegative at baseline. In Year 2, additional juvenile animals of each species will be captured to reach a cohort of 150 seronegative individuals. This same cohort will be sampled again six months later in Year 2 to measure the change in serostatus. If any members of the cohort seroconvert or cannot be captured due to disappearance or death, a new seronegative animal will be identified and added to the cohort. The same procedure of serological testing on 150 cohort animals per site of all five species every six months will continue through Years 3 and 4 (**Figure 5**). Juveniles will be targeted for two primary reasons: 1) juvenile animals are more likely to be immunologically naïve to Nipah virus than adults, so seroconversion will be easy to interpret; and 2) young animals are more likely to remain alive throughout the duration of the study. The latter is especially true for livestock animals, which are typically slaughtered for meat after about a year. The same serological assay as was used in the baseline survey will be used. Seroincidence during Years 2–4 will be measured for each animal species as the number of individuals seroconverting in a six month period and over the entire year.

Commented [36]: Rajib, how do you think we should mark captured animals in order to identify individuals upon recapture? Would microchips work? Or do we need some external marking, like an ear tag or paint?

Commented [spl37]: Will be important to know how long animals live.

Commented [spl38]: May not want to have animals that are too young, because they may have Nipah IgG from their mothers.

Bat serology: In addition to domesticated animals, we will measure changes in seroprevalence in the fruit bat populations living in the focal roosts in Faridpur and Chittagong. A target of 50 bats per site will be captured for blood serum sampling every other month in Years 1–4 (**Figure 5**) and tested for Nipah antibodies using the same bead-based assay as the domesticated animals. Changes in the proportion of juvenile and adult bats in the roosts will be monitored over time to compare with any observed changes in seroprevalence. All captured bats will be marked with microchips so that any change in serostatus (conversion or reversion) can be recorded for recaptured individuals.

Commented [39]: Emphasize this part a little more, because without force of infection from bats, no spillovers to humans and domesticated animals.

Human seroprevalence: In Year 1, during the interviews on household consumption of date palm sap and animal interactions as part of Aim 1, blood samples will be taken from household members that report drinking the most fresh sap to test for Nipah antibodies, with a target of 200 tests per site (**Figure 5**). Responses to quantitative survey questions will be used to analyze if any ecological interactions (e.g., consuming date palm sap) or history of contact with a human Nipah cases can explain seropositivity.

Limitations:

Commented [40]: Add in section for anticipated challenges and their solutions.

Aim 3 Strategy

Commented [41]: Reiterate the main goal, the hypotheses that will be tested.

Make sure we make super clear everything we are going to measure, how we are going to evaluate it, and what the implications are.

Date palm tree physiology: To understand how date palm sap production relates to patterns of human consumption, we will enroll a cohort of 20 date palm trees per site to measure sap yield and quality over the winter production period in Years 1–4. For two nights each week over the 22 weeks of the typical sap production season (mid-November to mid-April), we will measure nightly sap yield, sap pH, sap sugar content (using a Brix refractometer), and sap clarity from each tree (**Figure 6**). In addition to sap measurements, each

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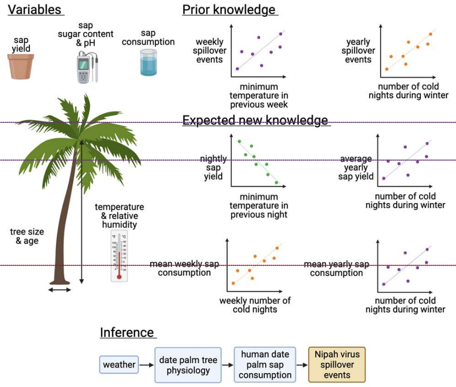

Figure 6. Inferential framework for understanding date palm sap production and consumption.

tree's height, trunk diameter, age, sex, amount of foliage, flowering status, and health status based on appearance will be recorded to explain individual variation in sap flow or quality.

Power calculation: With 20 date palm trees per site followed for two nights over 22 weeks, this sample size (880 observations) will be more than sufficient to detect a small effect size (d = 0.2) for the difference in mean sap yields between years or between sites with a power of 0.8 and a significance level of 0.05.

Commented [spl43]: Again, not clear to me what breakthrough knowledge this would generate.

Household surveys on sap consumption: Households reporting date palm sap consumption during the baseline survey of 200 households at each site in Year 1 as part of Aim 1 will be enrolled into a cohort whose fresh sap consumption behavior will be enumerated throughout winter over Years 2–4. Households will be given a paper log sheet to tally the daily volume of date palm sap consumed by each household member and the price that was paid per volume. Teams will then return to each household monthly to switch out paper logs and to perform a short survey on household sap consumption behavior. The survey will ask household members about the factors influencing their choice to buy sap that month, including sap availability, price, and quality (e.g., sweetness, flavor).

Power calculations: Assuming that 60% of households consume sap in Faridpur and 30% consume sap in Chittagong [7], 120 households in Faridpur and 60 households in Chittagong will be enrolled in the longitudinal assessments of sap consumption behavior. When daily sap consumption records from households at each site are combined over the 22 weeks of winter (mid-November to mid-April, covering the typical sap production period), this will produce sample sizes (15400 observations in Faridpur, 9240 in Chittagong) that are more than sufficient to identify a small effect size (d = 0.2) for the difference between the average number of household members consuming sap in a given year with 0.8 power at a significance level of 0.05.

Commented [spl44]: We anticipate that there will be zero incident human Nipah infections during this observation. Not sure what we will learn here. Are you trying to make the point that these two single sites, one in the high incidence area and one in a low incidence area are representative of high incidence and low incidence areas across all of Bangladesh? If so, then this should be stated explicitly and defended.

Weather patterns: Weather variables that could influence sap production and quality will be measured concurrently with tree physiology measurements. Based on anecdotal reports of their effects on sap yield or quality [22], measured variables will include ambient temperature, relative humidity, solar illuminance, and precipitation (**Figure 6**). All variables from throughout the sap production season will be downloaded from the nearest weather station; cloud cover and solar illuminance will be recorded during daytime prior to tree observations using a handheld light meter.

Inferential models: These data will build on existing knowledge within the system, including increased nightly *Pteropus* fruit bat visits to taller date palm trees and more visits during winter months (Islam et al., in review), more Nipah virus spillover events during cooler weeks [4], more Nipah spillover events in cooler years [5], and anecdotal reports from sap producers that date palm sap is sweeter and flows more freely during cooler weather [8,22,23]. These data on sap production and consumption, along with weather variables, will then be used to develop inferential models to disentangle the interactions between weather and date palm sap consumption patterns, including weekly variation within winter months and inter-annual variation between winters, and then relate these patterns with what has been previously observed in Nipah virus spillover risk in humans (**Figure 6**).

Commented [45]: Need to edit this section

Limitations:

Commented [46]: Add in section for anticipated challenges and their solutions.

Study Team

Commented [47]: This section should include:
- data management/cross-site communication
- project leadership/management
- justification/feasibility/background

Conclusion

Commented [48]: Include statements about the implications of the study and the potential benefits to human health.

References

1. Nikolay B, Salje H, Hossain MJ, Khan AKMD, Sazzad HMS, Rahman M, et al. Transmission of Nipah Virus - 14 Years of Investigations in Bangladesh. N Engl J Med. 2019;380: 1804–1814. doi:10.1056/NEJMoa1805376
2. Hsu VP, Hossain MJ, Parashar UD, Ali MM, Ksiazek TG, Kuzmin I, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerging Infect Dis. 2004;10: 2082–2087. doi:10.3201/eid1012.040701
3. Hegde ST, Sazzad HMS, Hossain MJ, Alam M-U, Kenah E, Daszak P, et al. Investigating Rare Risk Factors for Nipah Virus in Bangladesh: 2001-2012. Ecohealth. 2016;13: 720–728. doi:10.1007/s10393-016-1166-0
4. Cortes MC, Cauchemez S, Lefrancq N, Luby SP, Jahangir Hossain M, Sazzad HMS, et al. Characterization of the Spatial and Temporal Distribution of Nipah Virus Spillover Events in Bangladesh, 2007-2013. J Infect Dis. 2018;217: 1390–1394. doi:10.1093/infdis/jiy015
5. McKee CD, Islam A, Luby SP, Salje H, Hudson PJ, Plowright RK, et al. The Ecology of Nipah Virus in Bangladesh: A Nexus of Land-Use Change and Opportunistic Feeding Behavior in Bats. Viruses. 2021;13. doi:10.3390/v13020169
6. Chowdhury S, Khan SU, Crameri G, Epstein JH, Broder CC, Islam A, et al. Serological evidence of henipavirus exposure in cattle, goats and pigs in Bangladesh. PLoS Negl Trop Dis. 2014;8: e3302. doi:10.1371/journal.pntd.0003302
7. Gurley ES, Hegde ST, Hossain K, Sazzad HMS, Hossain MJ, Rahman M, et al. Convergence of humans, bats, trees, and culture in nipah virus transmission, bangladesh. Emerging Infect Dis. 2017;23: 1446–1453. doi:10.3201/eid2309.161922
8. Khan MSU, Hossain J, Gurley ES, Nahar N, Sultana R, Luby SP. Use of infrared camera to understand bats' access to date palm sap: implications for preventing Nipah virus transmission. Ecohealth. 2010;7: 517–525. doi:10.1007/s10393-010-0366-2
9. Nahar N, Sultana R, Gurley ES, Hossain MJ, Luby SP. Date palm sap collection: exploring opportunities to prevent Nipah transmission. Ecohealth. 2010;7: 196–203. doi:10.1007/s10393-010-0320-3
10. Chua KB, Chua BH, Wang CW. Anthropogenic deforestation, El Niño and the emergence of Nipah virus in Malaysia. Malays J Pathol. 2002;24: 15–21.
11. Openshaw JJ, Hegde S, Sazzad HMS, Khan SU, Hossain MJ, Epstein JH, et al. Bat Hunting and Bat-Human Interactions in Bangladeshi Villages: Implications for Zoonotic Disease Transmission and Bat Conservation. Transbound Emerg Dis. 2017;64: 1287–1293. doi:10.1111/tbed.12505
12. Nahar N, Uddin M, Sarkar RA, Gurley ES, Khan MSU. Exploring pig raising in Bangladesh: implications for public health interventions. Vet Ital. 2013;
13. Ching PKG, de los Reyes VC, Sucaldito MN, Tayag E, Columna-Vingno AB, Malbas FF, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerging Infect Dis. 2015;21: 328–331. doi:10.3201/eid2102.141433
14. Chua KB. Nipah virus outbreak in Malaysia. J Clin Virol. 2003;26: 265–275. doi:10.1016/s1386-6532(02)00268-8
15. Mungall BA, Middleton D, Crameri G, Bingham J, Halpin K, Russell G, et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. J Virol. 2006;80: 12293–12302. doi:10.1128/JVI.01619-06
16. Middleton DJ, Westbury HA, Morrissy CJ, van der Heide BM, Russell GM, Braun MA, et al. Experimental Nipah virus infection in pigs and cats. J Comp Pathol. 2002;126: 124–136. doi:10.1053/jcpa.2001.0532
17. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science. 2000;288: 1432–1435. doi:10.1126/science.288.5470.1432
18. Mills JN, Alim ANM, Bunning ML, Lee OB, Wagoner KD, Amman BR, et al. Nipah virus infection in dogs, Malaysia, 1999. Emerging Infect Dis. 2009;15: 950–952. doi:10.3201/eid1506.080453
19. Nor MNM, Gan CH, Ong BL. Nipah virus infection of pigs in peninsular Malaysia. Revue Scientifique et …. 2000;
20. Hayman DTS, Wang L-F, Barr J, Baker KS, Suu-Ire R, Broder CC, et al. Antibodies to henipavirus or henipa-like viruses in domestic pigs in Ghana, West Africa. PLoS ONE. 2011;6: e25256. doi:10.1371/journal.pone.0025256
21. Olufemi OT, Umoh JU, Dzikwi AA, Wang L, Crameri G, Morrissy C, et al. Serological evidence of henipavirus among horses and pigs in Zaria and environs, Kaduna State Nigeria. Int J Infect Dis. 2016;45: 189. doi:10.1016/j.ijid.2016.02.439
22. Annett HE, Amin BM, Lele GK. The date sugar industry in Bengal: an investigation into its chemistry and agriculture. 1913;
23. Islam MS, Sazzad HMS, Satter SM, Sultana S, Hossain MJ, Hasan M, et al. Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011-2014. Emerging Infect Dis. 2016;22: 664–670. doi:10.3201/eid2204.151747
24. Olival KJ, Latinne A, Islam A, Epstein JH, Hersch R, Engstrand RC, et al. Population genetics of fruit bat reservoir informs the dynamics, distribution and diversity of Nipah virus. Mol Ecol. 2020;29: 970–985. doi:10.1111/mec.15288
25. Epstein JH, Anthony SJ, Islam A, Kilpatrick AM, Ali Khan S, Balkey MD, et al. Nipah virus dynamics in bats and implications for spillover to humans. Proc Natl Acad Sci USA. 2020;117: 29190–29201. doi:10.1073/pnas.2000429117
26. Khan SU, Salje H, Hannan A, Islam MA, Bhuyan AAM, Islam MA, et al. Dynamics of Japanese encephalitis virus transmission among pigs in Northwest Bangladesh and the potential impact of pig vaccination. PLoS Negl Trop Dis. 2014;8: e3166. doi:10.1371/journal.pntd.0003166

From: Steve Luby on behalf of Steve Luby <sluby@stanford.edu>
To: Clif McKee
Cc: Emily Gurley; mzrahman@icddrb.org; Plowright, Raina; Ausraful Islam; hs743@cam.ac.uk; Munster, Vincent (NIH/NIAID) [E]; Broder, Christopher; Eric Laing; rebeca@icddrb.org; Titu Chowdhury
Subject: Re: Updated draft of Nipah R01 proposal
Date: Sunday, May 16, 2021 3:41:51 PM
Attachments: Nipah R01 pitch 2021 05 16 sl.docx

Hi Clif,

It is exciting to see this develop further. I downloaded the Google doc, so that I had the most recent version, but then made my comments in the attached MS Word document.

Steve

Clif McKee wrote on 5/1/2021 10:49 PM:

> Dear colleagues,
>
> Attached is a new version of the science proposal. Your comments on the last draft were great and I've made the suggested changes or written a response. I have proposed sample sizes using prior data and assumptions to do power calculations, plus a little bit of brainstorming about logistical effort. Please make or suggest changes as you see fit, especially when it comes to staying within our budget.
>
> I still have some edits to do on the inferential models section of the Aim 3 strategy, but that isn't vital to budgeting, so I will get to it tomorrow.
>
> The main document is on Google Docs in case folks would prefer to do their comments there as I continue to make changes.
>
> [redacted]
>
> All the best,
> Clif

--

Stephen Luby, MD
Professor of Medicine (Infectious Diseases & Geographic Medicine)
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October 16, 2020

MEMORANDUM FOR BRIAN K AGAN, MD , P and R - Uniformed Services University of the Health

Sciences (USUHS) SUBJECT: USUHS IRB (FWA 00001628; DoD Assurance P60001) Approval

Modification of Protocol 409136 (IDCRP-085) for Human Subjects Participation

The modification to your Minimal Risk 32 CFR 219 / 21 CFR 56 human subjects research protocol 409136 (IDCRP-085) (ref # 931762) titled “***Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)”*** was reviewed and approved for execution on October 15, 2020 by Dr. Edmund G. Howe, M.D., J.D., chair USUHS IRB under the provision of 32 CFR 219.110(b)(2). This approval will be reported to the USUHS IRB scheduled to meet on October 22, 2020.

The protocol outlines a systematic approach to collection of clinical specimens and data by adapting standard procedures for conducting clinical characterizations of severe emerging infections as published by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) network (ISARIC/WHO Clinical Characterization Protocol for Severe Emerging Infections, version 3.1). .

This action approves the modification to update Appendix A of the protocol. This will reflect recent changes approved in the ICD/HIPAA document and set forth in the recent partial HIPAA waiver authorization indicating what information may be obtained from MDR or J6.

Authorization to conduct protocol 409136 (IDCRP-085) will automatically expire on July 25, 2021. If you plan to continue data collection or analysis beyond this date, IRB approval for continuation is required. Please submit a Continuing Review Form via EIRB to the IRB Office 60 days prior to your expiration date.

You are required to submit amendments to this protocol, changes to the informed consent document (if applicable), adverse event reports, and other information pertinent to human research for this project. No changes to this protocol may be implemented prior to IRB approval. If you have questions regarding this IRB action or questions of a more general nature concerning human participation in research, please contact Chris Murphy at christopher.murphy.ctr@usuhs.edu or (301) 319-0444.

(b) (6)

EIRB Protocol Template (Version 1.15)

1.0 General Information

***Please enter the full title of your study:**

Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)

***Please enter the Protocol Number you would like to use to reference the protocol:**

IDCRP-085 Version 11.0, 14-SEP-2020, eIRB v1.15
* This field allows you to enter an abbreviated version of the Protocol Title to quickly identify this protocol.

Is this a multi-site study (i.e. Each site has their own Principal Investigator)?

Yes

Does this protocol involve the use of animals?

○ Yes ● No

2.0 Add Site(s)

2.1 List sites associated with this study:

Primary Dept?	Department Name
●	**P and R** - Uniformed Services University of the Health Sciences (USUHS)

3.0 Assign project personnel access to the project

3.1 *Please add a Principal Investigator for the study:

AGAN, BRIAN K, MD

Select if applicable

☐ Student	☐ Site Chair
☐ Resident	☐ Fellow

3.2 If applicable, please select the Research Staff personnel:

A) Additional Investigators

AGAN, BRIAN K, MD
Associate Investigator
BRODY, DAVID L, MD PhD

Associate Investigator
BURGESS, TIMOTHY H, MD, MPH CAPT
Associate Investigator
BYRNE, Celia, PhD
Associate Investigator
Broder, Christopher C
Associate Investigator
Chung, Kevin Kee, MD COL
Associate Investigator
Coles, Christian Logan, PhD
Associate Investigator
DUMLER, JOHN S, MD
Associate Investigator
Dalgard, Clifton Lee
Associate Investigator
HAIGNEY, MARK C, MD
Associate Investigator
Hickey, Patrick Wilson, MD COL
Associate Investigator
Laing, Eric Daniel
Associate Investigator
Lanteri, Charlotte Anne, PhD LTC
Associate Investigator
Livezey, Jeffrey Robert, LTC
Associate Investigator
MALLOY, Allison M, MD
Associate Investigator
Mcleroy, Robert Dustin
Associate Investigator
Oliver, Thomas George, MD COL
Associate Investigator
Rajnik, Michael, MD Col
Associate Investigator
Rusiecki, Jennifer A.
Associate Investigator
SCHER, ANN
Associate Investigator
Simons, Mark Paul, PhD, D(ABMM), MScPH CDR
Associate Investigator
TRIBBLE, DAVID R, MD DrPH
Associate Investigator

B) Research Support Staff

Austin, Anissa Dawn
Research Coordinator
Chapo, Elisa Wago
Research Coordinator
English, Caroline E
Research Coordinator
Fox, Christina joy
Research Coordinator
Fraser, Jamie Alexandra
Research Coordinator

Hadley, Arile
Research Coordinator
Illinik, Luca, MPH
Research Coordinator
Law, Natasha N
Research Coordinator
Morales, Carlos Enrique
Research Coordinator
Moreno, Nicole C
Research Coordinator
Nevo, Lev N/A, M.D.
Research Coordinator
Rozman, Julia Sofia
Research Coordinator
Sanchez Edwards, Margaret Anne, MS
Research Coordinator
Spevak, Marianne V, BSHS
Research Coordinator

3.3 *Please add a Protocol Contact:

AGAN, BRIAN K, MD
BURGESS, TIMOTHY H, MD, MPH CAPT
Chapo, Elisa Wago
English, Caroline E
Fox, Christina joy
Illinik, Luca, MPH
Morales, Carlos Enrique
Moreno, Nicole C
Nevo, Lev N/A, M.D.
Rozman, Julia Sofia
Sanchez Edwards, Margaret Anne, MS

The Protocol Contact(s) will receive all important system notifications along with the Principal Investigator. (i.e. The protocol contact(s) are typically either the Protocol Coordinator or the Principal Investigator themselves).

3.4 If applicable, please select the Designated Site Approval(s):

Add the name of the individual authorized to approve and sign off on this protocol from your Site (e.g. the Site Chair).

4.0
Project Information

4.1 Is this a research study?

● Yes ○ No

4.2 What type of research is this?

☑ Biomedical Research

☐ Clinical trial (FDA regulated)
☐ Behavioral Research
☐ Educational Research
☐ Psychosocial Research
☐ Oral History
☐ Other

4.4 Is this human subjects research (Activities that include both a systematic investigation designed to develop or contribute to generalizable knowledge AND involve a living individual about whom an investigator conducting research obtains data through intervention or interaction with the individual or identifiable private information. Activities covered by 32 CFR 219.101(a) (including exempt research involving human subjects) and DoDI 3216.02)?

● Yes ○ No

4.5 Do you believe this human subjects research is exempt from IRB review?

○ Yes ● No

5.0 Personnel Details

5.1 Will you have a Research Monitor for this study?

○ Yes
● No
○ N/A

Research Monitor Role:

If applicable, you may nominate an individual to serve as the Research Monitor:

No Users have been selected.

6.0 Data/Specimens

6.1 Does the study involve the use of existing data or specimens only (no interaction with human subjects)?

○ Yes ● No

7.0 Funding and Disclosures

7.1 Source of Funding:

Funding Source	Funding Type	Amount

NIAID IAA	:Other	56632

Total amount of funding:

56632

7.2 Do you or any other Investigator(s) have a disclosure of a personal interest or financial nature significant with sponsor(s), product(s), instrument(s) and/or company(ies) involved in this study?

○ Yes ● No

8.0 Study Locations

8.1 List any Research Team members without EIRB access that are not previously entered in the protocol:

Name: (Last, First, M.I.) Clark, Danielle Role on Protocol: Associate Investigator	Phone Number: 508-471-7655	Email Address: dclark@aceso-sepsis.org	Associated Institution: Henry M Jackson Foundation
Name: (Last, First, M.I.) Blair, Paul Role on Protocol: Associate Investigator	Phone Number: 301-256-2165	Email Address: pblair@aceso-sepsis.org	Associated Institution: Henry M Jackson Foundation/USUHS
Name: (Last, First, M.I.) Koehler, Jeff Role on Protocol: Associate Investigator	Phone Number: 301-619-8342	Email Address: jeffrey.w.koehler4.civ@mail.mil	Associated Institution: US Army Med Rsch Inst of Infectious Diseases
Name: (Last, First, M.I.) Fries, Anthony Role on Protocol: Associate Investigator	Phone Number: 937-938-2847	Email Address: anthony.fries.tr@us.af.mil	Associated Institution: US Air Force Sch Aerospace Med
Name: (Last, First, M.I.) Hansen, Erin Role on Protocol: Associate Investigator	Phone Number: 619-857-5219	Email Address: erin.a.hansen.ctr@mail.mil	Associated Institution: Naval Health Research Center

Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Peel, Sheila	301-319-2297	sheila.a.peel2.civ@mail.mil	Walter Reed Army of Institute Research
Role on Protocol:			
Associate Investigator			
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Darden, Janice	301-275-1190	jdarden@hivresearch.org	Walter Reed Army of Institute Research
Role on Protocol:			
Associate Investigator			
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Letizia, Andrew	240-507-6326	andrew.g.letizia.mil@mail.mil	Naval Medical Research Center
Role on Protocol:			
Associate Investigator			
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Snow, Andrew	301-295-3267	andrew.snow@usuhs.edu	USUHS
Role on Protocol:			
Associate Investigator			
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Pollett, Simon	301-319-2059	simon.d.pollett.ctr@mail.mil	Walter Reed Army of Institute Research
Role on Protocol:			
Associate Investigator			
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Berry, Irina	301-319-2032	irina.maljkovicberry.ctr@mail.mil	Walter Reed Army of Institute Research
Role on Protocol:			
Associate Investigator			
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Friberg, Heather	301-319-9224	heather.l.friberg-robertson.civ@mail.mil	Walter Reed Army of Institute Research
Role on Protocol:			
Associate Investigator			
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Currier, Jeffrey	240-994-3911	jeffrey.r.currier.civ@mail.mil	Walter Reed Army of Institute Research
Role on Protocol:			

Associate Investigator			

8.2 Has another IRB reviewed this study?

○ Yes ● No

IRB Name	Review Date	Determination	
No records have been added			

8.3 Is this a collaborative or multi-site study? (e.g., are there any other institutions involved?)

● Yes ○ No

8.4 Study Facilities and Locations:

Institution	Site Name	Site Role	FWA or DoD Assurance Number	Assurance Expiration Date	Is there an agreement?	IRB Reviewing for Site
Navy	Naval Health Research Center	Laboratory analyses	FWA00013516	03/02/2021	: IAIR	: USUHS IRB #1
Army	US Army Med Rsch Inst Infectious Dis	Laboratory analyses	FWA00007368	10/02/2024	: MOU	: USUHS IRB #1
Air Force	US AF Sch Aerospace Med	Laboratory analyses	FWA00027236	08/29/2023	: IAIR	: USUHS IRB #1
Army	Walter Reed Army of Institute Research	Laboratory analyses	FWA00000015	11/20/2023	: Other	: USUHS IRB #1
Navy	Naval Medical Research Center	Laboratory analyses	FWA00000152	06/05/2024	: IAIR	: USUHS IRB #1

Other:

Other Institution Site	Site Role	FWA or DoD Assurance Number	FWA or DoD Expiration Date	Is there an agreement?	IRB Reviewing for Site	
No records have been added						

8.5 Are there international sites?

Attach international approval documents, if applicable, when prompted. Note: Ensure local research context has been considered

○ Yes ● No

8.6 Is this an OCONUS (Outside Continental United States) study?

○ Yes ● No

Select the area of responsibility:

Have you obtained permission from that area of responsibility? (This is a requirement prior to study approval)

○ Yes ○ No

9.0 Study Details

9.1 Abstract/ Summary:

Summarize the proposed study in 500 words or less, to include the purpose, the subject population, the study's design type, and procedures

The overarching objective of the Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC) protocol is to provide the Military Health System (MHS) with a contingency protocol intended for activation at Military Treatment Facilities (MTFs) during outbreaks to allow clinical investigation of severe or potentially severe acute infections with pathogens of concern to public health and U.S. military medical readiness. The protocol outlines a systematic approach to collection of clinical specimens and data by adapting standard procedures for conducting clinical characterizations of severe emerging infections as published by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) network (ISARIC/WHO Clinical Characterisation Protocol for Severe Emerging Infections, version 3.1).
The EPICC protocol can be conducted as a multi-site study at participating MTFs, and overall protocol oversight will be coordinated centrally by the Uniformed Services University's Infectious Disease Clinical Research Program (IDCRP). MHS-wide enrollment for online participation will be coordinated by the IDCRP Program Coordinating Center (PCC).
EPICC activities will be executed by clinical study teams comprising existing IDCRP clinical research investigators and staff located at each MTF and protocol investigators from relevant hospital departments. The protocol is designed to be modified to accommodate longitudinal observational study of specific emerging or re-emerging pathogens. Protocol modifications will describe methods for serial collection and clinical characterization of specimens and associated demographic, laboratory, clinical, and other data specific for the pathogen of interest and associated study population(s).

9.2 Key Words:

Provide up to 5 key words that identify the broad topic(s) of your study

Epidemiology, Immunology, Emerging Infectious Diseases

9.3 Background and Significance:

Include a literature review that describes in detail the rationale for conducting the study. Include descriptions of any preliminary studies and findings that led to the development of the protocol. The background section should clearly support the choice of study variables and explain the basis for the research questions and/or study hypotheses. This section establishes the relevance of the study and explains the applicability of its findings

The EPICC protocol is intended to serve as a foundation for modification to allow the observational, longitudinal study of specific emerging or re-emerging pathogens causing disease with pandemic potential within the MHS. Modifications responsive to specific diseases will be described to include a schedule for serial collection of clinical specimens and data and methods for recruiting, enrolling, and observational study within the desired MHS study population(s). In general, enrolled participants (eligible adults or children) who are known or suspected cases at MTFs as inpatients or in isolation settings will undergo serial collections of biological specimens and clinical and laboratory data at day of enrollment and during the duration of hospitalization or isolation, with follow-up specimen and data collection convalescent and long-term follow-up timepoints. The study design can also include clinical observational investigation in outpatients. The major focus of this protocol is to conduct clinical observational study in symptomatic individuals; however, there is also the opportunity to enroll and follow (potentially at a reduced specimen and data collection frequency relative to symptomatic participants) individuals who are asymptomatic but who are at high/some risk for exposure. Inclusion of an asymptomatic comparator cohort could provide insight on risk factors or biomarkers associated with symptomatic versus asymptomatic presentations. The protocol supports observational, longitudinal collection of clinical specimens and data with a goal of identifying host biomarkers that predict severity of illness, resistance to infection, treatment failure, etc. If volunteers receive an experimental treatment product under eIND, the data collected through the EPICC protocol may help inform about the safety and efficacy of that product.

In subsequent sections, relevant background information on acute respiratory infections (ARI) of pandemic potential is provided, followed by specific schedule of events and other details to allow observational study of an ongoing outbreak of the 2019 novel coronavirus (COVID-19).

Acute Respiratory Infections of Pandemic Potential: Epidemiology, Immunology and Clinical Characteristics

ARI Case Definitions

To characterize the epidemiology, immunology and clinical features of novel pathogens associated with severe ARI, case definitions appropriate to emerging infection scenarios will be utilized. These definitions will vary by the availability and result of laboratory-based diagnostic tests.

I. *For laboratory-confirmed cases*, the case definition includes the presence of laboratory-confirmed infection with an agent of severe ARI (e.g., SARS-CoV-2, influenza A/H7N9, MERS-CoV, SARS, etc.).

OR

II. For persons under investigation (PUI), the case definition includes:

1. The current CDC case definition for a PUI for a respiratory pathogen under study.
2. An unknown or pending laboratory confirmation of an agent of severe ARI.

III. For individuals who are asymptomatic and have had recent high-risk exposure to the pathogen of interest such that they have increased their risk for infection, 1 or more of the following criteria must be met:

1. Unprotected exposure (for e.g., < 14 days of illness onset, modified commensurate with evolving epidemiologic information) with confirmed or suspected cases of human infection with a novel respiratory pathogen. Close contact is typically defined as coming within about 6 feet (2 meters) of a confirmed or suspected case while the case was ill (beginning 1 day prior to illness onset and continuing until resolution of illness).
2. Unprotected exposure to infectious pathogens in a laboratory setting.
3. If exigencies warrant, then asymptomatic/high exposure risk individuals in isolation may be considered for enrollment.

Acute respiratory infections (ARI) are among the most common infectious diseases. Due to conditions and circumstances of military training and deployment (e.g., crowding, inadequate hygiene practices, physical stress, etc.), military personnel in congregate settings are at increased risk for outbreaks due to ARI. There are a number of emerging viral respiratory pathogens to which military forces may be exposed, that carry substantial importation risk, and that also have significant pandemic potential. Recent and current examples are described below.

1. Influenza A/H5N1 was first identified in the human population in Hong Kong in 1997. Since 2003, 16 countries have reported 668 confirmed cases and 393 deaths due to influenza A/H5N1 (as of 1 Nov 2014). The global case fatality rate for influenza A/H5N1 infection is 60%, and the estimates range widely (0% to 100%) by country. While the significant morbidity and mortality of influenza A/H5N1 infection is readily apparent, its occurrence among humans has been relatively infrequent to date. Circulation of H5N1 predominates in poultry, currently the main reservoir, in Asia and northeast Africa. Host-to-human (i.e., zoonotic transmission of A/H5N1) infections are unpredictable and “remain rare

despite frequent and widespread contact with infected poultry and contaminated environments" (1). The majority (71%) of cases of influenza A/H5N1 have had recent exposure to live birds or contaminated areas. Cases of influenza A/H5N1 to date have been relatively young (median age: 19 years) (2). Exposure to other cases of influenza A/H5N1 has not been a major risk factor to date, suggesting that the likelihood of human-to-human transmission of this particular strain remains low (R0< 1) (3). The severity of influenza A/H5N1 is dependent upon the strain associated with infection /disease. Among strains with low pathogenicity, generally mild, non-fatal illnesses have been observed, ranging from conjunctivitis to influenza-like illness (e.g., fever, cough, sore throat, muscle aches) to lower respiratory disease (pneumonia). Among strains with high pathogenicity, the disease spectrum has ranged from a milder febrile respiratory illness to severe disease (e.g., dyspnea, hypoxemia, pneumonia, acute respiratory failure) with multi-organ disease, sometimes accompanied by nausea, abdominal pain, diarrhea, vomiting and delirium. The high mortality rate (~60%) associated with pathogenic strains of influenza A/H5N1 has been reported from multiple studies (2).

2. Influenza A/H7N9 infection of humans was first identified in China in March 2013. Since then China has reported 453 confirmed cases and 175 deaths due to influenza A/H7N9 (WHO 2014). As with influenza A/H5N1, most of the cases had recent exposures to live poultry or had visited areas where live poultry was sold (75%) (3). By contrast to influenza A/H5N1, the host reservoir, main exposures, routes of transmission, and distribution and prevalence of influenza A/H7N9 among humans and animals are unknown. Influenza A/H7N9 does not appear to transmit easily from birds or the environment to humans. In addition, reported cases of Influenza A/H7N9 have not demonstrated high transmissibility between humans to date, suggesting that the risk of pandemic potential currently remains low (4). Though limited in its reach, influenza A/H7N9 infection has been associated with significant mortality: approximately 39% of cases resulted in a fatal outcome (4).
3. The coronavirus associated with the Middle Eastern Respiratory Syndrome (MERS-CoV) was first identified in Saudi Arabia in 2012 (5). Several outbreaks have since occurred, originating in Middle Eastern countries and spreading globally, albeit to a limited extent, by international travelers. Symptoms including fever, rigors, malaise, cough, myalgia, headache, shortness of breath, anorexia, diarrhea and abdominal pain were observed in MERS-CoV cases (6,7). As of October 2014, a total of 897 confirmed cases and 325 deaths (case fatality rate [CFR]: 36%) have been reported worldwide (WHO 2014). In 2013, two studies concluded that MERS-CoV did not yet exhibit pandemic potential, with an estimated basic reproduction number less than 1.0 (8, 9). Some research indicates that camels may act as the host reservoir for MERS-CoV, although evidence is inconclusive (10). Human-to-human transmission of MERS-CoV has been observed in healthcare settings, possibly due to greater infectiousness in the later stages of illness; however, evidence that may further elucidate risk factors for transmission is limited (6, 7).
4. The 2002-3 pandemic of Severe Acute Respiratory Syndrome (SARS), also due to a novel Coronavirus, originated in China's Guangdong Province and resulted in 8,422 probable cases and 916 deaths reported from 29 countries (mostly in mainland China, where 63% of cases and 38% of deaths occurred) (11). The global case fatality rate due to SARS was estimated to be 11% (11). Although the host reservoir remains unknown, some studies have found coronaviruses similar to the SARS coronavirus circulating in bat populations (12). Transmission in human populations occurs by direct contact with the infectious respiratory droplets and/or fomites via the eyes, nose, and mouth (13). The incubation period ranges from 1-14 days (mean 4-6 days) and transmissibility appears to increase with severity of illness (greatest at day 10) (14). Attack rates ranged widely by geographic region from 2.2 per 100,000 in Guangdong Province to 25.6 per 100,000 in Hong Kong (14). Risk factors for infection include occupation as a healthcare worker (21% of all probable cases), household contact with a probable case, older age, male sex and existing illnesses (14). Symptoms include fever, dry cough, dyspnea, headache and hypoxemia (14).
5. Since December 2019 through March 2020, the **2019 novel coronavirus (SARS-CoV-2) causing "coronavirus disease 2019" (COVID-19)** has spread from Wuhan, China to 64 countries and has led to more than 88,948 confirmed cases globally, including 80,174 confirmed cases in China and 8,774 confirmed cases outside of China (15). To date, there have been 2,915 deaths in China and 128 deaths outside of China. As of 03 March 2020, in the United States there are 60 confirmed cases (22 travel-related, 11 person-to-person spread, 27 under investigation) and a total of 6 deaths (16). Individuals with COVID-19 can present with fever, and cough, ground-glass opacity on chest computed tomography (CT), and lymphocytopenia (17). The case-fatality rate (CFR) appears to be highly variable with early reports of 8-15% in older adults infected in Hubei Province (CFR is increased in adults with comorbid conditions who develop respiratory symptoms) but likely no higher than 1-2% outside of Hubei (though data is limited and this will depend on accurate detection of those with mild disease) (18). Several risk factors, including age, history of smoking, maximum body temperature on hospital admission, respiratory failure, albumin, and C-reactive protein, appear associated with increased risk for progression to pneumonia among COVID-19 hospitalized patients (19). Risk factors of disease progression and magnitude of clinical severity are incompletely understood and require further study. SARS-CoV-2 is believed to be transmitted primarily via person-to-person, such as through close

contact (within about 6 feet) and exposure to respiratory droplets from an infected individual who coughs or sneezes (20). Evidence exists for transmission of SARS-CoV-2 via the oral-fecal route, as viral nucleotide was detected in rectal swabs and blood later in the course of infection when virus was not detected in oral swabs (21). On the population level, there is a poorly-defined risk of transmission via asymptomatic infection (22). The occurrence of asymptomatic infections has raised concerns about the rapid spread of transmission to vulnerable populations (21).

9.4 Objectives/Specific Aims/Research Questions:

Describe the purpose and objective(s) of the study, specific aims, and/or research questions/hypotheses

General Goals and Objectives:

1. To describe the natural history of clinical disease
2. To identify risk factors for infection and severe clinical course
3. To characterize the host immune response to infection
4. To evaluate the correlation between symptom severity, virologic/microbiologic characteristics, the host immune response and clinical course and outcome
5. To develop and/or operationally assess diagnostic and prognostic tools for the study of novel pathogens / emerging infectious diseases
6. To establish a clinical and laboratory database, and related specimen repository, for future studies of novel pathogens / emerging infectious diseases
7. To investigate efficacy of emergency Investigational New Drug (eIND) therapies that may be administered to enrolled study participants during the course of clinical care

For COVID-19 / SARS-CoV-2 infection, clinical and demographic data will be evaluated, along with laboratory findings as appropriate, to assess key endpoints of interest to include, but not limited to, the following:

- Incubation period
- Duration of illness / symptoms
- Duration of functional disability
- Duration of shedding from respiratory and gastrointestinal tract
- Incidence and duration of viremia
- Predictors of severe disease: clinical signs, symptoms, laboratory features; host factors, eg smoking, vaping, alcohol consumption, comorbid illness/conditions, occupational health factors influencing lung disease; host biomarkers; virologic (strain/virulence) attributes
- Supportive management required: O_2, HFNC, NIPPV, ventilation; hemodynamics
- Complications: e.g., bacterial pneumonia; bacteremia; AKI; liver injury; CNS involvement, etc.
- Outcomes: resolution of symptoms; return to pre-illness functional status; requirement for O_2; development / exacerbation of reactive airway disease; pulmonary function; resolution of medical complications; new Rx requirements, eg bronchodilators; other sequelae
- Development of immunity (i.e., correlates), kinetics, duration of antibody detection
- Relationship between acute illness (or pre-illness) HCoV titer and infection, outcome, virologic parameters
- Costs of care, costs of duty days lost

9.5 Study Design:

Describe study design in one to two sentences (e.g., prospective, use of existing records/data /specimens, observational, cross-sectional, interventional, randomized, placebo-controlled, cohort, etc.). Specify the phase – Phase I, II, III, or IV – for FDA-regulated investigational drug research

A prospective, longitudinal cohort study of novel pathogens / emerging infections with pandemic potential

9.6 Target Population:

Describe the population to whom the study findings will be generalized

Study findings will be generalized and applicable to all populations that travel to or reside in disease endemic areas or live within an area that has been exposed to infectious pathogens with pandemic potential; some of which include military personnel and the general public.

9.7 Benefit to the DoD:

State how this study will impact or be of benefit to the Department of Defense

Infectious diseases are a significant source of morbidity among military forces. Infectious disease outbreaks compromise force readiness and interfere with training programs. Strategies to characterize and ultimately to treat and prevent the major infectious disease threats to military personnel are urgently needed.

10.0

Study Procedures and Data management

10.1 Study Procedures:

Describe step-by-step how the study will be conducted from beginning to end

Recruitment and enrollment strategies will be dynamically responsive to the outbreak as the situation evolves. Study volunteers may be recruited from areas of the hospital where they present for care or in the inpatient and isolation settings. Informed consents and enrollments will be conducted by study staff included on the IRB approved protocol, including staff named on delegation log, and trained in study procedures. Clinical evaluations will be performed by the MTF healthcare team and may also be conducted by qualified study team members.
In addition to MTF enrollment, MHS beneficiaries who have been clinically tested for COVID-19 will be invited to participate in a limited schedule of assessments for EPICC, including completion of a series of online questionnaires and at-home capillary blood sample collection at three timepoints. See Appendix A for further details.
At the time of a participant’s initial consultation at an MTF, study activities will consist of explanation of study goals and purpose, and obtaining informed consent (may include consultation with Legally Authorized Representative). The day of enrollment will be defined as Study Day 0. The team will subsequently conduct serial collection of data (demographics, vital signs, clinical findings, diagnostics and other laboratory results, etc.) through study participant interview and from medical record review from time of disease onset throughout study duration. Specific risk factors linked to pathogen exposure (eg., travel history to disease endemic regions, contact with sick individuals or known reservoirs of disease, etc.) will also be acquired from study volunteers, mainly through interview at Day 0. Medical records collected prior to day of enrollment will also be evaluated to ascertain medical history information pertinent to understanding baseline health and analysis of underlying risk factors associated with disease progression and other outcomes. Study team members will collect for research purposes biological specimens (e.g., nasopharyngeal and oropharyngeal swabs, rectal swabs, and blood) from enrolled participants who are laboratory-confirmed or suspected cases at time of enrollment, during the duration of hospitalization and/or isolation, with follow-up specimen collection up to approximately 1 year post-enrollment.

When possible, the study team will also obtain residual volumes of specimens collected for clinical care that would otherwise be discarded. Sample types include but are not limited to bronchoalveolar lavage, blood, urine, sputum, saliva, stool, cerebrospinal fluid, semen (adults only), or vomit.As available, these residual samples will be obtained from the time of onset of the disease under study. For active duty servicemembers, the study team will also request a pre-illness (up to 2 years prior to enrollment) serum sample from the DoD Serum Repository (DoDSR).

Some subjects may undergo clinical imaging studies as part of regular patient care or as part of this protocol using clinical imaging devices. For example, point-of-care portable ultrasound may be used to image areas such as the lungs, heart, and lower extremity veins, to explore the prognostic use of ultrasound in early detection of pneumonia and cardiovascular sequelae associated with severe disease. These imaging studies, when available, will be part of the collected subject data.

Study participants recruited and enrolled at MTFs who are ambulatory may also be evaluated via serial self-collection of samples such as nasopharyngeal swabs, oropharyngeal swabs, capillary blood samples, and/or other specimens appropriate for self-collection.

Recruitment of individuals who are asymptomatic at high/some risk of exposure may involve study team members approaching family or friends who escorted the sick individual to the hospital as well as healthcare providers who have potentially been exposed to known or suspected cases. Enrollees who are asymptomatic high/some risk will undergo serial collections of clinical and laboratory data as well as biological specimens (e.g., nasopharyngeal, oropharyngeal, and rectal swabs and blood) at enrollment, with follow-up specimen collection up to approximately 12 months post-enrollment. The study team may consider implementing a plan for asymptomatic study volunteers to conduct self-collection of clinical specimens (e.g. nasopharyngeal swabs, oropharyngeal swabs, rectal swabs, capillary blood smaples) through the follow-up period. Demographic, clinical, and other data as described above for symptomatic volunteers may also be acquired for asymptomatic high/some risk participants.

The Flu- PRO questionnaire (24-27) may be used to record the patient-reported outcome (PRO) on severity of clinical symptoms and the impact of clinical interventions on infection. A web link to the Flu-PRO questionnaire may be provided to participants to record the severity of ILI symptoms daily for 14 days from either the day of enrollment, or the date of onset of symptoms (for subjects who were asymptomatic at enrollment). Paper versions of the Flu-PRO questionnaire may be administered as needed.

In addition, an online questionnaire will be administered at enrollment and follow-up timepoints to collect data on medical history, comorbidities, behavioral risk factors, symptoms and recovery status.

If a participant is dually enrolled in a treatment protocol and scheduled to receive investigational products under an eIND, an additional sample may be collected in the first 48 hours after test article administration. If eligible, study participants may also be considered for enrollment concurrently in an interventional clinical trial evaluating efficacy of treatment regimens. The priority of collection of clinical specimens will be as follows: 1. specimens collected for clinical care; 2. specimens collected in support of an interventional clinical trial; and 3. specimens collected for observational study (i.e., EPICC protocol).

The study team may consider discontinuation of study participants who have a negative test result for the pathogen of interest (based on current standard of care diagnostics) and/or are diagnosed with another respiratory pathogen other than the one of interest and/or the disease of interest is highly unlikely based on the primary clinical team assessment. This approach may need to be taken to conserve study staff time and resources depending upon local epidemiologic circumstances, e.g. very high incidence and subject accrual.

Results obtained from research analyses will not be entered into the participant’s medical record. However, the study team will communicate relevant findings to the participant’s primary care provider overseeing care of the indicated infection of study. The study volunteer’s primary healthcare provider is likely to be a member of the protocol study team, and as such will be directly aware of study findings. If the primary healthcare provider is not a part of the protocol team, relevant findings from the study will be communicated to the healthcare provider verbally in a private setting to protect patient confidentiality or via a secure encrypted electronic communication.

Pregnant participants will be followed monthly until delivery to collect information on pregnancy outcome and health of the child with regards to the disease under study, to the extent practicable. A cord blood sample may be collected for research purposes following childbirth.

Specimens collected through this observational study will be evaluated in various laboratory assays to characterize the epidemiology, immunology, clinical characteristics, and other important outcomes of interest. Associated clinical and demographic data will also be evaluated, along with laboratory findings as appropriate, to assess key endpoints of interest as described in Section 9.4.

Schedule of Patient Evaluation and Data Collection

The table below shows the schedule of assessments for study participants enrolled in EPICC. Given the possibility of critical illness of the patient and the potential increased risk of exposure to medical personnel, assessments that cannot be completed due to patient care considertaions, indection control measures, or other logistical challenges will not constitute protocol deviations. In addition, the frequency of specimen and data collection may be reduced to accommodate logistics of acquiring specimens and data among study volunteers who are outpatients.
Ambulatory subjects who do not have restrictions of movement for infection control purposes (i.e., not advised by their care provider/local health authority to remain home in isolation) will be invited to return to the hospital for follow-up assessment and collection of specimens.

If an asymptomatic and/or COVID-19-negative subject develops symptoms or receives a positive test result for COVID-19, they will be asked to contact study staff to report their symptoms/test result and will restart the schedule of assessments from Day 0.

Inpatient MTF-Enrolled Subjects

Specimen/ Assessment	Enrollment (Day 0)	Days 3, 7 (± 1 day)	Once Weekly until discharge (± 2 days)	Day 14 if Discharged (± 3 days)	Day 28 (± 7 days)	6 Months (± 14 days)	12 Montl (± 28 day
Virology Sample: Nasal, Throat, and/or Oral specimen*	√	√	√	√			
Virology Sample: Rectal Swab	√	√	√		√		
Blood	√	√	√	√¶	√¶	√¶	√¶
Residual Clinical Samples #	√	√	√	√	√	√	√
Radiologic images **	√	√					
Flu-PRO Symptom Diary ***	√	√	√	√			
Online Questionnaire	√				√	√	√

* To detect and analyze virus in the nose, throat, and/or mouth, the specimen collection method employed may change to follow updated best practices as reported in the scientific literature. Example sample types are swabs (nasal, nasopharyngeal, oropharyngeal) and/or saliva that may be collected by study staff or self-collected by subjects.

¶ Blood collection after discharge may be done using a commercial at-home microsampling blood collection kit.

Residual samples from clinically-indicated lab tests will be collected for research. Sample types may include sputum, saliva, urine, stool, blood, semen (adults only) or vomit. Residuals may be obtained from samples collected starting at the date of disease onset, which may be prior to Study Day 0.

** Lung, cardiac, and/or venous ultrasound may be conducted on hospitalized subjects on days 0, 1, and 3 as resources and local patient care policy allows. Findings from any additional radiologic images obtained during routine clinical care will be collected for research.

*** Subjects aged 7 and older who are experiencing symptoms will be asked to complete the Flu-PRO diary for 14 days.

Outpatient MTF-Enrolled Subjects

Specimen/ Data	Enrollment (Day 0)	Day 7 (± 1 day)	Day 14 (± 3 days)	Day 28 (± 7 days)	6 Months (± 14 days)	12 Months (± 28 days)
Virology Sample: Nasal, Throat, and/or Oral specimen*	√‡		√			
Blood¶	√‡	√	√	√	√	√
Flu-PRO Symptom Diary **	√	√	√			
Online Questionnaire	√			√	√	√
Residual clinical samples #	√	√	√	√	√	√

* To detect and analyze virus in the nose, throat, and/or mouth, the specimen collection method employed may change to follow updated best practices as reported in the scientific literature. Example sample types are swabs (nasal, nasopharyngeal, oropharyngeal) and/or saliva that may be collected by study staff or self-collected by subjects.

¶ Blood collection may be done using a commercial at-home microsampling blood collection kit.

Residual samples from clinically-indicated lab tests will be collected for research. Sample types may include sputum, saliva, urine, stool, blood, semen (adults only) or vomit. Residuals may be obtained from samples collected starting at the date of disease onset, which may be prior to Study Day 0.

** Subjects aged 7 and older who are experiencing symptoms will be asked to complete the Flu-PRO diary for 14 days.

‡ There is a +3 day window for Day 0 research sample collection to allow for distribution of self-collection kits to participants whose samples could not be collected at enrollment.

Online Cohort: See Appendix A

Specimen/ Data		Day 28		

	Enrollment (Day 0)	(± 7 days)	6 Months (± 14 days)	12 Months (± 28 days)
Online Questionnaire	√	√	√	√
Self-collected blood¶		√	√	√

¶ All blood collection for this cohort will be done using a commercial at-home microsampling blood collection kit.

Considerations for Pediatric Participants

- Flu-PRO symptom diary may be completed by subjects aged **7 and older**.
- Pediatric subjects will not be asked to complete the online questionnaires.
- Self-collection of specimens is an option for subjects aged **8 and older**.
- Blood collection volume and frequency may be reduced to comply with safety limits. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.

Research Laboratory Methods

Clinical specimens collected through this observational study will be evaluated in a variety of laboratory assays developed by laboratory partners. Specific laboratory assays to study the virologic, immunologic, and other relevant aspects of infections are unlikely to be available at the time of specimen collection (i.e., during the outbreak) given the time required to establish assays specific for novel, emerging pathogens. Below is a general description of laboratory assay methods and endpoints that may be used; however, this does not represent an exhaustive list to include new methodologies that are later developed in response to assaying novel pathogens.

To characterize virologic correlates (e.g., viremia) of clinical symptoms and disease severity, serum levels of viral genomes can be assessed by real time-PCR at various time points. Serum concentration of viral antigen can be evaluated by enzyme-linked immunosorbent assay (ELISA). These same assays may be utilized in the longitudinal detection of virus in body fluids (e.g., nasal mucosa, sputum, urine, stool, etc.) throughout the course of evaluation/illness.

In addition, serum may be assayed for concentrations of virus-specific IgM and IgG in the acute and convalescent stages of evaluation/illness. Cytokine profiles can be conducted via multiplex assay to describe the pro- versus anti-inflammatory cytokine responses of study volunteers throughout the course of illness. PBMCs may be collected to investigate cell-mediated immune responses in the acute and convalescent phases of illness.

Novel biomarkers, including transcriptomic and proteomic signatures, are promising for diagnosis and prognosis. Collection of appropriate specimens from individuals with well-characterized illnesses is required to inform assay development. Immune factors have been used as biomarkers for predicting clinical course and outcome, as well as the severity of clinical symptoms. For example, higher levels of cytokines IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNFα were observed in patients with SARS-CoV-2 infection requiring ICU-level care compared to those receiving non-ICU care (21). Host specific responses may be evaluated using real-time PCR and other molecular methods to identify biomarkers, transcriptomic signatures, or other lab-based endpoints indicative of severity of clinical course of disease progression and other risk factors associated with clinical outcomes, etc. In addition, evaluation of host transcriptomic responses among symptomatic versus asymptomatic, high risk exposure individuals may identify an early warning means for identifying asymptomatic people who eventually develop disease. RNA sequencing of blood specimens may also be conducted to assess viral transcription signatures as biomarkers for clinical outcomes. Blood specimens may also be used in phosphoproteomic assessments by measuring the proportion of host immune system proteins that are phosphorylated to evaluate novel biomarkers associated with activation of protein signaling pathways in response to infection.

10.2 Data Collection:

Describe all the data variables, information to be collected, the source of the data, how the data will be operationally measured, and approvals needed for use of information from DoD databases

Diagnostic Tests and Laboratory Analysis to be Obtained:

As available, laboratory data elements, such as but not limited to hematologic, biochemical, and other clinical parameters; clinical images and associated reports; and clinical diagnosis of the pathogen of interest (SARS-CoV-2) and

/or alternate or co-infective pathogens, will be abstracted from the medical record of study volunteers on the day of enrollment (Study Day 0), throughout the period of hospitalization (inpatients) and through duration of follow-up. In addition, laboratory and diagnostic data available in medical records prior to Day 0 will be abstracted for study of baseline health, existing co-morbidities, etc.

Data Elements

Case report forms (CRFs) for this protocol have been adapted from those of the International Severe Acute Respiratory Infection Consortium (ISARIC).

There are five main components of the data collection forms, encompassing study participant information at:

1. The time of enrollment (at time of presentation at MTF, hospital admission, or being placed in isolation),
2. Period prior to Day 0, as appropriate, to assess recent medical history,
3. Medical encounter data (inpatient or outpatient),
4. The time of discharge or death
5. Follow-up period (following hospital discharge or being released from isolation setting, or otherwise as an out-patient)

Major data variable categories to be collected include, but are not limited to the following:

- Day of enrollment
    - Demographics (Residence, Occupation, Military Status)
    - Signs and Symptoms at onset of illness and presentation for care
    - Epidemiological Risk Factors and Exposures
    - Pre-admission Medications
    - Review of recent medical history via medical record review
    - Flu-PRO symptom severity questionnaire
- Hospitalization or Isolation Period
    - Daily Observations (i.e., vital signs) and Treatments (i.e., receipt of IV fluids)
    - Daily Laboratory Results
    - Medications and Blood Products
    - Microbiology Results
    - Critical Care
    - Severe Symptoms and Complications
    - Flu-PRO symptom severity questionnaire
- Outcome
    - Final Diagnosis
    - Patient Status at Outcome
    - Follow-up period

Data Collection

Study data will be copied onto electronic CRFs at the study site. These transcriptions will occur on an ongoing basis during the study in accordance with Good Clinical Practice (GCP) guidelines. Data entered on CRFs, as well as corrections made, shall be performed by authorized individuals. This study will make use of electronic case report forms (eCRFs) using tablets with the Research Electronic Data Capture (REDCap). For cases where a tablet cannot be used because of infection control concerns or logistical issues, authorized personnel will capture CRF information using a paper based version and later electronically record results into REDCap. All data transmitted to and from the application is done using a secure, encrypted transmission (SSL/HTTPS). For increased security, the application additionally verifies the SSL certificate of the REDCap server that it is communicating with in order to validate the server’s identity. The REDCap mobile application employs encryption-at-rest on the mobile device’s hard drive, so all data and information stored on the device is protected from unauthorized users. REDCap has built-in safeguards such as username and PIN, and remote lockout. When resources are available, data from clinical devices including de-identified ultrasound or radiograph images, continuous vital signs, and/or cardiac telemetry will be locally stored on password-protected devices, and if possible, transmitted wirelessly to a secure Cloud backup. Henry Jackson Foundation (HJF) headquarters IT will host deidentified study data on HJF HQ secured servers. HJF is responsible for the purchase, setup, maintenance, and service of any its servers and technology.

The supervising principal investigator, or designated associate investigator, will be responsible for regular maintenance and review of CRFs for accuracy. Access to study files (e.g., CRFs) is restricted to care providers and research team members given specific tasks to perform. The files are kept in a secured location in each study site. Some participant-completed forms such as questionnaires may be completed through the web-based program. These will be transmitted directly into the study database. Research labs will transmit their testing data to the DCC using standard procedures for data handling and transmission.

Data Management/Quality Control

During the study, the investigator will review study data from their site, including medical records, records detailing the progress of the study for each volunteer, laboratory reports, CRFs, documentation of informed consent and authorization forms for each study volunteer, correspondence with the IRB, adverse event (AE) reports and information regarding volunteer discontinuation of the study. All required study data will be clearly and accurately recorded on source documents or CRFs by either the participant themselves or authorized study personnel. Only designated study personnel shall record or change data on a CRF. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded on the CRFs. Original observations entered directly onto the CRFs are defined as source data. PHI will be removed from clinical devices, such as those used for radiology, and associated data will be labeled with the study participant's ID. For each pivotal analysis (e.g., for a sub-study, manuscript, or presentation), the 'snapshot' (or 'data freeze') database will be made and archived in the study data repository. After the study analysis ends, the database will be locked.

10.3 At any point in the study, will you request, use, or access PII from the Military Health System (MHS)?

● Yes ○ No

10.4 Have you consulted with an MHS data expert to determine the data elements to be extracted or the information system(s) to access?

Consulting with a data expert often saves time later in the compliance process because the data expert can advise on the data available in the numerous MHS information systems, the quality of that data and the methods for encrypting and collapsing data. To schedule a consult with an MHS data expert, send an email to: (**dha.ncr.pcl.mbx.privacyboard@mail.mil**)

● Yes, then complete the questions below according to the data consult

○ No, then complete the questions below according to the best of your knowledge (NOTE: It is highly recommended that you work with an MHS data expert)

10.5 Indicate whether you plan to receive a data extract from the MHS or plan to access an information system directly to create a data set:

A data extract is when the MHS or a contractor provides the data set directly to the researcher. When receiving a data set through data extract, the researcher may indicate whether the data elements should be provided as is, encrypted or collapsed. In contrast to a data extract, access to an information system means that the researcher may directly access an MHS information system and create a data set for the research study

☐ Data Extract

☑ Access

10.6 Do you intend to use only de-identified data from the MHS in your research study?

There are different two methods for de-identifying data pursuant to HIPAA:
1) Safe Harbor Method: Removing all of the identifiers listed in Table 1 below, provided that the researcher does not have actual knowledge that the remaining data can be used alone or in combination with other information to identify the individual who is the subject of the information
2) Statistical Method: An expert, with appropriate knowledge of and experience with generally accepted statistical and scientific principles and methods for rendering information not individually identifiable, determines that the data is not individually identifiable

○ Yes ● No

10.7 If your research study requires access to an MHS information system, please indicate the system to obtain data:

If you do not know which system(s) contain the data elements you need, refer to the Guide for DoD Researchers on Using MHS Data or seek guidance from an MHS data expert:

PHI Systems:

MHS Information System	Requesting Data

: AHLTA	: Yes
: CHCS	: Yes
: ESSENTRIS	: Yes
: MDR	: Yes

PII-Only Systems:

MHS Information System	Requesting Data
No records have been added	

De-Identified Data & Other Systems:

Information System	Requesting Data
Expense Assignment System	
MHS Genesis; JLV (accessed via AHLTA or MHS Genesis)	: Yes
DMSS, DRSi	: Yes

10.8 Do you intend to merge or otherwise associate the requested data with data from any sources outside of the MHS, including other DoD systems that are not part of the MHS?

● Yes, will merge data
○ No, will not merge data

10.9 Indicate the categories of data that you will request from MHS systems or MHS health care providers about research participants or relatives, employers, or household members of the research participants.

Data Element(s)	MHS	Non-MHS Systems
1. Names	☑	☐
2. Postal address with only town, city, state and zip code	☑	☐
3. Postal address with all geographic subdivisions smaller than a state, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of Census: 1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and 2) the initial three digits of a zip code for all such geographic units containing	☑	☐

20,000 or fewer people is changed to 000		
4. Dates including all elements (except year) directly related to an individual, including birth date, admission date, discharge date, and date of death	☑	☐
5. Ages over 89 and all elements of dates (including year) indicative of such age, unless you will only request a single category of “age 90 or older”	☑	☐
6. Telephone numbers	☑	☐
7. Fax numbers	☑	☐
8. Electronic mail addresses	☑	☐
9. Social Security numbers (SSNs)	☑	☐
10. Medical record numbers	☑	☐
11. Health plan beneficiary numbers	☑	☐
12. Account numbers	☐	☐
13. Certificate/license numbers	☐	☐
14. Vehicle identifiers and serial numbers, including license plate numbers	☐	☐
15. Device identifiers and serial numbers	☐	☐
16. Web Universal Resource Locators (URLs)	☐	☐
17. Internet Protocol (IP) address numbers	☐	☐
18. Biometric identifiers, including finger and voice prints	☐	☐
19. Full-face photographic images and any comparable images	☐	☐

20. Any other unique identifying number, characteristic, or code (DEERs ID, EDIPN, Rank)	☑	☐

If you are obtaining SSNs, provide a justification as to why and explain why a substitute cannot be used

We request to access potential subjects' medical records in order to evaluate them for recruitment for this protocol. PHI to be screened include (name, date of birth, social security number and health beneficiary numbers). These risks are no more than minimal because they are not greater than those ordinarily encountered by the patient during a medical visit.

Study investigators and personnel will review potential subjects' medical history to assess protocol eligibility. If the person consents to participate in the research study, the PHI will be stored in accordance with the protocol.

10.10 Is it possible that the data will become identifiable because of triangulation, a small cell size, or any unique data element(s)?

Triangulation means using different data elements that are not themselves identifiable but that when combined can be used to identify an individual. For example, triangulation would be using rank and race together to determine the identity of an individual with a particular health condition
Small cell size means that there are only a small number of eligible individuals that satisfy the category description. Guidance for acceptable cell size is available from the Centers for Medicare and Medicaid Services. For example, the rank category of four star generals with a particular diagnosis may be less than 30 so the rank category may need to be expanded to include lower ranks
A unique data element includes any unique features that are not explicitly enumerated in the categories of data in rows 1 – 19 of Table 1 above, but that could be used to identify an individual. Examples of unique data elements include: 1) a unique number, such as a medical record number or EDIPN; 2) a unique code, such as a diagnosis code or a bar code on an electronic health record; and 3) any unique characteristic, such as the rank of general or admiral, or a race or gender combined with another unique characteristic

● Yes, there is a reasonable possibility the data will become identifiable
○ No, there is no reasonable possibility the data will become identifiable

10.11 HIPAA Privacy Rule and Use of Protected Health Information in Research:

○ N/A – will not use or disclose protected health information (PHI)
○ HIPAA Authorization will be obtained
○ Use of a limited data set where a data use agreement will be obtained
● Waiver/alteration of HIPAA Authorization is being requested

10.12 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for this Study:

Include in this section the plan for acquiring data (both electronic and hard copy), access during the study, data/specimen storage and length of time stored, shipment/transmission, and the plan for storage and final disposition at the conclusion of the study. Describe any data agreements in place for accessing data within and/or outside of your institution (e.g., Data Sharing Agreement, Data Use Agreement, Business Agreements, etc.)

Human Biological Specimens

1. Intended Use of the Samples/Specimens/Data

Specimens will be processed and handled as appropriate following applicable MTF enhanced precaution and Infection Control SOPs. Methods for specimen collection, handling, and processing are described in their respective SOPs. Details of specimen storage, maintenance, and QA/QC are described in their respective SOPs.

Samples and data collected under this protocol will be analyzed to study the pathogenesis of disease and the host immune response to infection. Genetic testing on human specimens
will be conducted to evaluate the host (subject's) immune response to SARS-CoV-2 infection and its association with clinical outcomes. Additional genetic testing will examine viral genetic markers and transcription signatures as predictors of infection severity, clinical course, and outcomes. Genetic testing results will not be provided to participants.

This is a purely observational study. Any experimental treatments will be done under other protocols for which separate IRB review and approval will be obtained.

2. How Samples/Specimens/Data Will Be Stored

Access to research samples will be limited using locked facilities and monitored freezers. Samples and data will be stored using codes assigned by the investigators or their designee(s). Data will be kept in password-protected computers. Only investigators or their designee(s) will have access to the samples and data.

3. How Samples/Specimens/Data Will Be Stored & Tracked

Samples from individuals found infected with a novel pathogen / emerging infectious disease of interest will be archived and stored at an appropriate biosafety level facility.

Samples acquired as part of the protocol will be tracked using appropriate software, such as Freezer Works, a sample tracking, biorepository management and freezer inventory program.

4. What Will Happen to the Samples/Specimens/Data at the Completion of the Protocol?

In the future, other investigators may wish to study these samples and/or data. IRB approval will be sought prior to any sharing of samples/data. Any clinical information associated with the sample, with or without patient identifiers, will be shared after IRB approval has been obtained.Upon study closure, all samples and data will be de-identified in accordance with the DHA-approved Data De-Identification Plan.

5. What Circumstances Would Prompt the PI to Report to the IRB Loss or Destruction of Samples/Specimens/Data

Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB.

6. Storage and Tracking of Samples/Specimens/Data

Research use of data collected through this protocol will be coded and will not contain identifiers. These data will be coded with access maintained by a custodian not affiliated with the study team per IDCRP policy. Much of the subsequent research using the coded materials collected under the auspices of this study will be accomplishable through approved exempt or non-human use sub-studies. All research using the materials collected under this protocol will be conducted in accordance with an IRB-reviewed protocol. Data and specimens released for research use will be labeled by PIN only; data will be shared without any identifiers or links to individuals. The DCC custodian, the DCC Chief, will not share identifying information with investigators without explicit IRB approval.

A Defense Health Agency (DHA) Data Sharing Agreement will be implemented as needed to access MHS data. Appropriate data sharing agreements and other legal agreements will be implemented prior to sharing of data and specimens with collaborators.

10.13 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for Future Research:

If the study involves collecting, storing, or banking human specimens, data, or documents (either by the Investigator or through an established repository) for FUTURE research, address. How the specimens /data will be used, where and how data/specimens will be stored (including shipping procedures, storage plan, etc.), whether and how consent will be obtained, procedures that will fulfill subjects' request as stated in the consent, whether subjects may withdraw their data/specimens from storage, whether and how subjects may be recontacted for future research and given the option to decline, whether there will be genetic testing on the specimens, who will have access to the data/specimens, and the linkage, the length of time that data/specimens will be stored and conditions under which data/specimens will be destroyed

Establishment of a specimen repository for future clinical and laboratory investigation for future emerging infectious disease research:

The study aims to build a repository of biological specimens (e.g. whole blood, serum, and PBMCs, as well as nasopharyngeal fluid, sputum, bronchioalveolar lavage fluid, oropharyngeal fluid, saliva, urine, semen, and rectal swab specimens, etc.) from an observational, longitudinal study of emerging infectious diseases. Specimens may be stored in an appropriate biosafety level facility at a protocol-approved biorepository. These specimen repositories will be linked to an extensive de-identified clinical database.

In the future, this repository might be used in the identification and validation of biomarkers for severe infection, identification and molecular characterization of novel pathogens, development and evaluation of new diagnostic platforms, and/or evaluation of host factors that may be associated with susceptibility and outcome. Coupled with detailed clinical history and risk factor information gleaned through electronic medical record review, this repository will ultimately be an invaluable resource for future investigations of emerging infectious diseases in clinical as well as laboratory settings. Moreover, the infrastructure developed through these activities will serve as a broad-based platform for a diverse portfolio of relevant sub-studies into emerging infectious diseases and strategies for its control and prevention.

Study Time Line

This natural history core protocol will be open indefinitely, although sites may close once an epidemic has passed.

11.0
Statistical/Data Analysis Plan

11.1 Statistical Considerations:

List the statistical methods to be used to address the primary and secondary objectives, specific aims, and/or research hypotheses. Explain how missing data and outliers will be handled in the analysis. The analysis plan should be consistent with the study objectives. Include any sub-group analyses (e.g., gender or age group). Specify statistical methods and variables for each analysis. Describe how confounding variables will be controlled in the data analysis

Statistical Considerations

This is an enhanced surveillance protocol using clinical observations and laboratory testing, supplemented by additional lab testing for research purposes. The frequency and expansion of such outbreaks is unknown, and hence this protocol will be activated on contingencies in many cases. Data from a given outbreak will be compiled into a dataset for descriptive analyses. Risk factors will be identified retrospectively using odds ratios. Comparison between similar outbreaks may be performed if sufficient data allows. It is recognized that a proportion (i.e., not all) of eligible subjects will consent to participate in this study. As such, the limitations and generalizability of the study findings will be acknowledged in associated reports and manuscripts.

11.2 Sample Size Estimation:

As determined by exigencies of the outbreak, and resource availability. Please see Appendix A for sample size considerations for the online cohort.

11.3 Data Analysis Plan:

Data Analysis Plan

Student’s t test (or Wilcoxon rank-sum test if appropriate) on continuous variables and Pearson’s chi-square tests (or Fisher’s exact test if appropriate) for categorical variable to summarize key outcomes of interest. Multivariate regression techniques will be used to assess relationships between key variables adjusted for potential confounders.

A data analysis plan will be created to meet the following reporting requirements:

- During an outbreak, descriptive clinical and laboratory data will be summarized per patient on a regular basis as appropriate for all investigators, involved hospitals, the respective service's public health agencies, AFHSB, and DHA leadership as a part of the epidemiologic reporting requirements. It is recognized this data may not be fully cleaned. Aggregate data report will be prepared monthly.
- At conclusion of an outbreak, a summary report per outbreak will be prepared once all data is cleaned. This will take an estimated 90 days.

With respect to the analysis of the study data, the following objectives will be addressed, and more fully articulated, in a statistical analysis plan:

1) Describe natural history of clinical disease.

Data items related to disease duration include duration of fever and other predominant symptoms, and days from onset of symptoms until hospital discharge/return to normal activity. Each variable will be summarized by mean, standard deviation, median, inter-quartile range, minimum and maximum overall and also for each group within several groupings: age categories, adult vs. minor, gender, different race groups, index cases vs. secondary cases, military rank and/or nature of duties for those with active duty military duty status. We will also use symptom severity data from the Flu-PRO questionnaire to explore the extent to which ARI symptom severity scores differs across the cohort.

Adverse binary outcomes where the time to event may also be of interest: These include requirement for ICU admission, development of any super infection, development of oxygen requirement, mortality, etc. For each of these, we will first give a simple summary of the number and percent experiencing each outcome. We will also summarize by different subgroups as above.

2) Identify risk factors for infection and severe clinical course.

One set of analyses will treat laboratory-confirmed diagnosis as the outcome in logistic regression models, with the following covariates: age, sex, race, military rank, service branch, type of exposure, use of personal protective equipment, etc. Similar logistic regression models will be developed to determine predictors of severe clinical course, such as mortality.

Another set of analyses will treat illness duration as a quantitative endpoint to be used as the outcome variable in multiple linear regressions. The key endpoint will be the total symptom score considered at three different time points: at symptom onset, at 48 hours post symptom onset, and at its maximum value whenever that occurs. Other potential confounders to be included as covariates are: age, sex, race, and number of days since presumed exposure.
Complementary methods of subject recruitment and data collection within the study will address the following specific aims:

1. Among COVID-19 confirmed cases, compare risk factors (comorbidities, lifestyle factors, medications, exposure opportunities, general symptoms) for:
    1. Those who get hospitalized vs. those who do not get hospitalized
    2. Those who get hospitalized with a ventilator vs. those who do not get hospitalized and those hospitalized but not placed on a ventilator
    3. Those who get hospitalized in the ICU vs. those who do not get hospitalized and those hospitalized but not in the ICU
    4. Those who go on to die vs. those who do not die

1. Compare risk factors (comorbidities, lifestyle factors, medications, exposure opportunities, general symptoms) for infection among the total COVID-19 tested study population of COVID-19 confirmed cases (test positive) vs. confirmed non-cases (test negative).
2. Compare symptoms between various groups:
    1. Among the total COVID_19 tested study population, COVID-19 confirmed cases vs. confirmed non-cases
    2. Among COVID-19 confirmed cases for those:
        1. who get hospitalized vs. those who do not get hospitalized
        2. who get hospitalized in the ICU vs. those who are hospitalized, but not in the ICU
        3. who go on to die vs. those who do not die
3. Among the COVID-19-confirmed cases and an appropriate and hypothesis-specific randomly selected comparison group, using medical record data, identify comorbidities and medications that are associated with reduced occurrence of COVID-19 symptoms as well as with becoming infected with COVID-19.

The proposed analytic strategy will be to report crude (non-adjusted), age and sex-adjusted, and multivariable adjusted probability of hospitalization in those with and without the exposures of interest.

1. Calculate crude (non-adjusted) likelihood of hospitalization in demographic sub-populations of interest (e.g. by age strata, sex, and race/ethnicity)

2. Determine the age-specific prevalence of the major comorbidities (obesity, hypertension, asthma) separately by sex and race/ethnicity
3. The primary model will be logistic regression, calculating adjusted predicted prevalence of hospitalization for those with each comorbidity alone and comorbidity clusters (since comorbidities are correlated)
    1. A priori, evaluate whether risk associated with each comorbidity is similar for men and women and for African Americans vs others (interaction by sex; interaction by race).

Conduct separate models for the active duty population and the beneficiary populations

3) Characterize host immune response.

We will examine the relationship between factors, such as the immune response (e.g., serum antibody concentration) and pathogen infection kinetics, and disease progression, symptom severity, and clinical outcomes of interest. We will look at both cross-sectional relationships (i.e., among people) and within person relationships. For cross-sectional relationships, we will start by plotting the total symptom severity score against the immune response, separately for each of the four blood samples. We anticipate a monotonic relationship, but it is possible for a U-shaped relationship in some cases, i.e., illness severity is greater if there is a too small immune response or a too large one. We will develop regression models with the outcome of the symptom severity or clinical course on the chosen function of immune response to see if there is a statistically significant relationship. We will add covariates to control for possible confounders, such as age, race, sex, military status, etc. and others mentioned above.

4) Evaluate correlations between symptom severity, virologic/microbiologic characteristics, host immune response and clinical course/outcome.

We will consider two groups: subjects with versus without a severe clinical course, such as mortality. We will compare the proportions with a significant serum antibody 'response' between the two groups by contingency table chi-square tests. We anticipate that the categorization of immune response will be binary (i.e., proportion of individuals exceeding a given concentration of serum antibody), in which case we will be comparing a binomial probability between the two groups. These analyses will form the backbone for more elaborate multivariate analyses.

5) Develop diagnostic and prognostic tools.

Data and specimens from this protocol may be used in the development of diagnostic and prognostic assays for novel pathogens. For example, the collection of blood and other body fluids from individuals with laboratory-confirmed infection may aid in the development of novel assays with higher sensitivity and specificity. Additionally, the collection of longitudinal clinical characteristics in addition to clinical laboratory data may aid in the development of prediction models to determine risk factors for severe clinical course, such as mortality.

12.0 Participant Information

12.1 Subject Population:

As this is an observational study, subjects of all ages will be enrolled. Military Treatment Facilities see a diverse population including all genders and races, and a wide range of ages. This study will examine the epidemiology of novel pathogens / emerging infectious diseases, seek to identify risk factors for severe disease and elucidate mechanisms of host immunity to these pathogens.

Given the possibility of MTFs being used as surge capacity to support the care of individuals affected by an infectious disease outbreak, this study also allows for enrollment of anyone meeting the inclusion criteria and obtaining care at a participating MTF. Males and females of all ages who are symptomatic or asymptomatic individuals (at high/some risk of exposure) meeting inclusion criteria are eligible for participation.

Participation in the online-only study group is limited to adults only.

12.2 Age Range:

☑ 0-17
☑ 18-24

☑ 25-34
☑ 35-44
☑ 45-54
☑ 55-64
☑ 65-74
☑ 75+

12.3 Gender:

☑ Male
☑ Female

12.4 Special categories:

☑ Minors /Children - “You must also consider the requirements of 45 CFR 46 Subpart D and DoDI 3216.02, Enclosure 3, paragraph 7.d.”
☐ Students
☐ Employees - Civilian - “You must also consider the requirements of DoDI 3216.02, paragraph 7.e.”
☐ Employees - Contractor
☐ Resident/trainee
☐ Cadets /Midshipmen - “You must also consider the requirements of DoDI 3216.02, Enclosure 3, paragraphs 7.e. and 12.”
☑ Active Duty Military Personnel - “You must also consider the requirements of DoDI 3216.02, Enclosure 3, paragraph 7.e.”
☐ Wounded Warriors - “Depending on your intended subjects’ status, you may also need to consider the requirements of DoDI 3216.02, Enclosure 3, paragraph 7.e.”
☐ Economically Disadvantaged Persons - “You must also consider the requirements of 32 CFR 219.111 (b).”
☐ Educationally Disadvantaged Persons - “You must also consider the requirements of 32 CFR 219.111 (b).”
☐ Physically Challenged (Physical challenges include visual and/or auditory impairment)
☑ Persons with Impaired Decisional Capacity - “You must also consider the requirements of 10 USC 980.”
☐ Prisoners - “You must also consider the requirements of 45 CFR 46 Subpart C and DoDI 3216.02, Enclosure 3, paragraphs 7.b. and 7.c.”
☑ Pregnant Women, Fetuses, and Neonates
☐ Non-English Speakers
☐ International Research involving Foreign Nationals - Headquarters Review is necessary

12.5 Inclusion Criteria:

Order Number	Criteria
1	Inclusion Criteria: Individuals of any age who are eligible for care at an MTF and meet at least one of the following criteria: 1. **Laboratory-confirmed presence of the pathogen of interest** (e.g., SARS-CoV-2, influenza A /H7N9, etc.) ***OR*** 2. **Meet criteria for testing for the pathogen of interest, as identified per current CDC guidelines** at **https://www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html**
	Additional Inclusion Criteria for **Online Enrollment:**

2	1. An MHS Beneficiary who has been clinically tested for the pathogen of interest 2. Able to receive email communications and respond to web-based questionnaires 3. ≥18 years of age

12.6 Exclusion Criteria:

Order Number	Criteria
1	Individuals who decline participation in the study
2	Individuals who the study investigators believe are unable to comply with the requirements of the study

13.0

Recruitment and Consent

13.1 Identification and Selection of Subjects:

Given the dynamic nature of novel pathogens/emerging infectious diseases, there is no pre-defined target enrollment for the protocol. The main objective of this protocol is to recruit and enroll a representative proportion of cases of emerging infectious diseases to characterize the epidemiology and clinical characteristics of disease. Site study team members will engage with relevant clinical staff to identify eligible patients in inpatient wards, intensive care units, acute care clinics, and emergency departments. Participants will be identified through screening of medical records to identify individuals with the recent exposures and clinical manifestations of interest, and will be approached following the initial medical evaluation. The study will be described to patients, and their eligibility and willingness to participate will be assessed. For those with confirmed or suspected infection, room access may be limited and consent will be performed by study staff members with patient access.

Individuals presenting for care at MTFs, such as at clinics or in the inpatient and isolation setting, will be recruited and enrolled and will participate in serial collection of biological specimens over a 12-month period, with more intensive (i.e., daily, weekly) data collection occurring during the inpatient or isolation period. Information on current and prior medical conditions and hospitalizations will be collected via medical chart review and/or MDR data extractions. Information on specific epidemiologic risk factors during or related to deployment (e.g., travel history, zoonotic exposures, etc.) will be ascertained through participant interview or review of available medical records (e.g., AHLTA-T, ALTHA, CHCS, Essentris, MHS Genesis, JLV (accessed via AHLTA or MHS Genesis), MDR, DMSS, and/or DRSi). Study participants evaluated at MTFs and who are either 1. ambulatory cases advised to return home and remain home in isolation or 2. classified as asymptomatic individuals with high/some risk for exposure may also be evaluated for follow up through a study design including serial self-collection of nasal swabs, oropharyngeal swabs, rectal swabs, or other specimens appropriate for self-collection. A similar schedule of data collection and methods used for enrolled inpatients will be applied for ambulatory cases (non-hospitalized) and asymptomatic individuals as feasible.

Details on the recruitment, consent, and enrollment of subjects participating through the MHS-wide online process are included in Appendix A.

13.2 Recruitment Process:

Recruitment of study volunteers at the MTFs will rely heavily on the direct interface of healthcare providers with potential eligible participants. The study team will develop an MTF communications plan, outlined in the protocol’s Manual of Procedures, that will be implemented by local site teams to facilitate identification of potential eligible participants. Study volunteers may be recruited from clinics and inpatient or isolation settings. In addition, recruitment of individuals who are asymptomatic at high/some risk of exposure may involve study team members approaching family or friends who escorted the sick individual to the hospital. Another group of individuals who may be approached for recruitment as asymptomatic high/some risk participants, are healthcare providers who are in close contact with and potentially exposed to known cases. Adults and children are eligible for participation.

The study team will use multiple means of communicating study awareness to enhance recruitment efforts, including periodic briefings delivered by the site PI's and their study teams to their critical care and infectious disease staff and inpatient nursing staff. Information notices with points of contacts will be posted in clinical sites such as inpatient units, acute care clinics, and several points in the emergency room. Research team members who are Active Duty military personnel will not wear visible signs of their rank during recruitment. Site PI's or their authorized designees will conduct daily queries of MTF electronic health heath records (including infectious disease reportables list, DRSi, and searching in CHCS for patients with coronavirus lab code) to identify individuals who tested positive for disease of interest and will review daily admissions into the ICU, general pediatric and internal medicine wards with their study personnel to identify new inpatients. Information from infection control, preventive medicine and infectious disease departments may be obtained to find patients who may be managed / followed outside of the MTF.

J6 Data: Staff at the J6 CarePoint MHS Information Portal will provide subject identifiers (including names, Active Duty status, DoD Identifier, DOB, Email addresses, Date of Initial COVID-19 Respiratory Swab PCR Test, and all available COVID-19 Respiratory Swab PCR Test results) of AD members and beneficiaries who have been tested for COVID-19 to the approved research team using DoD SAFE. These data will be used to contact subjects for potential enrollment in the study and to contact subjects while enrolled. The original data file is retained only until a QC check determines the data have been uploaded successfully to REDCap, after which the file is destroyed. At study end, all PHI and PII shall be de-identified per the De-identification Plan.

Depending on age, severity of infection, and MTF requirements, study staff will determine if a potential subject is capable and/or allowed to provide a signed consent, verbal consent, or if an LAR is required according to IRB, HIPAA, and local MTF requirements.

13.3 Compensation for Participation:

In accordance with 24 USC 30:

MTF-enrolled subjects will be provided compensation in the form of a gift card. Subjects enrolled in the online-only group will receive compensation in the form of an electronic gift code sent via email.

Subjects will receive:

- $50 for each outpatient venipuncture
- $10 for each self-collected sample completed at home
- $10 for completion of each online questionnaire
- $10 for completion of 14-day Flu-PRO symptom diary

13.4 Eligibility Assessment Process:

As part of medical record review, the team will assess the eligibility of potential subjects. Individuals identified as having known risk factors for an emerging infectious disease (e.g., recent travel to a disease-endemic region, or close contact with a known or suspected infected individual or a returning traveler) who present to a participating MTF for medical care (e.g., febrile illness) or are placed into isolation are eligible for study participation. Additionally, asymptomatic high/some risk for exposure individuals may be enrolled at MTFs. Given the possibility of MTFs being used as surge capacity to support the care of individuals affected by an infectious disease outbreak, this study allows for enrollment of anyone meeting the inclusion criteria and obtaining care at a participating MTF. Eligibility will be assessed and a final determination of subject eligibility will be made during the consent process. Please refer to section 12.5 for the inclusion criteria.

13.5 Consent Process:

Are you requesting a waiver or alteration of informed consent?

● Yes ○ No

What type?

☑ Waiver of documentation of informed consent
☐ Waiver or alteration of informed consent

Please explain why you qualify for a waiver or alteration of informed consent:

Due to the contagious nature of the emergent infectious diseases being studied under this protocol, study staff may not always be allowed work near the potential and/or active study subjects or handle any items that they may have come in contact with per local MTF infection control policies. Therefore, obtaining signed ICDs and HIPAA Authorizations for subjects enrolled in this study may not always be feasible or safe for study staff. The study team is requesting a waiver of documentation of informed consent to allow for verbal subject/LAR informed consent, an Alteration of Authorization of HIPAA to allow for verbal subject/LAR HIPAA authorization, as well as a partial Waiver of HIPAA Authorization to identify eligible potential subjects and make initial contact with individuals to notify them regarding the study. These will be used as appropriate based on the circumstances of the disease being studied and MTF requirements. This study falls under the following criteria noted in 32 CFR 219.117: “That the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context."

Please explain the consent process:

ICD and Assent Regulatory Requirements

The Informed Consent Document (ICD) and Assent Form, including a HIPAA authorization, will be reviewed and approved by the IRB prior to initiation of the study. The ICD will contain a full explanation of the possible advantages and risks of study participation. The ICD will be compliant with applicable DoD regulations, 32 CFR 219, and the Belmont Principles, and the Health Insurance Portability and Accountability Act (HIPAA) Authorization. Informed consent includes the principle that it is critical the participant be informed about the principal potential risks and benefits. This information will allow the participant to make a personal risk versus benefit decision and understand the following general principles:

- Participation is entirely voluntary,
- Participants may withdraw from participation at any time,
- Refusal to participate involves no penalty; and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

An ICD or Assent Form (with parental permission) will be signed by the participant before any study-related procedures are initiated for each participant. The investigators or their designees will present the protocol in lay terms to the participants. If access is limited, an authorized member of the study team accompanied by a witness may conduct the informed consent process. Questions on the nature of the protocol, the means by which it is to be accomplished, and the risks to the participants will then be solicited. Any question that cannot be answered will be referred to the principal investigator (PI). All participants will be given the opportunity to ask questions. No participant should grant consent until questions have been answered to his/her satisfaction. Documented parental permission will be received prior to obtaining assent from a minor. If rules allow, a copy should be provided to the volunteer or their guardian.

Minors:

An assent form (with parental permission) will be used for children aged 7-17. The ICD and Assent Form (with parental permission) indicate that by signature, the participant permits access to relevant medical records by the study investigators and regulatory authorities. Documented parental permission is required prior to assent by a minor using the ICD. A Waiver of Documentation of Assent will be used for children 6 and under, documenting that the study was explained to the child in age-appropriate terms and that the child has either expressed verbal assent or does not have the capacity to do so.

For adults who are incapacitated by the illness:

Consent may be obtained by a Legally Authorized Representative (LAR). The patients should be consented themselves at such time that they are able to provide consent.

For Hospital Staff who are Active Duty Military:

Hospital staff who are active duty military, and therefore eligible for care at a military treatment facility, may be approached for consenting to the study due to their potential exposure to coronavirus. If hospital staff who are active duty military are approached for participation in the study, supervisors will not be present at any human subject recruitment sessions or during the consent process in which hospital staff who are active duty military under their supervision are afforded the opportunity to participate as human subjects. Supervisors (e.g., military and civilian supervisors or anyone in the supervisory structure) will not influence the decisions of their subordinates regarding their participation as subjects in this study.

Verbal Informed Consent:

Due to the contagious nature of the emergent infectious diseases being studied under this protocol, study staff may not always be allowed work near the potential and/or active study subjects or handle any items that they may have come in contact with per local MTF infection control policies. Therefore, obtaining signed ICDs and HIPAA Authorizations for subjects enrolled in this study may not always be feasible or safe for study staff.

The study team is requesting a waiver of documentation of informed consent, an Alteration of HIPAA Authorization, as well as a partial Waiver of HIPAA Authorization to use as appropriate based on the circumstances of the disease being studied and MTF requirements. When possible, the subject will provide signed ICD and HIPAA authorization. If the subject is unable to provide signed authorizations, the Alteration of HIPAA Authorization will be utilized, allowing the subject/LAR to provide verbal, rather than written, HIPAA Authorization. If study personnel are unable to work near the potential and/or active subject for safety reasons, the study personnel will contact the patient according to the requirements of the MTF. Options may include but are not limited to speaking with the patient from a safe distance in person, contacting the patient by phone in person with a barrier in between the potential and/or active subject and the study personnel, contacting the patient by phone from a separate location, or having a study physician trained in infectious disease safety procedures meet with the potential and/or active subject in close proximity (if allowed by the MTF).

Regardless of the method of delivery, the potential and/or active subject would be thoroughly briefed on the study’s Informed Consent and HIPAA information. They would also be provided with an opportunity to ask any questions and an unsigned copy of the informed consent if permissible by the MTF. In turn, study staff will thoroughly document the informed consent process and the subject’s verbal consent in the study chart. The study personnel will sign and file the ICD. The study personnel will also include a note in the study chart summarizing the informed consent process.

13.6 DoDI 3216.02 requires an ombudsman to be present during recruitment briefings when research involves greater than minimal risk and recruitment of Service members occurs in a group setting. If applicable, you may nominate an individual to serve as the ombudsman.

● N/A
○ Propose ombudsman

13.7 Withdrawal from Study Participation:

Explain the process for withdrawal and specify whether or not the subjects will be given the opportunity to withdraw their data their data/specimens in the event they wish to withdraw from the study

A subject may voluntarily end participation in the study at any time. If a subject withdraws, the investigator will make a reasonable effort to determine the reason for the subject’s withdrawal from the study. Telephone calls, registered letters, and/or e-mail correspondence will be considered reasonable effort. Data and specimens collected up to the time of withdrawal will be retained and de-identified in accordance with IRB, HIPAA, and Safe Harbor requirements.

Subjects may be discontinued by the study team if there is a determination by the on-site physician that the volunteer may be unable to comply with the requirements of the study, or in the interest of the military mission.

The study team may consider discontinuation of study participants who have a negative test result for the pathogen of interest (based on current standard of care diagnostics) *and/or* are diagnosed with another respiratory pathogen other than the one of interest *and/or* the disease of interest is highly unlikely based on the primary clinical team assessment. This approach may need to be taken to conserve study staff time and resources depending upon local epidemiologic circumstances, e.g. very high incidence and subject accrual.

14.0
Risks and Benefits

14.1

Risks of Harm:

Identify all research-related risks of harm to which the subject will be exposed for each research procedure or intervention as a result of participation in this study. Consider the risks of breach of confidentiality, psychological, legal, social, and economic risks as well as physical risks. Do not describe risks from standard care procedures; only describe risks from procedures done for research purposes

Risks associated with obtaining specimens:

- Obtaining specimens could be associated with discomfort from a needle stick, swab, or other procedure, and occasionally associated with bleeding, bruising, local or systemic infection.
- Genetic testing may reveal sensitive information that subjects may not want disclosed, and may cause some emotional distress for the participant.

Risks associated with privacy:

- Despite meticulous precautions, breaches of confidential information (including genetic information) do occasionally occur, although the chances of such a breach are unlikely.

Expected Adverse Events from Research Risks and Reporting

a. Rare (Event Rate < 1%)
Secondary infection and syncope from vasovagal reactions occur from blood collection in rare cases. Risks of nasopharyngeal swab, rectal swab, cheek swab could include bleeding or mucous membrane irritation.

b. Less Likely (1% ≤ Event Rate < 5%)
No known risks

c. Likely (5% ≤ Event Rate < 10%)
Bruising and bleeding may occur as a result of blood collection procedures.

d. More likely (Event Rate ≥10%)
No known risks

14.2

Measures to Minimize Risks of Harm (Precautions, safeguards):

For each research procedure or intervention, describe all measures to minimize and/or eliminate risk of harms to subjects and study personnel

Participation in this protocol will impose minimal risks to the participant. As feasible, the study team will coordinate the collection of research purpose blood draws during timing of blood collection for clinical care to minimize discomfort to the patient. Blood volume collection per visit will be approximately 25 mL total. Total blood volumes collected over the 12 month duration of study will not exceed limitations for adults and children outlined in the CFR §46.110: Expedited review procedures for certain kinds of research involving no more than minimal risk, and for minor changes in approved research. Blood draw volumes will be reduced for pediatric subjects, small adults, and pregnant women in accordance with minimal risk and safety guidelines, and as needed per guidance from the primary care provider overseeing care of enrolled patients.

Staff safety in sample collection and handling is a concern that will be closely monitored by the Infection Control staff at the facility. Risk to staff is mitigated by minimizing an additional phlebotomy beyond that being done for clinical care. Specimen collection should be harmonized with the clinician’s requirements, and missed samples will not be re-drawn.

Unanticipated problems involving risk to subjects or others (UPIRTSO) may also occur that are not related to a described harm (adverse event) to study subject, rather a potential harm (such as loss of accountability of PII/PHI) or risks to others including medical staff. Specifically in this study is a concern that collection of infection fluids may cause harm to study personnel. Collection of samples for this study must only be conducted by individuals trained and authorized to collect and handle serious biohazardous specimens wearing proper personal protective equipment (PPE). PPE training will be conducted and documented for all personnel who may interact directly with patients and/or samples in order to mitigate any UPIRTSO related to accidental exposure of study staff and other individuals in the facility.

14.3
Confidentiality Protections (for research records, data and/or specimens):

Describe in detail the plan to maintain confidentiality of the research data, specimens, and records throughout the study and at its conclusion (e.g., destruction, long term storage, or banking). Explain the plan for securing the data (e.g., use of passwords, encryption, secure servers, firewalls, and other appropriate methods). If data will be shared electronically with other team members/collaborators outside the institution, describe the method of transmission and safeguards to maintain confidentiality. Explain whether this study may collect information that State or Federal law requires to be reported to other officials or ethically requires action, e.g., child or spouse abuse

A certificate of confidentiality will not be used in this protocol.

The only electronic links established between the participant name, SSN, and the Study ID number will be in the data management system managed by DCC and on a tracking spreadsheet used for scheduling purposes, which will both require a password to access and will only be accessed by authorized study personnel. During the study, source documents will be held in a secured cabinet with restricted access. Team members with access to the documents are counseled on the importance of confidentiality of participant medical records. All staff involved in this study have completed the required human subjects protection and ethics training. In all electronic records for data analysis, the participant is not referenced by name, but only Study ID number. This method is designed to protect the privacy of study participant medical information. All CRFs will be held at the individual study sites in the research spaces in secured cabinets with restricted access.

The only hard copy link between participant identifiers and Study ID will be on the Registry Form and Contact Information Form. This will be kept in a secure location separate from source document files. Any copies faxed to DCC personnel acting as a backup Registrar will be shredded after registration is complete.

In order to determine whether an individual has been enrolled previously at another study site, study staff at all active sites will have the ability to query DCC's data management system to locate an individual. The data management system is secure and can only be accessed via authorized individuals using a password.

What precautions will you take to protect the confidentiality of research source documents (Case Report Forms, questionnaires, etc.), the research data file, and the master code (if any)?

The only hard copy link established between the participant's name, SSN, and the study code number will be held in a secured cabinet with access restricted to research team members with a need to use the Registry Form or Contact Information Form. Team members with access to CRFs are counseled on the importance of confidentiality of participant medical records. In all electronic records for data analysis, the participant is not referenced by name, but only study code number.

The only electronic link will be stored on a secure password protected server. The file is only accessible directly to the DCC data custodian. The designated CRC at each site and two backups at DCC will also have access via DCC's data management system.

Will research data including Identifiable Protected Health Information be sent outside of Department of Defense facilities?

The study team may share coded data with collaborators at non-DoD facilities, and may work with participating MTFs to assist with appropriate public health data reporting requirements.

14.4
Potential Benefits:

Describe any real and potential benefits of the research to the subject and any potential benefits to a specific community or society

If the individuals in the research are considered experimental subjects (per 10 USC 980), and they cannot provide their own consent, the protocol must describe the intent to directly benefit all subjects

This is an observational study that does not test interventions that would offer direct benefit to study volunteers.

Study volunteers may indirectly benefit from enhanced clinical testing, physical examination, radiographic studies, and longitudinal follow up, or improved access to related trials of therapeutics.

14.5 Privacy for Subjects:

Describe the measures to protect subject's privacy during recruitment, the consent process, and all research activities, etc.

When possible, all means necessary will be taken to ensure participant privacy. This may entail conducting study procedures in a private area and keeping study documents coded. Non-coded study documents will be kept in a locked drawer and/or locked file in a locked room. Subjects will disclose information at their consent, if subjects choose not to disclose information, the study team will respect subject privacy and not inquire further.

14.6 Incidental or Unexpected Findings:

Describe the plan to address incidental findings and unexpected findings about individuals from screening to the end of the subject's participation in the research. In cases where the subject could possibly benefit medically or otherwise from the information, state whether or not the results of screening, research participation, research tests, etc., will be shared with subjects or their primary care provider. State whether the researcher is obligated or mandated to report results to appropriate military or civilian authorities and explain the potential impact on the subject

The study team will share relevant findings from research labs/imaging with the patient's healthcare provider(s).

Aspects of this study may include epidemiologic reporting to AFHSB and public health authorities at the respective services, state and local health departments, and CDC. That data transfer is not subject to Data Use Agreement or HIPAA requirements.

15.0 Study Monitoring

15.1 Data Monitoring Plan:

Describe the plan to monitor the data to verify that data are collected and analyzed as specified in the protocol. Include who will conduct the monitoring, what will be monitored and the frequency of monitoring

The supervising principal investigator, or designated associate investigator, will be responsible for regular maintenance and review of CRFs for accuracy. Access to study files (e.g., CRFs) is restricted to care providers and research team members given specific tasks to perform. The files are kept in a secured location in each study site. Some participant-completed forms such as questionnaires may be completed through the web-based program. These will be transmitted directly into the study database. Research labs will transmit their testing data to the DCC using standard procedures for data handling and transmission.

During the study, the investigator will review study data from their site, including medical records, records detailing the progress of the study for each volunteer, laboratory reports, CRFs, signed ICD and authorization forms for each study volunteer, correspondence with the IRB, adverse event (AE) reports and information regarding volunteer discontinuation of the study. All required study data will be clearly and accurately recorded on source documents or CRFs by either the participant themselves (questionnaires) or authorized study personnel. Only designated study personnel shall record or change data on a CRF. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded on the CRFs. Original observations entered directly onto the CRFs are defined as source data. A Quality Management Plan (QMP) will be used for this study. The QMP will include a QC subject data review checklist, a QC Subject data review checklist, as well as automatic queries from the electronic database based on expected parameters for data entry, noting possible inconsistencies or omissions. The QC subject data review checklist will be completed at each site on 100% of subject’s study files. This will be completed by another member of the study team who did not complete the original source document or CRF. A QA subject data review checklist will also be used on a small subset of study records at each site to ensure that quality management processes are being conducted appropriately for the study. QA review will be conducted by an individual on the study who is not involved in the day-to-day activities of the study.

Responsibilities of the Data Coordination Center (DCC)

1. Provides review of protocol, CRFs, data security plan and data management SOPs
2. Complies with protocol, SOPs, and all federal and local regulations and policies
3. Develops clinical database
4. Entry and quality control of CRF based study data
5. Receipt and management of specimen tracking and laboratory data
6. Provides study database to IDCRP Program Coordination Center (PCC)
7. Locks final study database

15.2 Safety Monitoring Plan:

Describe the plan to monitor the data to ensure the safety of subjects

Participation in this protocol will impose minimal risks to the participant. Staff safety in sample collection and handling is a concern that will be closely monitored by the Infection Control staff at the facility. Risk to staff is mitigated by minimizing an additional phlebotomy beyond that being done for clinical care. Specimen collection must be harmonized with the clinician’s requirements, and missed samples will not be re-drawn. Proper safety training, following hospital Infection Control requirements, of study team members who come in contact with study participants will be implemented to mitigate disease transmission risk.

Termination of study participation will take place through 1) an expressed desire by the volunteer to terminate participation, 2) a determination by the on-site physician that the volunteer risks his/her health by further participation in the study, or 3) in the interest of the military mission.

15.3 Does your study require independent data and safety monitoring?

○ Yes ● No

16.0 Reportable Events

16.1 Reportable Events:

Consult with the research office at your institution to ensure requirements are met

• Describe plans for reporting expected adverse events. Identify what the expected adverse events will be for this study, describe the likelihood (frequency, severity, reversibility, short term management and any long term implications of each expected event)
• Describe plans for reporting unexpected adverse events and unanticipated problems. Address how unexpected adverse events will be identified, who will report, how often adverse events and unanticipated problems will be reviewed to determine if any changes to the research protocol or consent form are needed and the scale that will be used to grade the severity of the adverse event

Reporting Protocol Deviations

The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or study-specific procedure. Investigators will report protocol deviations to the USU IRB within 2 business days that:

- relate to participant safety
- relate to the informed consent process
- are any other protocol deviations which, in the opinion of the PI, should be promptly reported to the IRB.

A log of all deviations will be reported annually in the continuing review report to the IRB and in the Final Study Report.

Reportable events will also be submitted to reporting site's regulatory authorities as required.

Reporting Adverse Events and Serious Adverse Events

In this observational, no-greater-than-minimal risk study, the reporting of adverse events is limited to an analysis at the time of annual reporting. Prompt reporting is required for any related serious adverse event or unanticipated problems involving risk to subject or others (UPIRTSO).

An adverse event is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

AEs that meet all of the following criteria will be recorded by the study team and reported to the IRB at the annual Continuing Review:

- At least possibly related to study procedures
- Unexpected (i.e. not included in the list of known risks in section 14)
- Moderate or greater severity

Additional AEs may be reported if the determined by the study site investigator to be of sufficient significance to require reporting to the IRB.

AEs are recorded and inspected by the supervising physician investigator to determine severity and relatedness to participation (definite, probable, possible, or unlikely). The physician investigator is responsible for directly managing or coordinating management of adverse events.

Reporting unanticipated problems involving risk to subjects or others

Unanticipated problems involving risk to subjects or others (UPIRTSO) may also occur that are not related to a described harm (adverse event) to study subject, rather a potential harm (such as loss of accountability of PII /PHI) or risks to others including medical staff. Specifically in this study is a concern that collection of infection fluids may cause harm to study personnel. Collection of samples for this study must only be conducted by individuals trained and authorized to collect and handle serious biohazardous specimens wearing proper personal protective equipment. Any sample collection for research purposes (as compared to clinical care) that results in an exposure to clinical staff is considered a UPIRTSO.

All UPIRTSOs will be collected and reported to the IRB within 24 hours of the PI recognition of the problem.

17.0

Equipment/non-FDA Regulated Devices

17.1 Does the study involve the use of any unique non-medical devices/equipment?

○ Yes ● No

18.0
FDA-Regulated Products

18.1 Will any drugs , dietary supplements, biologics, or devices be utilized in this study?

☐ Drugs
☐ Dietary Supplements
☐ Biologics
☐ Devices
☑ N/A

18.5 Sponsor (organization/institution/company):

☐ N/A

If applicable, provide sponsor contact information:

Infectious Diseases Clinical Research Program
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences
11300 Rockville Pike, Suite 1211
Rockville, MD 20852

19.0
Research Registration Requirements

19.1 ClinicalTrials.gov Registration:

● Registration is not required
○ Registration pending
○ Registration complete

19.2 Defense Technical Information Center Registration (Optional):

● Registration is not required
○ Registration pending
○ Registration complete

20.0
References and Glossary

20.1 References:

1. Van Kerkhove MD, Mumford E, Mounts AW, Bresee J, Ly S, Bridges CB, et al. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. PloS one. 2011;6 (1):e14582. Epub 2011/02/02. doi: 10.1371/journal.pone.0014582. PubMed PMID: 21283678; PubMed Central PMCID: PMC3025925.

2. WHO. Weekly Epidemiological Record: Update on human cases of highly pathogenic avian influenza A (H5N1) virus infection, 2011. Geneva, Switzerland: World Health Organization, 2012 30 Mar. Report No.
3. Cowling BJ, Jin L, Lau EH, Liao Q, Wu P, Jiang H, et al. Comparative epidemiology of human infections with avian influenza A H7N9 and H5N1 viruses in China: a population-based study of laboratory- confirmed cases. Lancet. 2013;382 (9887):129-37. Epub 2013/06/28. doi: 10.1016 /s0140-6736 (13) 61171-x. PubMed PMID: 23803488; PubMed Central PMCID: PMC3777567.
4. WHO. WHO Risk Assessment: Human infections with avian influenza A (H7N9) virus. Geneva, Switzerland: World Health Organization, 2014 Oct 2. Report No.
5. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England journal of medicine. 2012;367 (19):1814-20. Epub 2012/10/19. doi: 10.1056/NEJMoa1211721. PubMed PMID: 23075143.
6. Assiri A, McGeer A, Perl TM, Price CS, Al Rabeeah AA, Cummings DA, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. The New England journal of medicine. 2013;369 (5):407-16. Epub 2013/06/21. doi: 10.1056/NEJMoa1306742. PubMed PMID: 23782161; PubMed Central PMCID: PMC4029105.
7. Memish ZA, Zumla AI, Al-Hakeem RF, Al-Rabeeah AA, Stephens GM. Family cluster of Middle East respiratory syndrome coronavirus infections. The New England journal of medicine. 2013;368 (26):2487- 94. Epub 2013/05/31. doi: 10.1056/NEJMoa1303729. PubMed PMID: 23718156.
8. Breban R, Riou J, Fontanet A. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. Lancet. 2013;382 (9893):694-9. Epub 2013/07/09. doi: 10.1016/s0140-6736 (13)61492-0. PubMed PMID: 23831141.
9. Cauchemez S, Fraser C, Van Kerkhove MD, Donnelly CA, Riley S, Rambaut A, et al. Middle East respiratory syndrome coronavirus: quantification of the extent of the epidemic, surveillance biases, and transmissibility. The Lancet Infectious diseases. 2014;14 (1):50-6. Epub 2013/11/19. doi: 10.1016/s1473-3099 (13)70304-9. PubMed PMID: 24239323; PubMed Central PMCID: PMC3895322.
10. Hemida MG, Chu DK, Poon LL, Perera RA, Alhammadi MA, Ng HY, et al. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis. 2014;20 (7):1231-4. Epub 2014/06/26. doi: 10.3201/eid2007.140571. PubMed PMID: 24964193; PubMed Central PMCID: PMC4073860.
11. WHO. Summary of probably SARS cases with onset of illness from 1 November 2002 to 31 July 2003 Switzerland, Geneva: World Health Organization; 2003 [cited 2014 Oct 31]. Available from: http://www. who.int/csr/sars/country/table2004_04_21/en/.
12. Ge X-Y, Li J-L, Yang X-L, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013;503 (7477):535-8. doi: 10.1038/nature12711.
13. WHO. Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). Geneva, Switzerland: World Health Organization, Response DoCDSa; 2003.
14. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. The New England journal of medicine. 2003;348 (20):1967-76. Epub 2003/04/12. doi: 10.1056/NEJMoa030747. PubMed PMID: 12690091.
15. WHO. Coronavirus Disease Situation Report, 2020. Geneva, Switzerland: World Health Organization, 2020 Mar 2. Report No 42. Available from: https://www.who.int/docs/default-source /coronaviruse/20200302-sitrep-42-covid-19.pdf?sfvrsn=d863e045_2
16. CDC. Coronavirus Disease 2019 (COVID-19) in the U.S. https://www.cdc.gov/coronavirus/2019-ncov/cases-in-us.html. Accessed 03 March 2020
17. Wei-jie G., Zheng-yi N., Yu H., Wen-hua L., Chun-quan O., Jian-xing H., et al. Clinical Characteristics of Coronavirus Disease 2019 in China. 2020. Epub 2020/02/28. The New England Journal of Medicine. doi: 10.1056/NEJMoa2002032
18. del Rio C, Malani PN. COVID-19—New Insights on a Rapidly Changing Epidemic. JAMA. Published online February 28, 2020. doi:10.1001/jama.2020.3072
19. Liu W, Tao ZW, Lei W, Ming-Li Y, Kui L, Ling Z, Shuang W, Yan D, Jing L, Liu HG, Ming Y, Yi H. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chin Med J (Engl). 2020 Feb 28. doi: 10.1097/CM9.0000000000000775.
20. CDC. How COVID-19 Spreads. 2020. https://www.cdc.gov/coronavirus/2019-ncov/about /transmission.html
21. Wei Zhang, Rong-Hui Du, Bei Li, Xiao-Shuang Zheng, Xing-Lou Yang, Ben Hu, Yan-Yi Wang, Geng-Fu Xiao, Bing Yan, Zheng-Li Shi & Peng Zhou (2020) Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes, Emerging Microbes & Infections, 9:1, 386-389, DOI: 10.1080/22221751.2020.1729071
22. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirglmaier K, Zange S, Wolfel R, Hoelscher M. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N Engl J Med. 2020. Epub 2020/02/01. doi: 10.1056/NEJMc2001468.
23. Chan JF Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet (London, England). 2020. Epub 2020/01/28. doi: 10.1016/s0140-6736 (20) 30154- 9

24. Powers JH, Bacci ED, Leidy NK, Poon JL, Stringer S, Memoli MJ, Han A, Fairchok MP, Coles C, Owens J, Chen WJ, Arnold JC, Danaher PJ, Lalani T, Burgess TH, Millar EV, Ridore M, Hernández A, Rodríguez-Zulueta P, Ortega-Gallegos H, Galindo-Fraga A, Ruiz-Palacios GM, Pett S, Fischer W, Gillor D, Moreno Macias L, DuVal A, Rothman R, Dugas A, Guerrero M. Performance of the inFLUenza Patient-Reported Outcome (FLU-PRO) diary in patients with influenza-like illness (ILI). PLoS One. 2018 Mar 22; 13(3):e0194180.
25. Coles C, Millar EV, Burgess T, Ottolini MG.The Acute Respiratory Infection Consortium: A Multi-Site, Multi-Disciplinary Clinical Research Network in the Department of Defense. Mil Med. 2019 Nov 1;184 (Supplement 2):44-50. doi: 10.1093/milmed/usz174.
26. Han A, Poon JL, Powers JH, Leidy NK, Yu R, Memoli MJ. Using the Influenza Patient-reported Outcome (FLU-PRO) diary to evaluate symptoms of influenza viral infection in a healthy human challenge model. BMC Infect Dis. 2018 Jul 28;18(1):353. doi: 10.1186/s12879-018-3220-8.
27. Yu J, Powers JH, Vallo D, Falloon J. Evaluation of Efficacy Endpoints for a Phase IIb Study of a Respiratory Syncytial Virus Vaccine in Older Adults Using Patient-Reported Outcomes With Laboratory Confirmation. Value Health. 2020 Feb;23(2):227-235. doi: 10.1016/j.jval.2019.09.2747.

20.2 Abbreviations and Acronyms:

CoV - Coronavirus

AE - Adverse Event

AFHSB - Armed Forces Health Surveillance Branch

AHLTA - Armed Forces Health Longitudinal Technology Application

AIDS/HIV - Acquire Immunodeficiency Syndrome/Human Immunodeficiency Virus

APTT - Activated Partial Thromboplastin Time

ARI - Acute Respiratory Infection

ALT/SGPT - Alanine Aminotransferase/Serum Glutamic Pyruvic Transaminase

AST/SGOT - Aspartate Aminotransferase/Serum Glutamic Oxaloacetic Transaminase

BSL - Biosafety Level

CDC - Centers for Disease Control and Prevention

COVID-19 - Coronavirus Disease 2019–

CRC - Clinical Research Coordinator

CRF - Case Report Form

DARPA - Defense Advanced Research Projects Agency

DCC - Data Coordination Center

DEERS - Defense Enrollment Eligibility Reporting System

DHA - Defense Health Agency

DoD - Department of Defense

DoDSR - DoD Serum Repository

eIND - Emergency Investigational New Drug

EVD - Ebola Virus Disease

FDA - Food and Drug Administration

GCP - Good Clinical Practice

GEIS - Global Emerging Infections Surveillance and Response System

HIPAA - Health Insurance Portability and Accountability Act

HRPP - Human Research Protection Program

ICD - Informed Consent Document

ICU - Intensive Care Unit

ICH - International Conference on Harmonization

IDCRP - Infectious Disease Clinical Research Program

IFN - Interferon

Ig - Immunoglobulin

IL - Interleukin

IND - Investigational New Drug

IRB - Institutonal Review Board

ISARIC - International Severe Acute Respiratory Infection Consortium

MCP - Macrophage Chemo-attractant Protein

MDR – Military Health System Data Repository

MERS-CoV - Middle East Respiratory Syndrome-Coronavirus

MHRP - Military HIV Research Program

MIP - Macrophage Inflammatory Protein

MTF - Military Treatment Facility

NIAID - National Institute of Allergy and Infectious Diseases

NIH - National Institutes of Health

NMCP - Naval Medical Center Portsmouth

NMCSD - Naval Medical Center San Diego

OCRPRO - Office of Clinical Research Policy and Regulatory Operations

OHRP - Office for Human Research Protections

OUA - Operation United Assistance

PBMC - Peripheral Blood Mononuclear Cell

PCC - Program Coordination Center

PCR - Polymerase Chain Reaction

PHD - Predicting Health and Disease

PI - Principal Investigator

PHI - Protected Health Information

PII - Personally Identifiable Information

PPE - Personal Protective Equipment

PTT - Partial Thromboplastin Time

PUI - Person under Investigation

RCT - Randomized Controlled Trial

RNA - Ribonucleic Acid

SAE - Serious Adverse Event

SARI - Secure Acute Respiratory Infection

SARS - Severe Acute Respiratory Syndrome

SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2

SOP - Standard Operating Procedure

SPL - Specimen Processing Laboratory

SSN - Social Security Number

Study ID - Study Identification Number

TNF - Tumor Necrosis Factor

UPIRTSO - Unanticipated Problems Involving Risk to Subjects or Others

US - United States

USU - Uniformed Services University of the Health Sciences

WHO - World Health Organization

INFORMED CONSENT DOCUMENT FOR AN OBSERVATIONAL COVID-19 RESEARCH STUDY: IDCRP-085: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)

Thank you for your interest in learning more about this study. Please read the following information to ensure that you would like to participate. The Department of Defense has sponsored this research study, led by the Infectious Disease Clinical Research Program at the Uniformed Services University of the Health Sciences (the military's medical school), to better understand risks and illness caused by the novel Coronavirus disease (**COVID-19**).The COVID-19 pandemic is a global public health emergency. We are asking you to consider volunteering for this research study because you may have COVID-19 and/or qualify for COVID-19 testing according to the Centers for Disease Control and Prevention (CDC) guidelines. We are asking you to complete questionnaires about your health history, possible exposures, symptoms and health care visits, and keep a two week daily diary of your symptoms. We also are asking to collect nose, mouth, rectum, and blood samples, as well as ultrasounds of your lungs, heart and veins. From your medical care, we would like to use any leftover samples, and review your medical records in the military health system now and in the future for information related to COVID-19 risks, treatments, and health outcomes.

Who can participate?

This study is available to DoD beneficiaries (active duty and non-active duty) of all ages who have confirmed COVID-19 or who meet current CDC criteria for testing for the virus that causes COVID-19. If you are unable to actively participate in this informed consent process, your legally authorized representative (LAR) may do that on your behalf. If your LAR consents on your behalf and you later become capable of consent, you will be asked to give your informed consent once you are able to do so.

KEY INFORMATION

- This is an observational study meaning we will look at your medical records and collect data and samples from you *but we will not give you any medicine or treatment*. Questions about your treatment should be directed to your doctor.
- Volunteering for this study does not limit your ability to participate in another study of an experimental drug or vaccine. That decision would be made in discussion with your doctor and the other study's investigators and is separate from this study. We may collect additional blood to assess the new treatment's effects.
- Your participation is voluntary, your decision will not affect your care or any other benefits to which you are entitled. Your alternative to volunteering is to not participate.
- Your participation will last about 1 year.
- You may end your participation at any time without any consequences.
- There is no cost for participating.
- All information and samples you provide will be handled securely and in confidence. Only those involved in conducting this study will know you are enrolled.
- The information collected about you is for research purposes only. This information will not be used to guide your medical care.
- Leftover samples from tests your doctor orders will be stored and may be shared for future analyses related to COVID-19 or other infections
- You may not personally benefit from this study. The information gained from this study may not be available in time to affect your care. The information we learn may help in caring for other future patients with COVID-19 or similar pandemics.
- Potential risks in participating include the following:
  - Blood draws can cause a risk of pain, bruising, bleeding, and (rarely) fainting or infection.
  - Nose, mouth, and rectal swabs may cause discomfort but are generally well tolerated.
  - There may also be unforeseen risks associated with this study.
- If you are pregnant, there are no additional known risks to you or your fetus.

(USUHS) IRB 1
IRB NUMBER: 409136 (IDCRP-085)
IRB APPROVAL DATE: 07/28/2020
IRB EXPIRATION DATE: 07/25/2021

- Once your identifying information has been removed from the collected data and specimens, additional consent will not be sought for future studies. This also applies if you withdraw from the study.

<u>What is involved in the study?</u>

<u>During your hospitalization:</u>

- ✓ We will review your medical records to collect information related to your health.
- ✓ Blood samples for research will be collected (~5 teaspoons each time from your vein) and/or you will be asked to collect a small blood sample (a few drops from your finger), using a specially designed device similar to blood glucose monitoring used by diabetic people.
- ✓ Nose, mouth and rectum samples will be collected using a swab or other simple collection method to identify and count the virus. You may also be asked to collect some samples yourself with our help. The type of sample may change during your participation in the study as we learn more about the virus.
- ✓ We may do an ultrasound to look at your lungs, heart, and veins.
- ✓ You will start a daily online symptom diary called Flu-PRO for 14 days.
- ✓ We will ask you to complete a small questionnaire about your medical history, illness, and vaccine history.

Samples will be collected following the below schedule as feasible, however it is possible that not all samples will be collected.

Procedure / Assessment	**Day 0 (today)**	**Days 3 and 7**	**If hospitalized longer than 7 days: samples obtained weekly until discharge**
Nose, mouth, and rectal samples	✓	✓	✓
Blood collection for research	✓	✓	✓
Ultrasound of your lungs, heart and veins *	✓	✓	
Flu-PRO Symptom Diary (14 days)**	✓	✓	✓
Online Questionnaire	✓		

* May be performed on or around hospital days 0 (admission), 1, and 3, based on availability.

** Does not all have to be completed while hospitalized.

<u>Follow-Up Visits:</u>

In 1 Week (If you've left the hospital)	• Continue the Flu-PRO online daily symptom diary (14-days total). • Blood collection – we will draw blood from your vein (about 25 mL or 5 teaspoons) and/or you will be asked to collect a small blood sample (a few drops from your finger), using a specially designed device similar to blood glucose monitoring used by diabetic people. • If you are at home, we will provide you with the materials and instructions to collect your own samples, including pre-paid mailing materials to return your samples.
In 2 Weeks (If you've left the hospital)	• Finish the Flu-PRO online daily symptom diary (14-day total). • To check for active virus, we will collect samples from your nose and/or mouth using a swab or other simple collection method. This method may change during your participation in the study as we learn more about the virus. • Blood collection (same as above - either at home or in clinic).
In 1 Month	• Complete an online questionnaire that takes about 10 minutes. We will send you an email reminder with the web link as it gets closer to the time. • Blood collection (same as above - either at home or in clinic). • If you return to the clinic for this visit, we will ask to collect a follow-up rectal swab sample to check for presence of the virus.
In 6 Months	The following procedures happen at each time point (6 and 12 months): • Complete an online questionnaire that takes about 10 minutes. We will send you an email reminder with the web link as it gets closer to the time. • Blood collection (same as above - either at home or in clinic).
In 12 Months	

(USUHS) IRB 1
IRB NUMBER: 409136 (IDCRP-085)
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IRB EXPIRATION DATE: 07/25/2021

Additional information

- You can choose not to provide blood samples or swabs and still participate in the study.
- The total amount of blood collected for research while hospitalized or during a return visit at the hospital at one sitting will be about 25 mL (5 teaspoons). The total number of required blood samples depends on the duration of your illness. During the 12 months that you participate in the study, no more than 550 mL (2⅓ cups) total of blood will be collected. For comparison, a typical blood drive donation is 470 mL (about 2 cups) in one day. To minimize discomfort to you, we will try to coordinate with your healthcare providers so that the collection of research blood samples happens at the same time as your standard blood draws.
- Based on your answers to the questionnaires, we may contact you to discuss your health if you have particular symptoms or any new illness that may be related to COVID-19.
- Throughout your participation in the study, we will collect leftover samples from tests your doctors order such as blood, swabs, urine, etc. We will collect these samples dating back to the time you became ill. We will study these materials for COVID-19 and related illnesses. You do not have to do anything additional for this.
- The US military regularly stores service members blood samples obtained for medical surveillance in the DoD Serum Repository. If you are Active Duty or former military (since the 1990s) we plan to obtain stored blood specimens prior to and after your COVID-19 illness. You do not have to do anything additional for this.
- If you are pregnant or become pregnant during the study participation period, we will follow up with you until your baby is born. We may collect a sample of umbilical cord blood at the time of birth, review your baby's medical records, and ask you questions about your baby's health. There are no additional known risks to you or your fetus for participating in the study.

Genetic Information

The study is focusing on risks related to COVID-19 including your genetic results as part of the analysis. The risks of studying your unique genetic code may be the identification of risk for developing certain diseases. We will not be studying general issues related to your genetic results because our research is focused on COVID-19 and related illnesses. We will not provide you with your genetic results or release any information regarding your results connected to your personal identification.
Please let us know your decision by signing your initials:

______ Yes, I give permission to use my **genetic information** for COVID-19-related research

______ No, I DO NOT give permission to use my **genetic information** for COVID-19 research

Compensation:

You will receive payment (compensation) in the form of gift cards as follows for completion of the following study procedures**:**

- Completion of each online questionnaire: $10 (if completed off-duty for service members)
- Completion of 14-day Flu-PRO Symptom Diary: $10 (if completed off-duty for service members)
- Completion of each at-home self-collected sample: $10 (if completed off-duty for service members)
- Blood draws at hospital/clinic after you leave the hospital: $50 per blood draw

Under 24 USC 30, payment to Federal Employees and Active Duty military personnel for participation in research while on duty is limited to blood donation. Military members may not receive any payment or non-monetary compensation for participation in a research study unless they are off duty or on leave during the time they are participating in the protocol. There are no restrictions on payment while off-duty.

HIPAA: AUTHORIZATION TO USE AND/OR DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

PI Name and Rank: Brian Agan, MD, DrPH

Corps and Service/Organization: Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences
Title of Research Study: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential

Purpose of this Document:
An Authorization is your signed permission to use or disclose your health information. The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, as implemented by the Department of Defense (DoD), permits the Military Health System (MHS) to use or disclose your health information with a valid Authorization. The MHS is defined as all DoD health plans and DoD health care providers that are organized under the management authority of, or in the case of covered individual providers, assigned to or employed by, the Defense Health Agency (DHA), the Army, the Navy, or the Air Force. A valid Authorization must include the core elements and required statements as contained in this document.

Please read the information below and ask questions about anything you do not understand before deciding to give permission for the use and disclosure of your health information.

The Health Insurance Portability & Accountability Act of 1996, Public Law 104-109 (also known as HIPAA), establishes privacy standards to protect your health information. This law requires the researchers to obtain your authorization (by signing this form) before they use or disclose your protected health information for research purposes in the study discussed in this form. Note: Protected health information of military service members may be used or disclosed for activities deemed necessary by appropriate military command authorities to ensure the proper execution of the military mission.

By signing this authorization, you give your permission for information gained from your participation in this study to be published in medical literature, discussed for educational purposes, and used generally to further medical science. You will not be personally identified; all information will be presented as anonymous data.

A. What health information will be used or disclosed about you?

- Names
- Address
- Date of birth
- Ages over 89 (can be grouped as age 90 or older)
- DoD identification number
- Health information
- Health plan beneficiary numbers
- Phone numbers
- Fax numbers
- E-mail addresses
- Social security numbers
- Medical record numbers

B. Who will be authorized to use or disclose (release) your health information?

MTFs who have treated you over the past ten years

C. Who may receive your health information?

- The Institutional Review Board (USU IRB)
- Infectious Disease Clinical Research Program (IDCRP)
- Military Treatment Facilities who have treated you or Department of Defense representatives
- Henry M. Jackson Foundation and their representatives
- Neoteryx and their representatives
- Local, State and Federal Government representatives, when required by law
- Defense Health Agency (DHA)
- Food and Drug Administration (FDA)
- Centers for Disease Control and Prevention (CDC)
- National Institute of Allergy and Infectious Disease (NIAID) and their representatives

The researchers and those listed above agree to protect your health information by using and disclosing it only as permitted by you in this Authorization and as directed by state and federal law.

You need to be aware that some parties receiving your protected health information may not have the same obligations to protect your protected health information and may re-disclose your protected health information to parties not named here. If your protected health information is re-disclosed, it may no longer be protected by state or federal privacy laws. This authorization does not expire.

D. What if you decide not to sign this Authorization?
You do not have to sign this Authorization. If you decide not to sign the Authorization it will not affect your treatment, payment or enrollment in any health plans or affect your benefit eligibility. You will not be allowed to participate in this research study because your health information is required in order for you to take part in this study. The MHS will not condition (withhold or refuse) treatment that is not part of this study, payment, enrollment, or eligibility for benefits on whether you sign this Authorization.

E. Is your health information requested for future research studies?
Yes, your health information is requested for future research studies as specified below: Your health information will not be used for future research studies unless you give your permission by initialing your choice below:

____ YES, I give permission to use my health information for future research studies

____ NO, I do not give permission to use my health information for future research studies

F. Can you access your health information during the study?
You may have access to your health information at any time, unless your identifiers are permanently removed from the data.

G. Can you revoke this Authorization?
You may change your mind and revoke (take back) your Authorization at any time. However, if you revoke this Authorization, any person listed above may still use or disclose any already obtained health information as necessary to maintain the integrity or reliability of this research.

- If you revoke this Authorization, you may no longer be allowed to participate in this research study.
- If you want to revoke your Authorization, you must write to the Study Doctor.

H. Does this Authorization expire?
No, it does not expire

I. What else may you want to consider?
- No publication or public presentation about the research described above will reveal your identity without another signed Authorization from you.
- If all information that does or can identify you is removed from your health information, the remaining de-identified information will no longer be subject to this Authorization and may be used or disclosed for other purposes.
- In the event your health information is disclosed to an organization that is not covered by HIPAA, the privacy of your health information cannot be guaranteed.

If you have not already received a copy of the brochure entitled "Military Health System Notice of Privacy Practices," you may request one. DD Form 2005, Privacy Act Statement - Military Health Records (located on your medical records jacket), contains the Privacy Act Statement for the records. If you have any questions or concerns about your privacy rights, you should contact the Uniformed Services University of the Health Sciences (USU) Human Research Protections Program at 301-295-9534.

Who can answer my questions?
Any questions about this research study or if you believe you have received a research-related injury, you may contact the lead for the study at your hospital or the study Principal Investigator.
PRINCIPAL INVESTIGATOR: Overall director of this research study is Dr. Brian Agan at the Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences (a DoD institution).

Questions? Call Dr. Agan at 1-833-475-0881 or contact at this address: Uniformed Services University of the Health Sciences (USU), Department of Preventive Medicine and Biostatistics, 4301 Jones Bridge Road, Bethesda, MD 20814-5119

LOCAL PRINCIPAL INVESTIGATOR (Study Doctor): – [Sites will fill in]

The USU Institutional Review Board is the research ethics committee responsible for safeguarding your rights as a research participant. If you have any questions about your rights as a research participant or any other concerns that cannot be addressed by the Study Doctor, you can contact the Director of the Human Research Protections Program at (301) 295-9534.

SIGNATURE PAGE:

Your signature is voluntary consent to participate in this study. Your signature also acknowledges that you authorize personnel to use and disclose your Protected Health Information (PHI) collected about you for research purposes as described above. A copy of this form will be offered to you.

____
Printed Name of Participant

____ ___________________
Participant Signature Today’s Date

____
Printed Name of Study Representative

____ ___________________
Study Representative Signature Today’s Date

Verbal/physical limitation section: use the section below only if applicable.

The participant, ____ (name), has verbally consented to participate in the study as described and also authorizes the use and disclosure of his/her health information as described. The participant is coherent and in the opinion of the advising research staff, the participant has understood the nature and consequences of participation in the study, but the participant is physically disabled or in isolation and is unable to sign the informed consent document.

The consent and authorization information were accurately explained. The participant was given the opportunity to ask questions. All questions were answered before giving verbal consent. The participant apparently understood the information. The informed consent and authorization were given freely by the participant.

____
Printed Name of Witness

____ ___________________
Witness Signature Today’s Date

Complete LAR section only if applicable. If the LAR has a copy of the power of attorney or court appointment, attach it to this consent document.

Printed Name of Legally Authorized Representative (LAR)	Relationship to Participant
LAR Signature	Today's Date
Printed Name of Witness (if applicable)	
Witness Signature (if applicable)	Today's Date

Consent of participant who regains ability to consent on their own behalf for continued participation. I understand the nature and consequences of my participation in this study. The consent and authorization information were accurately explained. I have been given the opportunity to ask questions and my questions have been answered.

_____ I agree to continue in this research.

_____ I do not agree to continue in this research.

Printed Name of Participant	
Participant Signature	Today's Date
Printed Name of Study Representative	
Study Representative Signature	Today's Date
Printed Name of Witness (if applicable)	
Witness Signature (if applicable)	Today's Date

(USUHS) IRB 1
IRB NUMBER: 409136 (IDCRP-085)
IRB APPROVAL DATE: 07/28/2020
IRB EXPIRATION DATE: 07/25/2021

INFORMED CONSENT DOCUMENT FOR AN OBSERVATIONAL COVID-19 RESEARCH STUDY: IDCRP-085: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)

Thank you for your interest in learning more about this study. Please read the following information to ensure that you would like to participate. The Department of Defense has sponsored this research study, led by the Infectious Disease Clinical Research Program at the Uniformed Services University of the Health Sciences (the military's medical school), to better understand risks and illness caused by the novel Coronavirus disease (**COVID-19**).

The COVID-19 pandemic is a global public health emergency. We are asking you to consider volunteering for this research study because you may have COVID-19 and/or qualify for COVID-19 testing according to the Centers for Disease Control and Prevention (CDC) guidelines. If you agree to participate, we will ask you to complete online questionnaires about your health history, possible exposures, symptoms, and health care visits. We will collect swabs from your nose and/or mouth to check for the virus, and draw blood to see how your body reacts to the infection. In the future we will ask you to return for more visits and/or collect some samples at home such as a nose and/or mouth swab and a few drops of blood from your fingertip, using a simple kit that you mail in. Your medical records in the military health system will be reviewed now and in the future for information related to COVID-19 risks, treatments, and health outcomes.

Who can participate?

This study is available to DoD beneficiaries (active duty and non-active duty) of all ages who have confirmed COVID-19 or who meet current Centers for Disease Control and Prevention (CDC) criteria for testing for the virus that causes COVID-19.

KEY INFORMATION

- This is an observational study, meaning we will look at your medical records and collect data and samples from you *but we will not give you any medicine or treatment*. Questions about your treatment should be directed to your doctor.
- Volunteering for this study does not limit your ability to participate in another study of an experimental drug or vaccine. That decision would be made in discussion with your doctor and the other study’s investigators and is separate from this study. We may collect additional blood to assess the new treatment’s effects.
- Your participation is voluntary, your decision will not affect your care or any other benefits to which you are entitled. Your alternative to volunteering is to not participate.
- Your participation will last about 1 year.
- You may end your participation at any time without any consequences.
- There is no cost for participating.
- All information and samples you provide will be handled securely and in confidence. Only those involved in conducting this study will know you are enrolled.
- The information collected about you is for research purposes only. This information will not be used to guide your medical care.
- Leftover samples from tests your doctor orders will be stored and may be shared for future analyses related to COVID-19 or other infections.
- You may not personally benefit from this study. The information gained from this study may not be available in time to affect your care. The information we learn may help in caring for future patients with COVID-19 or similar pandemics.
- Potential risks in participating include the following:
  - Blood draws can cause a risk of pain, bruising, bleeding, and (rarely) fainting or infection.
  - Nose and mouth swabs may cause discomfort but are generally well tolerated.
  - There may also be unforeseen risks associated with this study.
- If you are pregnant, there are no additional known risks to you or your fetus.

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- Once your identifying information has been removed from the collected data and specimens, additional consent will not be required for future studies. This also applies if you withdraw from the study.

What is involved in the study?
The following schedule describes the research study procedures and when they occur.

Today	• You will be given a link to a questionnaire to complete online (takes about 20 minutes). • Blood collection – we will draw blood from your vein (about 25 mL or 5 teaspoons) and/or you will be asked to collect a small blood sample (a few drops from your finger), using a specially designed device similar to blood glucose monitoring used by diabetic people. • We will collect samples from your nose and/or mouth using a swab or other simple collection method. You may also be asked to collect some samples yourself with our help. The type of sample may change during your participation in the study as we learn more about the virus. • If you are at home, we will provide you with the materials and instructions to collect your own samples, including pre-paid mailing materials to return your samples. • You will start an online daily symptom diary called Flu-PRO.
In 1 Week	• Blood collection (same as above - either at home or in clinic). • Continue to complete the Flu-PRO online daily symptom diary.
In 2 Weeks	• Finish the Flu-PRO online daily symptom diary (14 days total). • Nose and/or mouth sample (same as above, either at home or in clinic). • Blood collection (same as above - either at home or in clinic).
In 1 Month	The following procedures happen at each of the time points (1, 6, and 12 months): • Blood collection (same as above - either at home or in clinic). • Online questionnaire (takes about 10 minutes).
In 6 Months	
In 12 Months	

Additional information

- Blood collection: During the 12 months that you participate in the study, approximately 180 mL (less than a cup) total of blood will be collected. For comparison, a typical blood drive donation is 470 mL (about 2 cups) in one day.
- You can choose to not provide samples and still participate in the study.
- If you are admitted to the hospital for COVID-19 symptoms, we may ask to collect additional samples.
- Based on your answers to the questionnaires, we may contact you to discuss your health if you have particular symptoms or new illness that may be related to COVID-19.
- Throughout your participation in the study, we will collect leftover samples from tests your doctors order such as blood, swabs, urine, etc. We will collect these samples dating back to the time you became ill. We will study these materials for COVID-19 and related illnesses. You do not have to do anything additional for this.
- The US military regularly stores service members blood samples obtained for medical surveillance in the DoD Serum Repository. If you are Active Duty or former military (since the 1990s) we plan to obtain stored blood specimens prior to and after your COVID-19 illness. You do not have to do anything additional for this.
- If you are pregnant or become pregnant during the study participation period, we will follow up with you until your baby is born. We may collect a sample of umbilical cord blood at the time of birth, review your baby's medical records, and ask you questions about your baby's health.

Genetic Information
The study is focusing on understanding risks related to COVID-19 including how genetic factors impact COVID-19. The risks of studying your unique genetic code may be the identification of risk for developing certain diseases. We will not be studying general issues related to your genetic results because our research is focused on COVID-

19 and related illnesses. We will not provide you with your genetic results or release any information regarding your results connected to your personal identification.

Please let us know your decision by signing your initials:

_______Yes, I give permission to use my **genetic information** for COVID-19-related research

_______ No, I DO NOT give permission to use my **genetic information** for COVID-19 research

Compensation:

You will receive payment (compensation) in the form of gift cards as follows for completion of the following study procedures:

- Completion of each online questionnaire: $10 (if completed off duty for servicemembers)
- Completion of 14-day Flu-PRO Symptom Diary: $10 (if completed off-duty for service members)
- Completion of each at-home self-collected sample: $10 (if completed off-duty for service members)
- Blood draws at hospital/clinic: $50 per blood draw

Under 24 USC 30, payment to Federal Employees and Active Duty military personnel for participation in research while on duty is limited to blood donation. Military personnel may not receive any payment or non-monetary compensation for participation in a research study unless they are off duty or on leave during the time they are participating in the protocol. There are no restrictions on payment while off-duty.

HIPAA: AUTHORIZATION TO USE AND/OR DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

PI Name and Rank: Brian Agan, MD, DrPH

Corps and Service/Organization: Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences

Title of Research Study: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential

Purpose of this Document:

An Authorization is your signed permission to use or disclose your health information. The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, as implemented by the Department of Defense (DoD), permits the Military Health System (MHS) to use or disclose your health information with a valid Authorization. The MHS is defined as all DoD health plans and DoD health care providers that are organized under the management authority of, or in the case of covered individual providers, assigned to or employed by, the Defense Health Agency (DHA), the Army, the Navy, or the Air Force. A valid Authorization must include the core elements and required statements as contained in this document.

Please read the information below and ask questions about anything you do not understand before deciding to give permission for the use and disclosure of your health information.

The Health Insurance Portability & Accountability Act of 1996, Public Law 104-109 (also known as HIPAA), establishes privacy standards to protect your health information. This law requires the researchers to obtain your authorization (by signing this form) before they use or disclose your protected health information for research purposes in the study discussed in this form. Note: Protected health information of military service members may be used or disclosed for activities deemed necessary by appropriate military command authorities to ensure the proper execution of the military mission.

By signing this authorization, you give your permission for information gained from your participation in this study to be published in medical literature, discussed for educational purposes, and used generally to further medical science. You will not be personally identified; all information will be presented as anonymous data.

A. What health information will be used or disclosed about you?

- Names
- Address
- Date of birth
- Health plan beneficiary numbers
- Phone numbers
- Fax numbers

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- Ages over 89 (can be grouped as age 90 or older)
- DoD identification number
- Health information
- E-mail addresses
- Social security numbers
- Medical record numbers

B. Who will be authorized to use or disclose (release) your health information?
MTFs who have treated you over the past ten years

C. Who may receive your health information?

- The Institutional Review Board (USU IRB)
- Infectious Disease Clinical Research Program (IDCRP)
- Other Military Treatment Facilities or Department of Defense representatives
- Henry M. Jackson Foundation and their representatives
- Neoteryx and their representatives
- Local, State and Federal Government representatives, when required by law
- Defense Health Agency (DHA)
- Food and Drug Administration (FDA)
- Centers for Disease Control and Prevention (CDC)
- National Institute of Allergy and Infectious Disease (NIAID) and their representatives

The researchers and those listed above agree to protect your health information by using and disclosing it only as permitted by you in this Authorization and as directed by state and federal law.
You need to be aware that some parties receiving your protected health information may not have the same obligations to protect your protected health information and may re-disclose your protected health information to parties not named here. If your protected health information is re-disclosed, it may no longer be protected by state or federal privacy laws. This authorization does not expire.

D. What if you decide not to sign this Authorization?
You do not have to sign this Authorization. If you decide not to sign the Authorization it will not affect your treatment, payment or enrollment in any health plans or affect your benefit eligibility. You will not be allowed to participate in this research study because your health information is required in order for you to take part in this study. The MHS will not condition (withhold or refuse) treatment that is not part of this study, payment, enrollment, or eligibility for benefits on whether you sign this Authorization.

E. Is your health information requested for future research studies?
Yes, your health information is requested for future research studies as specified below: Your health information will not be used for future research studies unless you give your permission by initialing your choice below:

____ YES, I give permission to use my health information for future research studies

____ NO, I do not give permission to use my health information for future research studies

F. Can you access your health information during the study?
You may have access to your health information at any time, unless your identifiers are permanently removed from the data.

G. Can you revoke this Authorization?
You may change your mind and revoke (take back) your Authorization at any time. However, if you revoke this Authorization, any person listed above may still use or disclose any already obtained health information as necessary to maintain the integrity or reliability of this research.

- If you revoke this Authorization, you may no longer be allowed to participate in this research study.
- If you want to revoke your Authorization, you must write to the Study Doctor.

H. Does this Authorization expire?
No, it does not expire

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I. What else may you want to consider?

- No publication or public presentation about the research described above will reveal your identity without another signed Authorization from you.
- If all information that does or can identify you is removed from your health information, the remaining de-identified information will no longer be subject to this Authorization and may be used or disclosed for other purposes.
- In the event your health information is disclosed to an organization that is not covered by HIPAA, the privacy of your health information cannot be guaranteed.

If you have not already received a copy of the brochure entitled "Military Health System Notice of Privacy Practices," you may request one. DD Form 2005, Privacy Act Statement - Military Health Records (located on your medical records jacket), contains the Privacy Act Statement for the records. If you have any questions or concerns about your privacy rights, you should contact the Uniformed Services University of the Health Sciences (USU) Human Research Protections Program at 301-295-9534.

Who can answer my questions?

Any questions about this research study or if you believe you have received a research-related injury, you may contact the lead for the study at your hospital or the study Principal Investigator.

PRINCIPAL INVESTIGATOR: Overall director of this research study is Dr. Brian Agan at the Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences (a DoD institution).

Questions? Call Dr. Agan at 1-833-475-0881 or contact at this address: Uniformed Services University of the Health Sciences (USU), Department of Preventive Medicine and Biostatistics, 4301 Jones Bridge Road, Bethesda, MD 20814-5119

LOCAL PRINCIPAL INVESTIGATOR (Study Doctor): – [Sites will fill in]

The USU Institutional Review Board is the research ethics committee responsible for safeguarding your rights as a research participant. If you have any questions about your rights as a research participant or any other concerns that cannot be addressed by the Study Doctor, you can contact the Director of the Human Research Protections Program at (301) 295-9534.

SIGNATURE PAGE:

Your signature is voluntary consent to participate in this study. Your signature also acknowledges that you authorize personnel to use and disclose your Protected Health Information (PHI) collected about you for research purposes as described above. provide your signature (in person or electronic). A copy of this form will be offered to you. purposes as described above. A copy of this form will be offered to you.

____
Printed Name of Participant

____ ____________________
Participant Signature Today's Date

____
Printed Name of Study Representative

____ ____________________
Study Representative Signature Today's Date

Verbal/physical limitation section: use the section below only if applicable.

The participant, ____ (name), has verbally consented to participate in the study as described and also authorizes the use and disclosure of his/her health information as described. The participant is coherent and in the opinion of the advising research staff, the participant has understood the nature and consequences of participation in the study, but the participant is physically disabled or in isolation and is unable to sign the informed consent document.

The consent and authorization information were accurately explained. The participant was given the opportunity to ask questions. All questions were answered before giving verbal consent. The participant apparently understood the information. The informed consent and authorization were given freely by the participant.

____
Printed Name of Witness

____ ____________________
Witness Signature Today's Date

INFORMED CONSENT DOCUMENT FOR AN OBSERVATIONAL COVID-19 RESEARCH STUDY: IDCRP-085: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)

Thank you for your interest in learning more about this study. Please read the following information to ensure that you would like to participate. The Department of Defense has sponsored this research study, led by the Infectious Disease Clinical Research Program at the Uniformed Services University of the Health Sciences (the military's medical school), to better understand risks and illness caused by the novel Coronavirus disease (**COVID-19**).

The COVID-19 pandemic is a global public health emergency. We are asking you to consider volunteering for this research study because you may have been tested for COVID-19. If you agree to participate, we are asking you to complete online questionnaires about your health history, possible exposures, symptoms and health care visits and to collect small blood samples using a simple in-home kit to look for immunity (antibodies against the virus). Your medical records in the military health system will be reviewed now and in the future for information related to COVID-19 risks, treatments, and health outcomes.

Who can participate?

The EPICC study is available to DoD beneficiaries of all ages who have confirmed COVID-19 or who meet current Centers for Disease Control and Prevention (CDC) criteria for testing for the virus that causes COVID-19.

This online-only section of the study is available to adult DoD beneficiaries who have been tested for COVID-19 and are able to send/receive email and complete online questionnaires. You do not have to know the result of your COVID-19 test to volunteer, nor does the test have to be positive.

KEY INFORMATION

- This is an observational study meaning we will look at your medical records and collect data and samples from you *but we will not give you any medicine or treatment*. Questions about your treatment should be directed to your doctor.
- Your participation is voluntary, your decision will not affect your care or any other benefits to which you are entitled. Your alternative to volunteering is to not participate.
- Your participation will last about 1 year.
- You may end your participation at any time without any consequences.
- There is no cost for participating.
- All information and samples you provide will be handled securely and in confidence. Only those involved in conducting this study will know you are enrolled.
- The information collected about you is for research purposes only. This information will not be used to guide your medical care.
- You may not personally benefit from this study. The information gained from this study may not be available in time to affect your care. The information we learn may help in caring for other future patients with COVID-19 or similar illnesses.
- Potential risks in participating include the following:
  - Blood draws can cause a risk of pain, bruising, bleeding, and rarely fainting or infection.
  - There may also be unforeseen risks associated with this study.
- If you are pregnant, there are no additional risks to you or your fetus.
- Once your identifying information has been removed from the collected data and specimens, additional consent will not be sought for future studies. This also applies if you withdraw from the study. Leftover samples will be stored and may be shared for future analyses related to COVID-19 or other infections.
- Volunteering for this study does not limit your ability to participate in another study of an experimental drug or vaccine. That decision would be made in discussion with your doctor and the other study’s investigators and is separate from this study. We may ask to collect additional blood to assess the new treatment’s effects.

What is involved in the study?
The following schedule describes the research study procedures and when they occur.

Today	• You will be asked to sign this informed consent form online that also includes HIPAA authorization (related to your privacy protections). • After consenting you will be provided a link to an online questionnaire that takes about 20 minutes to complete.
In 1 Month	The following procedures happen at each of the time points (1, 6 and 12 months): • Complete an online questionnaire that takes about 10 minutes to complete. We will send you an email reminder with the web link as it gets closer to the time. • Collect a small blood sample at home consisting of a few drops of blood from your finger, similar to blood glucose monitoring used by diabetic people. You will use a simple kit that we provide. You will then mail the kit into our lab using a pre-paid mailer.
In 6 Months	
In 12 Months	

You can choose to not provide these blood samples and still participate in the study. If you agree to participate in this portion of the study, we will inform you if you will be asked to provide the samples. Due to availability of study resources, not all participants who agree to provide a blood sample will be invited.
Please let us know your decision by signing your initials:

______ Yes, I DO want to participate in the at-home blood collection part of the study.

______ No, I DO NOT want to participate in the at-home blood collection part of the study.

Additional information

- Based on your answers to the questionnaires, we may contact you to discuss your health if you have particular symptoms or new illness that may be related to COVID-19.
- The US military regularly stores servicemembers blood samples obtained for medical surveillance in the DoD Serum Repository. If you are Active Duty or former military (since the 1990s) we plan to obtain stored blood specimens prior to and after your COVID-19 illness. You do not have to do anything additional for this.
- If you are pregnant or become pregnant during the study participation period, we will follow up with you until your baby is born. We may review your baby's medical records, and ask you questions about your baby's health.

Genetic Information
The study will be evaluating how a person's genes are associated with risk and outcomes related to COVID-19. The risks of studying your unique genetic code may be the identification of risk for developing certain diseases. We will not be studying general issues related to your genetic results because our research is focused on COVID-19 and related illnesses. We will not provide you with your genetic results or release any information regarding your results connected to your personal identification.
Please let us know your decision by signing your initials:

______Yes, I give permission to use my **genetic information** for COVID-19-related research.

______ No, I DO NOT give permission to use my **genetic information** for COVID-19 research.

Compensation:
You will receive payment (compensation) in the form of Amazon gift codes as follows for completion of the following study procedures:

- Completion of each online questionnaire: $10 (if completed off duty for servicemembers)
- Completion of each self-collected blood sample: $10

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Under 24 USC 30, payment to Federal Employees and Active Duty military personnel for participation in research while on duty is limited to blood donation. They may not receive any payment or non-monetary compensation for participation in a research study unless they are off duty or on leave during the time they are participating in the protocol. There are no restrictions on payment while off-duty.

HIPAA: AUTHORIZATION TO USE AND/OR DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

PI Name and Rank: Brian Agan, MD, DrPH
Corps and Service/Organization: Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences
Title of Research Study: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential

Purpose of this Document:
An Authorization is your signed permission to use or disclose your health information. The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, as implemented by the Department of Defense (DoD), permits the Military Health System (MHS) to use or disclose your health information with a valid Authorization. The MHS is defined as all DoD health plans and DoD health care providers that are organized under the management authority of, or in the case of covered individual providers, assigned to or employed by, the Defense Health Agency (DHA), the Army, the Navy, or the Air Force. A valid Authorization must include the core elements and required statements as contained in this document.

Please read the information below and ask questions about anything you do not understand before deciding to give permission for the use and disclosure of your health information.

The Health Insurance Portability & Accountability Act of 1996, Public Law 104-109 (also known as HIPAA), establishes privacy standards to protect your health information. This law requires the researchers to obtain your authorization (by signing this form) before they use or disclose your protected health information for research purposes in the study discussed in this form. Note: Protected health information of military service members may be used or disclosed for activities deemed necessary by appropriate military command authorities to ensure the proper execution of the military mission.

By signing this authorization, you give your permission for information gained from your participation in this study to be published in medical literature, discussed for educational purposes, and used generally to further medical science. You will not be personally identified; all information will be presented as anonymous data.

A. What health information will be used or disclosed about you?

- Names
- Address
- Date of birth
- Ages over 89 (can be grouped as age 90 or older)
- DoD identification number
- Health information
- Health plan beneficiary numbers
- Phone numbers
- Fax numbers
- E-mail addresses
- Social security numbers
- Medical record numbers

B. Who will be authorized to use or disclose (release) your health information?

MTFs who have treated you over the past ten years.

C. Who may receive your health information?

- The Institutional Review Board (USU IRB)
- Infectious Disease Clinical Research Program (IDCRP)
- Other Military Treatment Facilities or Department of Defense representatives
- Henry M. Jackson Foundation and their representatives
- Neoteryx and their representatives
- Local, State and Federal Government representatives, when required by law
- Defense Health Agency (DHA)

- Food and Drug Administration (FDA)
- Centers for Disease Control and Prevention (CDC)
- National Institute of Allergy and Infectious Disease (NIAID) and their representatives

The researchers and those listed above agree to protect your health information by using and disclosing it only as permitted by you in this Authorization and as directed by state and federal law.

You need to be aware that some parties receiving your protected health information may not have the same obligations to protect your protected health information and may re-disclose your protected health information to parties not named here. If your protected health information is re-disclosed, it may no longer be protected by state or federal privacy laws. This authorization does not expire.

D. What if you decide not to sign this Authorization?

You do not have to sign this Authorization. If you decide not to sign the Authorization it will not affect your treatment, payment or enrollment in any health plans or affect your benefit eligibility. You will not be allowed to participate in this research study because your health information is required in order for you to take part in this study. The MHS will not condition (withhold or refuse) treatment that is not part of this study, payment, enrollment, or eligibility for benefits on whether you sign this Authorization.

E. Is your health information requested for future research studies?

Yes, your health information is requested for future research studies as specified below: Your health information will not be used for future research studies unless you give your permission by initialing your choice below:

____ YES, I give permission to use my health information for future research studies

____ NO, I do not give permission to use my health information for future research studies

F. Can you access your health information during the study?

You may have access to your health information at any time, unless your identifiers are permanently removed from the data.

G. Can you revoke this Authorization?

You may change your mind and revoke (take back) your Authorization at any time. However, if you revoke this Authorization, any person listed above may still use or disclose any already obtained health information as necessary to maintain the integrity or reliability of this research.

- If you revoke this Authorization, you may no longer be allowed to participate in this research study.
- If you want to revoke your Authorization, you must write to the Study Doctor.

H. Does this Authorization expire?

No, it does not expire

I. What else may you want to consider?

- No publication or public presentation about the research described above will reveal your identity without another signed Authorization from you.
- If all information that does or can identify you is removed from your health information, the remaining de-identified information will no longer be subject to this Authorization and may be used or disclosed for other purposes.
- In the event your health information is disclosed to an organization that is not covered by HIPAA, the privacy of your health information cannot be guaranteed.

If you have not already received a copy of the brochure entitled “Military Health System Notice of Privacy Practices,” you may request one. DD Form 2005, Privacy Act Statement - Military Health Records (located on your medical records jacket), contains the Privacy Act Statement for the records. If you have any questions or concerns about your privacy rights, you should contact the Uniformed Services University of the Health Sciences (USU) Human Research Protectio at 301-295-9534.

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Who can answer my questions?

Any questions about this research study or if you believe you have received a research-related injury, you may contact the study Principal Investigator (overall director based at USU).

PRINCIPAL INVESTIGATOR (Study Doctor): Overall director of this research study is Dr. Brian Agan at the Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences (a DoD institution).

Questions? Call Dr. Agan at 1-833-475-0881 or contact at this address: Uniformed Services University of the Health Sciences (USU), Department of Preventive Medicine and Biostatistics, 4301 Jones Bridge Road, Bethesda, MD 20814-5119

The USU Institutional Review Board is the research ethics committee responsible for safeguarding your rights as a research participant. If you have any questions about your rights as a research participant or any other concerns that cannot be addressed by the Study Doctor, you can contact the Director of the Human Research Protections Program at (301) 295-9534.

Your digital signature (signed using your mouse or finger) is voluntary consent to participate in this study. Your signature also acknowledges that you authorize personnel to use and disclose your Protected Health Information (PHI) collected about you for research purposes as described above.

A copy of this form will be sent to you by email.

____
Printed Name of Participant

____ __________________
Participant Signature Today's Date

(USUHS) IRB 1
IRB NUMBER: 409136 (IDCRP-085)
IRB APPROVAL DATE: 07/28/2020
IRB EXPIRATION DATE: 07/25/2021

ASSENT FORM FOR PARTICIPANTS AGED 7-17 YEARS

IDCRP-085: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)

We are doing a research study about people who are sick with a new illness called COVID-19. A research study is a way to find out about something. We are talking to you today because you may have had COVID-19, or you may have been close to people who are sick with COVID-19. We want to ask you to help us find out more about this illness.

You can decide if you want to be in this research study or not. We have discussed this research with your parent(s)/guardian, and they know that we are also asking you if you want to join. **If you want to participate in the study, your parent(s)/guardians need to give permission. If you do not want to be in the study, you do not have to, even if your parents have given permission.**

If you do want to be in the study, this is what will happen:

- We will ask you and your parent or guardian to sign a form letting us know that you want to be in the study.
- A staff member may rub the inside of your nose and mouth with a swab. This may be uncomfortable and may cause you to cough, sneeze, or bleed a little bit.
- A staff member may draw a small amount of blood from you. When the blood is drawn, it will be done using a clean needle that is stuck into your arm or a tube that is already connected to you. When your blood is drawn, it may hurt, but the hurt will go away before very long. You may bleed a little bit or get a bruise. Sometimes getting blood drawn can make some people faint, but this doesn't happen very often.
    - We will try our best to draw blood at the same time as your doctor so you don't have to be stuck with a needle as often.
- If there are any leftover samples from other tests that your doctors do, we will keep them so the leftovers can be tested.
- We will ask you to answer some questions about how you're feeling every day for the next 2 weeks using an online diary called Flu-PRO.
- ***If you are 8 or older***, we may ask you to use a simple kit to collect a sample from your nose and/or mouth, and take a small blood sample from your finger using a different simple kit. Your parent/guardian can help.
- ***If you are in the hospital***:
    - We may use ultrasound to look at your lungs, heart, arms and legs using a wand and gel.
    - We may ask to swab the inside of your bottom/butt. You can do this yourself, or your parent/guardian or a staff member can help. This may be uncomfortable.

Some of these procedures will be done while you are in a hospital or clinic. We may ask you to return there after you have left to do more tests and ask you more questions about how you are feeling. If you can't come back, we may ask you to collect your own samples with help from your parent/guardian.

Being in the study may not benefit you or help you. What we learn about the sickness from you may help other people not get sick in the future or help them get better faster. If we learn anything that will help you, we will let your doctors know right away.

For your participation in this research, you will receive $50 for each sample of blood that is collected from your arm after you leave the hospital, $10 for each sample you collect at home, and $10 for completing the 14-day Flu-PRO diary.

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We will not tell other people that you are in this study. The only people that will know you are in the study are those people that work on the research study. Any information about you will have a number on it instead of your name. Only the researchers will know what your number is, and we will lock up that information. It will not be shared with or given to anyone except the hospital and people who make sure that research is done in a way that is safe.

You can ask questions if you don’t understand something about the study. You don’t have to be in this study. **The choice is yours**. You will still be taken care of even if you decide that you don’t want to be in the study. If you say okay now, but change your mind later, that’s okay too. Just tell one of the study doctors.

To be in this study, both you and your parent must agree.

If you want to be in this study, please sign and print your name. You will be offered a copy of this form to keep.

☐ **Yes, I will be in this research study.**

☐ **No, I do not want to be in this research study.**

____
PRINT your name here

____ __________________
SIGN your name here Today’s Date

(USUHS) IRB 1
IRB NUMBER: 409136 (IDCRP-085)
IRB APPROVAL DATE: 07/28/2020
IRB EXPIRATION DATE: 07/25/2021

INFORMED CONSENT DOCUMENT FOR AN OBSERVATIONAL COVID-19 RESEARCH STUDY: IDCRP-085: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)

Thank you for your interest in learning more about this study. Please read the following information to ensure your child would like to participate. The Department of Defense has sponsored this research study, led by the Infectious Disease Clinical Research Program at the Uniformed Services University of the Health Sciences (the military's medical school), to better understand risks and illness caused by the novel Coronavirus disease (COVID-19).

The COVID-19 pandemic is a global public health emergency. We are asking you to consider allowing your child to participate in this research study because your child may have COVID-19 and/or qualify for COVID-19 testing according to the Centers for Disease Control and Prevention (CDC) guidelines.

Who can participate?

This study is available to DoD beneficiaries of all ages (active duty and non-active duty) who have confirmed COVID-19 or who meet current Centers for Disease Control and Prevention (CDC) criteria for testing for the virus that causes COVID-19.

KEY INFORMATION

- This is an observational study, meaning we will look at your child's medical records and collect data and samples from your child, *but we will not give your child any medicine or treatment*. Questions about your child's treatment should be directed to your child's doctor.
- Your child's participation is voluntary, you and your child's decision will not affect your child's care or any other benefits to which he or she is entitled. Your child's alternative to volunteering is to not participate.
- Your child's participation will last about 1 year. If your child is not sick now but later develops symptoms, we will follow up with them for 1 year from the time they became ill.
- If your child is unable to actively participate in the assent process, for example if they are incapacitated due to illness, you may do that on their behalf. If they later become capable of assent, they will be approached to give their assent.
- You or your child may end his or her participation at any time without any consequences.
- There is no cost for participating.
- All information and samples your child provides will be handled securely and in confidence. Only those involved in conducting this study will know your child is enrolled.
- The information collected about your child is for research purposes only. This information will not be used to guide your child's medical care.
- Your child may not personally benefit from this study. The information gained from this study may not be available in time to affect your child's care. The information we learn may help in caring for other future patients with COVID-19 or similar pandemics.
- Leftover samples from tests ordered by your child's doctor will be stored and may be shared for future analyses related to COVID-19 or other infections.
- Potential risks in participating include the following:
    - Blood draws can cause a risk of pain, bruising, bleeding, and rarely fainting or infection.
    - Nose, mouth, and rectal swabs may cause discomfort but are generally well tolerated.
    - There may also be unforeseen risks associated with this study.
- If your child is pregnant, we will ask to follow your child until your grandchild is born. There are no known additional risks to your child or her fetus.
- Once your child's identifying information has been removed from the collected data and specimens, additional consent will not be required for future studies. This also applies if your child withdraws from the study.
- Volunteering for this study does not limit your child's ability to participate in another study of an experimental drug or vaccine. That decision would be made in discussion with your child's doctor and the other study's investigators and is separate from this study. If your child receives an experimental drug or vaccine, we may collect an additional blood sample to study the new treatment's effects.

IF YOUR CHILD IS CURRENTLY HOSPITALIZED:

During your child's hospitalization:

- ✓ We will review your child's medical records now and in the future for information related to your child's health.
- ✓ Blood samples for research will be collected.
- ✓ Nose, mouth and rectum sample will be collected for identifying and counting the virus.
  If your child is 8 years old or older, we may ask your child to collect some of their own samples with your help.
- ✓ We may do an ultrasound to look at your child's lungs, heart, and veins.
- ✓ If your child is 7 or older, we will ask them to complete a daily symptom diary for 14 days.

Samples will be collected following the below schedule as feasible, however it is possible that not all samples will be collected.

Procedure / Assessment	**Day 0 (Today)**	**Days 3 and 7**	**If hospitalized longer than 7 days: samples obtained weekly until discharge**
Nose, mouth, and rectal samples	✓	✓	✓
Blood collection for research	✓	✓	✓
Ultrasound of lungs, heart and veins *	✓	✓	
Flu-PRO Symptom Diary (14 days)**	✓	✓	✓

* May be performed on or around hospital days 0 (admission), 1, and 3, based on availability.
** For participants 7 years of age and older. Does not all have to be completed while hospitalized.

IF YOUR CHILD IS NOT HOSPITALIZED:

If your child is 8 years old or older, we may request self-collection of a nose and/or mouth sample to check for the virus, as well as self-collection of blood using a sample kit to perform blood collection to look for immunity (antibodies against the virus). Your child may collect the nose and/or mouth samples and blood (few droplets from fingerstick) by themselves, or you may assist them with sample collection. *For at-home collections you will be given instructions and pre-addressed mailing materials to return the specimens.* Your child's medical records in the military health system will be reviewed now and in the future for information related to COVID-19. If your child is admitted to the hospital for COVID-19 symptoms, we may ask to collect additional samples.

Today	• Blood collection – we will draw your child's blood, from a vein (about 25 mL or 5 teaspoons; total blood collected may be less based on body weight, age, and other factors). We may also collect another blood sample (a few drops from finger/heel prick) using a simple device similar to blood glucose monitoring used by diabetic people. • We will collect samples from your child's nose and/or mouth using a swab or other simple collection method. The type of sample may change during your participation in the study as we learn more about the virus. • *For children 8 years of age and older*: your child may be asked to collect their own samples with your help. • *For children age 7 and older*: you will assist your child with starting a daily online symptom diary called Flu-PRO.
In 1 Week	• Blood collection (same as above - either at home or in clinic). • *For children age 7 and older:* Continue to assist your child with the Flu-PRO symptom diary.

FOLLOW-UP VISITS FOR ALL PARTICIPANTS

In 2 Weeks	• *For children age 7 and older:* Finish the Flu-PRO daily online symptom diary (14 days total). • Nose and/or mouth sample (same as above, either at home or in clinic). • Blood collection (same as above - either at home or in clinic).
In 1 Month	• Blood collection (same as above - either at home or in clinic). • *If your child was hospitalized,* we will ask to collect a follow-up rectal swab sample to check for presence of the virus, if you return to the hospital/clinic for this visit.
In 6 Months	• Blood collection (same as above - either at home or in clinic).
In 12 Months	

Additional information

- The total amount of blood collected for research while hospitalized or during a return visit at the hospital at one sitting will be up to 25 mL (5 teaspoons). Blood collection volume and frequency may be reduced to comply with safety limits. The amount of blood drawn for research will not exceed the lesser of 50 mL or 3 mL per kg in any 8 week period, and collection will not occur more frequently than 2 times per week. For comparison, a typical blood drive donation is 470 mL (about 2 cups) in one day. To minimize discomfort to your child, we will try to coordinate with your healthcare providers so that the collection of research blood samples happens at the same time as standard blood draws.
- Your child can choose not to provide samples at the time points listed above and still participate in the study.
- You will be asked to read, sign, and date this consent form after the study has been verbally explained to you by one of the research staff members and all your questions have been answered. Your child will be asked to read and sign an assent form if he or she is between the ages of 7 and 17 years old.
- Throughout your child's participation in the study, we will collect leftover samples from tests their doctors order such as blood, swabs, urine, etc. We will collect samples dating back to the time your child became ill. We will study these materials for COVID-19 and related illnesses. You do not have to do anything additional for this.

Genetic Information

The study is focusing on understanding risks related to COVID-19, including how genetic factors impact COVID-19. The risks of studying your child's unique genetic code may be the identification of risk for developing certain diseases. We will not be studying general issues related to your child's genetic results, our research is focused on COVID-19 and related illnesses. We will not provide you or your child with your child's genetic results or release any information regarding your child's results connected to his or her personal identification.
Please let us know your decision by signing your initials:

_______ Yes, I give permission to use my child's **genetic information** for COVID-19-related research

_______ No, I DO NOT give permission to use my child's **genetic information** for COVID-19 research

Compensation:

Your child will not receive any compensation while hospitalized. Your child will receive payment (compensation) in the form of gift cards as follows for completion of the following study procedures:

- Completion of each at-home self-collected sample: $10
- Completion of 14-day Flu-PRO Symptom Diary: $10 total *(for participants 7 years of age and older)*
- Blood draws at hospital/clinic after your child leaves the hospital: $50 per blood draw

HIPAA: AUTHORIZATION TO USE AND/OR DISCLOSE PROTECTED HEALTH INFORMATION FOR RESEARCH

PI Name and Rank: Brian Agan, MD, DrPH

Corps and Service/Organization: Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences
Title of Research Study: Epidemiology, Immunology, and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential

Purpose of this Document:
An Authorization is your signed permission to use or disclose your child's health information. The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule, as implemented by the Department of Defense (DoD), permits the Military Health System (MHS) to use or disclose your child's health information with a valid Authorization. The MHS is defined as all DoD health plans and DoD health care providers that are organized under the management authority of, or in the case of covered individual providers, assigned to or employed by, the Defense Health Agency (DHA), the Army, the Navy, or the Air Force. A valid Authorization must include the core elements and required statements as contained in this document.
Please read the information below and ask questions about anything you do not understand before deciding to give permission for the use and disclosure of your child's health information.
The Health Insurance Portability & Accountability Act of 1996, Public Law 104-109 (also known as HIPAA), establishes privacy standards to protect your child's health information. This law requires the researchers to obtain your authorization (by signing this form) before they use or disclose your child's protected health information for research purposes in the study discussed in this form. Note: Protected health information of military service members may be used or disclosed for activities deemed necessary by appropriate military command authorities to ensure the proper execution of the military mission.
By signing this authorization, you give your permission for information gained from your child's participation in this study to be published in medical literature, discussed for educational purposes, and used generally to further medical science. Your child will not be personally identified; all information will be presented as anonymous data.

A. What health information will be used or disclosed about your child?

- Names
- Address
- Date of birth
- DoD identification number
- Health information
- Health plan beneficiary numbers
- Phone numbers
- Fax numbers
- E-mail addresses
- Social security numbers
- Medical record numbers

B. Who will be authorized to use or disclose (release) your child's health information?
MTFs who have treated your child over the past ten years

C. Who will be authorized to use or disclose (release) your child's health information and who may receive your child's health information?

- The Institutional Review Board (USU IRB)
- Infectious Disease Clinical Research Program (IDCRP)
- Other Military Treatment Facilities or Department of Defense representatives
- Henry M. Jackson Foundation and their representatives
- Neoteryx and their representatives
- Local, State and Federal Government representatives, when required by law
- Defense Health Agency (DHA)
- Food and Drug Administration (FDA)
- Centers for Disease Control and Prevention (CDC)
- National Institute of Allergy and Infectious Disease (NIAID) and their representatives

The researchers and those listed above agree to protect your child's health information by using and disclosing it only as permitted by you in this Authorization and as directed by state and federal law.
You need to be aware that some parties receiving your child's protected health information may not have the same obligations to protect your child's protected health information and may re-disclose your child's protected health information to parties not named here. If your child's protected health information is re-disclosed, it may no longer be protected by state or federal privacy laws.

D. What if you decide not to sign this Authorization?

You do not have to sign this Authorization. If you decide not to sign the Authorization it will not affect your child's treatment, payment or enrollment in any health plans or affect your child's benefit eligibility. Your child will not be allowed to participate in this research study because your child's health information is required in order for them to take part in this study. The MHS will not condition (withhold or refuse) treatment that is not part of this study, payment, enrollment, or eligibility for benefits on whether you sign this Authorization.

E. Is your child's health information requested for future research studies?

Yes, your child's health information is requested for future research studies as specified below: Your child's health information will not be used for future research studies unless you give your permission by initialing your choice below:

____ YES, I give permission to use my child's health information for future research studies

____ NO, I do not give permission to use my child's health information for future research studies

F. Can you access your child's health information during the study?

You may have access to your child's health information at any time, unless their identifiers are permanently removed from the data.

G. Can you revoke this Authorization?

You may change your mind and revoke (take back) your Authorization at any time. However, if you revoke this Authorization, any person listed above may still use or disclose any already obtained health information as necessary to maintain the integrity or reliability of this research.

- If you revoke this Authorization, your child may no longer be allowed to participate in this research study.
- If you want to revoke your Authorization, you must write to the Study Doctor.

H. Does this Authorization expire?

No, it does not expire

I. What else may you want to consider?

- No publication or public presentation about the research described above will reveal your child's identity without another signed Authorization from you.
- If all information that does or can identify your child is removed from your health information, the remaining de-identified information will no longer be subject to this Authorization and may be used or disclosed for other purposes.
- In the event your child's health information is disclosed to an organization that is not covered by HIPAA, the privacy of your child's health information cannot be guaranteed.

If you have not already received a copy of the brochure entitled "Military Health System Notice of Privacy Practices," you may request one. DD Form 2005, Privacy Act Statement - Military Health Records (located on your child's medical records jacket), contains the Privacy Act Statement for the records. If you have any questions or concerns about your child's privacy rights, you should contact the USU Human Research Protections Program at 301-295-9534.

<u>Who can answer my questions?</u>

Any questions about this research study or if you believe your child's child has received a research-related injury, you may contact the lead for the study at your child's hospital (overall director based at USU) or the study Principal Investigator.

PRINCIPAL INVESTIGATOR: Overall director of this research study is Dr. Brian Agan at the Infectious Disease Clinical Research Program, Uniformed Services University of the Health Sciences (a DoD institution).

Questions? Call Dr. Agan at 1-833-475-0881 or contact at this address: Uniformed Services University of the Health Sciences (USU), Department of Preventive Medicine and Biostatistics, 4301 Jones Bridge Road, Bethesda, MD 20814-5119

LOCAL PRINCIPAL INVESTIGATOR (Study Doctor): – [Sites will fill in]

The USU Institutional Review Board is the research ethics committee responsible for safeguarding your child's rights as a research participant. If you have any questions about your child's rights as a research participant or any other concerns that cannot be addressed by the <u>Study Doctor</u>, you can contact the Director of the Human Research Protections Program at (301) 295-9534.

SIGNATURE PAGE:

You have been asked to provide permission for your child, for whom you are either the parent or legal representative, to participate in this research study. Your signature indicates that you have had this information presented to you and your child, have had the opportunity to ask questions about the research and your child's participation, and agree to permit them to participate in the study. Your signature also acknowledges that you authorize personnel to use and disclose your child's Protected Health Information (PHI) collected about your child for research purposes as described above. Further, your signature indicates that you have been offered a copy of this consent document. You will also be offered a copy of your child's assent document.

Printed Name of Parent/Guardian	Relationship to Participant
Parent/Guardian Signature	Today's Date
Printed Name of Study Representative	
Study Representative Signature	Today's Date

Verbal/physical limitation section: use the section below only if applicable

The subject's parent/guardian, ____ (name), has verbally consented for their child to participate in the study as described and also authorizes the use and disclosure their child's health information as described. The subject's parent/guardian is coherent and has understood the nature and consequences of participation in the study, but the subject's parent/guardian is physically disabled or in isolation and is unable to sign.

The consent and authorization information were accurately explained. The subject's parent/guardian was given the opportunity to ask questions. All questions were answered before giving verbal consent. The subject's parent/guardian apparently understood the information. The informed consent and authorization were given freely by the subject's parent/guardian.

Printed Name of Witness (if applicable)	
Witness Signature (if applicable)	Today's Date

Complete LAR section only if applicable. If the LAR has a copy of the power of attorney or court appointment, attach it to this consent document.

Printed Name of Legally Authorized Representative (LAR)	Relationship to Participant
LAR Signature	Today's Date
Printed Name of Witness (if applicable)	
Witness Signature (if applicable)	Today's Date

(USUHS) IRB 1
IRB NUMBER: 409136 (IDCRP-085)
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From: Simon Pollett on behalf of Simon Pollett <spollett@idcrp.org>
To: Munster, Vincent (NIH/NIAID) [E]; Eric Laing; Brian Agan; Caroline English
Cc: Broder, Chris (USU-DoD); De wit, Emmie (NIH/NIAID) [E]; Cammarata, Stephanie M CTR USN NAVMEDCEN SAN CA (USA)
Subject: Statement of work for Munster Lab; ethics approval
Date: Tuesday, November 3, 2020 1:17:12 PM
Attachments: IDCRP-085_CORE_AM14_Approval_20201016.pdf
IDCRP-085_CORE_Protocol_v11_EIRB v1.15_20200914.pdf
IDCRP-085_CORE_Adult_ICD_HIPAA_MTF Inpatient_v2.0_20200715_STAMPED.pdf
IDCRP-085_CORE_Adult_ICD_HIPAA_MTF Outpatient_v2.0_20200715_STAMPED.pdf
IDCRP-085_CORE_Adult_ICD_HIPAA_Online_v2.0_15JUL2020_STAMPED.pdf
IDCRP-085_CORE_Assent_v4.0_15JUL2020_STAMPED.pdf
IDCRP-085_CORE_Parental ICD_v4.0_20200715_STAMPED.pdf

Dr Munster and all,

We are enthused to start this collaboration on the EPICC project. See below for the next two steps:

1. Below is a description of the proposed scope of this work so we can get the agreements in place to send specimens and data to the Munster Lab. Is the below accurate?
    a. IDCRP/USU sends up to 500 uL of sera from 50 subjects and associated metadata from the EPICC study.
    b. Munster Lab perform wild type MERS, SARS-CoV-1 and SARS-COV2 neutralization on up to 50 SARS-CoV-2 positive sera with and without cross binding to MERS and SARS-1 (as determined by the Broder lab) with an objective to examine how cross-binding correlates with wildtype cross-neutralization.
2. For your NIH NHSR determination, please find the protocol, CV, and ICF attached.

Thanks, let me know if you have any questions

Simon

Dr. Simon Pollett, MBBS
Associate Scientific Director, HJF
Infectious Disease Clinical Research Program
Department of Preventive Medicine and Biostatistics
Uniformed Services University of the Health Sciences
6720A Rockledge Drive, Suite 250
Rockville, MD 20817
Phone: 310-895-8048
Email: spollett@idcrp.org

From: Simon Pollett
Sent: Wednesday, October 21, 2020 11:54 AM
To: 'Munster, Vincent (NIH/NIAID) [E]' <vincent.munster@nih.gov>; Eric Laing <eric.laing@usuhs.edu>; Brian Agan <BAgan@idcrp.org>; Caroline English <CEnglish@idcrp.org>

Cc: 'Broder, Chris (USU-DoD)' <christopher.broder@usuhs.edu>; 'De wit, Emmie (NIH/NIAID) [E]' <emmie.dewit@nih.gov>; 'Cammarata, Stephanie M CTR USN NAVMEDCEN SAN CA (USA)' <stephanie.m.cammarata.ctr@mail.mil>
Subject: RE: nhps and CoV-2 question

Hi all

Following up on the below:

1/ See current protocol and IRB approval to inform NIAID NHSR determination

2/ CCing in Stephanie Cammarata for agreements

3/ Eric and Chris: to confirm, is the proposed project synopsis essentially wt VNT of n = ?25 SARS-CoV-2 positive sera with and without cross binding to MERS and SARS-1 with an aim to examine how cross-binding correlates with wildtype cross-neutralization?

Thanks

Simon

From: Simon Pollett
Sent: Monday, October 5, 2020 7:59 AM
To: 'Munster, Vincent (NIH/NIAID) [E]' <vincent.munster@nih.gov>; Eric Laing <eric.laing@usuhs.edu>; Brian Agan <BAgan@idcrp.org>; Caroline English <CEnglish@idcrp.org>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
Subject: RE: nhps and CoV-2 question

Dr Munster,

I am one of the AIs on the EPICC study and the director of the COVID19 research area at IDCRP. Pleased to meet you and looking forward to this EPICC subproject. Dr Brian Again is the EPICC PI and deputy science director of IDCRP and Caroline English the CRM of EPICC. Stephanie Cammarata is our IDCRP Agreements Officer (among other roles). Below are the relatively simple next logistic steps required to execute this really interesting serology subproject.

1. Ethics review (NIAID NHSR determination) - Attached is the EPICC protocol v 9 for your preliminary review. I will send ICF and protocol for version 10. Caroline are you able to send the relevant DoD determination?

2. I discussed with Stephanie Cammarata and this should just require a SLA between USU and NIAID. We can initiate this on our side.

3. Study synopsis – for the purposes of #2, we'd need a brief synopsis. Eric and Chris, this sounds like wt VNT of n = ?25 SARS-CoV-2 positive sera with and without cross binding to MERS and

> SARS-1 with an aim to examine how cross-binding correlates with wildtype cross-neutralization?

Happy to assist on all the above as needed

Simon

Dr. Simon Pollett, MBBS
Associate Scientific Director, HJF
Infectious Disease Clinical Research Program
Department of Preventive Medicine and Biostatistics
Uniformed Services University of the Health Sciences
Phone: 310-895-8048
Email: spollett@idcrp.org

From: Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>
Sent: Tuesday, September 29, 2020 3:09 PM
To: Eric Laing <eric.laing@usuhs.edu>; Simon Pollett <spollett@idcrp.org>; Brian Agan <bagan@idcrp.org>; Caroline English <CEnglish@idcrp.org>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
Subject: [EXTERNAL] RE: nhps and CoV-2 question

Thanks Eric,

Sounds good, would be easy to determine any neutralizing cross-reactivity with MERS, SARS-2/2

Cheers,

Vincent Munster, PhD
Chief Virus Ecology Section
Rocky Mountain Laboratories
NIAID/NIH

From: Laing, Eric <eric.laing@usuhs.edu>
Sent: Tuesday, September 29, 2020 12:42 PM
To: Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>; Simon Pollett <spollett@idcrp.org>; Agan, Brian (IDCRP) <bagan@idcrp.org>; Caroline English

<CEnglish@idcrp.org>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
Subject: Re: nhps and CoV-2 question

Hi Vincent,

Looping back to this topic regarding MERS/SARS-CoV VNs/PRNTs and bringing in the IDCRP leadership that can begin an approval process for non-human research determination.

We have a handful of SARS-CoV-2 IgG positive serum samples (~15 - 20) with fairly high cross-reactivity to SARS-CoV-1 and MERS-CoV. Some of these serum samples are longitudinal collections from the same subject; over time, the cross-reactivity decreases as the IgG response matures and becomes monotypic for SARS-CoV-2. It would be interesting to write a short letter about cross-reactivity and cross-neutralization between SARS-CoV-2 IgG antibodies and high priority zoonotic bCoVs.

- Eric

Eric D. Laing, Ph.D.
Research Assistant Professor
Department of Microbiology and Immunology
Uniformed Services University
4301 Jones Bridge Road
Bethesda, MD 20814
cell: (301) 980-8192
office: (301) 295-9884
lab: (301) 295-9618

eric.laing@usuhs.edu

On Thu, Sep 10, 2020 at 9:47 AM Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov> wrote:

> Hi Chris,
>
> Would be easy to do some MERS and SARS-CoV-2 VNs or prnts.
>
> Cheers,
>
> Vincent Munster, PhD
> Chief Virus Ecology Section
> Rocky Mountain Laboratories
> NIAID/NIH

From: Laing, Eric <eric.laing@usuhs.edu>
Sent: Wednesday, September 9, 2020 8:34 PM
To: Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
Subject: Re: nhps and CoV-2 question

Hi Vincent and Emmie,

Any interest or bandwidth for testing SARS-CoV-2 patient serum samples that are highly cross-reactive with MERS-CoV and SARS-CoV-1 spike proteins to assess cross-neutralization potential? We have a handful of SARS-CoV-2 seroconverts that have a strong polyclonal response to SARS-CoV-1 and MERS-CoV, could be an interesting short translational letter if the antisera retains neutralizing antibodies.

- Eric

Eric D. Laing, Ph.D.
Research Assistant Professor
Department of Microbiology and Immunology
Uniformed Services University
4301 Jones Bridge Road
Bethesda, MD 20814
cell: (301) 980-8192
office: (301) 295-9884
lab: (301) 295-9618

eric.laing@usuhs.edu

On Fri, Sep 4, 2020 at 9:42 AM Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov> wrote:

> Hi Chris no plans for that,
>
> Housing the animals for 6 months after challenge into high containment would make it unfeasible. There are reports of back challenge after a month or so, but I think we should start getting data from humans soon,
>
> Cheers,
>
> Vincent Munster, PhD
> Chief Virus Ecology Section
> Rocky Mountain Laboratories

> NIAID/NIH
>
> ---
>
> **From:** "Broder, Christopher" <christopher.broder@usuhs.edu>
> **Date:** Thursday, September 3, 2020 at 10:01 AM
> **To:** "vincent.munster@nih.gov" <vincent.munster@nih.gov>, Emmie De wit <emmie.dewit@nih.gov>
> **Cc:** Eric Laing <eric.laing@usuhs.edu>
> **Subject:** nhps and CoV-2 question
>
> hi Vincent / Emmie.
>
> hope all is continuing to go so great.
>
> are you all going to try an NHP SARS-2 back challenge exp after
> waiting 6 or more months after primary infection and recovery? so expensive i know,
> might provide very informative data on the ab response / longevity / anamnestic response ect..
>
> chris

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIEN

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October 16, 2020

MEMORANDUM FOR BRIAN K AGAN, MD , P and R - Uniformed Services University of the Health

Sciences (USUHS) SUBJECT: USUHS IRB (FWA 00001628; DoD Assurance P60001) Approval

Modification of Protocol 409136 (IDCRP-085) for Human Subjects Participation

The modification to your Minimal Risk 32 CFR 219 / 21 CFR 56 human subjects research protocol 409136 (IDCRP-085) (ref # 931762) titled "***Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)"*** was reviewed and approved for execution on October 15, 2020 by Dr. Edmund G. Howe, M.D., J.D., chair USUHS IRB under the provision of 32 CFR 219.110(b)(2). This approval will be reported to the USUHS IRB scheduled to meet on October 22, 2020.

The protocol outlines a systematic approach to collection of clinical specimens and data by adapting standard procedures for conducting clinical characterizations of severe emerging infections as published by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) network (ISARIC/WHO Clinical Characterization Protocol for Severe Emerging Infections, version 3.1). .

This action approves the modification to update Appendix A of the protocol. This will reflect recent changes approved in the ICD/HIPAA document and set forth in the recent partial HIPAA waiver authorization indicating what information may be obtained from MDR or J6.

Authorization to conduct protocol 409136 (IDCRP-085) will automatically expire on July 25, 2021. If you plan to continue data collection or analysis beyond this date, IRB approval for continuation is required. Please submit a Continuing Review Form via EIRB to the IRB Office 60 days prior to your expiration date.

You are required to submit amendments to this protocol, changes to the informed consent document (if applicable), adverse event reports, and other information pertinent to human research for this project. No changes to this protocol may be implemented prior to IRB approval. If you have questions regarding this IRB action or questions of a more general nature concerning human participation in research, please contact Chris Murphy at christopher.murphy.ctr@usuhs.edu or (301) 319-0444.

(b) (6)

EIRB Protocol Template (Version 1.15)

1.0 General Information

***Please enter the full title of your study:**

Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)

***Please enter the Protocol Number you would like to use to reference the protocol:**

IDCRP-085 Version 11.0, 14-SEP-2020, eIRB v1.15
* This field allows you to enter an abbreviated version of the Protocol Title to quickly identify this protocol.

Is this a multi-site study (i.e. Each site has their own Principal Investigator)?

Yes

Does this protocol involve the use of animals?

○ Yes ● No

2.0 Add Site(s)

2.1 List sites associated with this study:

Primary Dept?	Department Name
○	**P and R** - Uniformed Services University of the Health Sciences (USUHS)

3.0 Assign project personnel access to the project

3.1 *Please add a Principal Investigator for the study:

AGAN, BRIAN K, MD

Select if applicable
☐ Student
☐ Resident
☐ Site Chair
☐ Fellow

3.2 If applicable, please select the Research Staff personnel:

A) Additional Investigators

AGAN, BRIAN K, MD
Associate Investigator
BRODY, DAVID L, MD PhD

Associate Investigator
BURGESS, TIMOTHY H, MD, MPH CAPT
Associate Investigator
BYRNE, Celia, PhD
Associate Investigator
Broder, Christopher C
Associate Investigator
Chung, Kevin Kee, MD COL
Associate Investigator
Coles, Christian Logan, PhD
Associate Investigator
DUMLER, JOHN S, MD
Associate Investigator
Dalgard, Clifton Lee
Associate Investigator
HAIGNEY, MARK C, MD
Associate Investigator
Hickey, Patrick Wilson, MD COL
Associate Investigator
Laing, Eric Daniel
Associate Investigator
Lanteri, Charlotte Anne, PhD LTC
Associate Investigator
Livezey, Jeffrey Robert, LTC
Associate Investigator
MALLOY, Allison M, MD
Associate Investigator
Mcleroy, Robert Dustin
Associate Investigator
Oliver, Thomas George, MD COL
Associate Investigator
Rajnik, Michael, MD Col
Associate Investigator
Rusiecki, Jennifer A.
Associate Investigator
SCHER, ANN
Associate Investigator
Simons, Mark Paul, PhD, D(ABMM), MScPH CDR
Associate Investigator
TRIBBLE, DAVID R, MD DrPH
Associate Investigator

B) Research Support Staff

Austin, Anissa Dawn
Research Coordinator
Chapo, Elisa Wago
Research Coordinator
English, Caroline E
Research Coordinator
Fox, Christina joy
Research Coordinator
Fraser, Jamie Alexandra
Research Coordinator

Hadley, Arile
Research Coordinator
Illinik, Luca, MPH
Research Coordinator
Law, Natasha N
Research Coordinator
Morales, Carlos Enrique
Research Coordinator
Moreno, Nicole C
Research Coordinator
Nevo, Lev N/A, M.D.
Research Coordinator
Rozman, Julia Sofia
Research Coordinator
Sanchez Edwards, Margaret Anne, MS
Research Coordinator
Spevak, Marianne V, BSHS
Research Coordinator

3.3 *Please add a Protocol Contact:

AGAN, BRIAN K, MD
BURGESS, TIMOTHY H, MD, MPH CAPT
Chapo, Elisa Wago
English, Caroline E
Fox, Christina joy
Illinik, Luca, MPH
Morales, Carlos Enrique
Moreno, Nicole C
Nevo, Lev N/A, M.D.
Rozman, Julia Sofia
Sanchez Edwards, Margaret Anne, MS

The Protocol Contact(s) will receive all important system notifications along with the Principal Investigator. (i.e. The protocol contact(s) are typically either the Protocol Coordinator or the Principal Investigator themselves).

3.4 If applicable, please select the Designated Site Approval(s):

Add the name of the individual authorized to approve and sign off on this protocol from your Site (e.g. the Site Chair).

4.0 Project Information

4.1 Is this a research study?

● Yes ○ No

4.2 What type of research is this?

☑ Biomedical Research

☐ Clinical trial (FDA regulated)
☐ Behavioral Research
☐ Educational Research
☐ Psychosocial Research
☐ Oral History
☐ Other

4.4 Is this human subjects research (Activities that include both a systematic investigation designed to develop or contribute to generalizable knowledge AND involve a living individual about whom an investigator conducting research obtains data through intervention or interaction with the individual or identifiable private information. Activities covered by 32 CFR 219.101(a) (including exempt research involving human subjects) and DoDI 3216.02)?

● Yes ○ No

4.5 Do you believe this human subjects research is exempt from IRB review?

○ Yes ● No

5.0 Personnel Details

5.1 Will you have a Research Monitor for this study?

○ Yes
● No
○ N/A

Research Monitor Role:

If applicable, you may nominate an individual to serve as the Research Monitor:

No Users have been selected.

6.0 Data/Specimens

6.1 Does the study involve the use of existing data or specimens only (no interaction with human subjects)?

○ Yes ● No

7.0 Funding and Disclosures

7.1 Source of Funding:

Funding Source	Funding Type	Amount

NIAID IAA	: Other	56632

Total amount of funding:

56632

7.2 Do you or any other Investigator(s) have a disclosure of a personal interest or financial nature significant with sponsor(s), product(s), instrument(s) and/or company(ies) involved in this study?

○ Yes ● No

8.0 Study Locations

8.1 List any Research Team members without EIRB access that are not previously entered in the protocol:

Name: (Last, First, M.I.) Clark, Danielle Role on Protocol: Associate Investigator	Phone Number: 508-471-7655	Email Address: dclark@aceso-sepsis.org	Associated Institution: Henry M Jackson Foundation
Name: (Last, First, M.I.) Blair, Paul Role on Protocol: Associate Investigator	Phone Number: 301-256-2165	Email Address: pblair@aceso-sepsis.org	Associated Institution: Henry M Jackson Foundation/USUHS
Name: (Last, First, M.I.) Koehler, Jeff Role on Protocol: Associate Investigator	Phone Number: 301-619-8342	Email Address: jeffrey.w.koehler4.civ@mail.mil	Associated Institution: US Army Med Rsch Inst of Infectious Diseases
Name: (Last, First, M.I.) Fries, Anthony Role on Protocol: Associate Investigator	Phone Number: 937-938-2847	Email Address: anthony.fries.tr@us.af.mil	Associated Institution: US Air Force Sch Aerospace Med
Name: (Last, First, M.I.) Hansen, Erin Role on Protocol: Associate Investigator	Phone Number: 619-857-5219	Email Address: erin.a.hansen.ctr@mail.mil	Associated Institution: Naval Health Research Center

Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Peel, Sheila Role on Protocol: Associate Investigator	301-319-2297	sheila.a.peel2.civ@mail.mil	Walter Reed Army of Institute Research
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Darden, Janice Role on Protocol: Associate Investigator	301-275-1190	jdarden@hivresearch.org	Walter Reed Army of Institute Research
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Letizia, Andrew Role on Protocol: Associate Investigator	240-507-6326	andrew.g.letizia.mil@mail.mil	Naval Medical Research Center
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Snow, Andrew Role on Protocol: Associate Investigator	301-295-3267	andrew.snow@usuhs.edu	USUHS
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Pollett, Simon Role on Protocol: Associate Investigator	301-319-2059	simon.d.pollett.ctr@mail.mil	Walter Reed Army of Institute Research
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Berry, Irina Role on Protocol: Associate Investigator	301-319-2032	irina.maljkovicberry.ctr@mail.mil	Walter Reed Army of Institute Research
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Friberg, Heather Role on Protocol: Associate Investigator	301-319-9224	heather.l.friberg-robertson.civ@mail.mil	Walter Reed Army of Institute Research
Name: (Last, First, M.I.)	Phone Number:	Email Address:	Associated Institution:
Currier, Jeffrey Role on Protocol:	240-994-3911	jeffrey.r.currier.civ@mail.mil	Walter Reed Army of Institute Research

Associate Investigator			

8.2 Has another IRB reviewed this study?

○ Yes ● No

IRB Name	Review Date	Determination	
No records have been added			

8.3 Is this a collaborative or multi-site study? (e.g., are there any other institutions involved?)

● Yes ○ No

8.4 Study Facilities and Locations:

Institution	Site Name	Site Role	FWA or DoD Assurance Number	Assurance Expiration Date	Is there an agreement?	IRB Reviewing for Site
Navy	Naval Health Research Center	Laboratory analyses	FWA00013516	03/02 /2021	IAIR	USUHS IRB #1
Army	US Army Med Rsch Inst Infectious Dis	Laboratory analyses	FWA00007368	10/02 /2024	MOU	USUHS IRB #1
Air Force	US AF Sch Aerospace Med	Laboratory analyses	FWA00027236	08/29 /2023	IAIR	USUHS IRB #1
Army	Walter Reed Army of Institute Research	Laboratory analyses	FWA00000015	11/20 /2023	Other	USUHS IRB #1
Navy	Naval Medical Research Center	Laboratory analyses	FWA00000152	06/05 /2024	IAIR	USUHS IRB #1

Other:

Other Institution Site	Site Role	FWA or DoD Assurance Number	FWA or DoD Expiration Date	Is there an agreement?	IRB Reviewing for Site	
No records have been added						

8.5 Are there international sites?

Attach international approval documents, if applicable, when prompted. Note: Ensure local research context has been considered

○ Yes ● No

8.6 Is this an OCONUS (Outside Continental United States) study?

○ Yes ● No

Select the area of responsibility:

Have you obtained permission from that area of responsibility? (This is a requirement prior to study approval)

○ Yes ○ No

9.0 Study Details

9.1 Abstract/ Summary:

Summarize the proposed study in 500 words or less, to include the purpose, the subject population, the study's design type, and procedures

The overarching objective of the Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC) protocol is to provide the Military Health System (MHS) with a contingency protocol intended for activation at Military Treatment Facilities (MTFs) during outbreaks to allow clinical investigation of severe or potentially severe acute infections with pathogens of concern to public health and U.S. military medical readiness. The protocol outlines a systematic approach to collection of clinical specimens and data by adapting standard procedures for conducting clinical characterizations of severe emerging infections as published by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) network (ISARIC/WHO Clinical Characterisation Protocol for Severe Emerging Infections, version 3.1).
The EPICC protocol can be conducted as a multi-site study at participating MTFs, and overall protocol oversight will be coordinated centrally by the Uniformed Services University's Infectious Disease Clinical Research Program (IDCRP). MHS-wide enrollment for online participation will be coordinated by the IDCRP Program Coordinating Center (PCC).
EPICC activities will be executed by clinical study teams comprising existing IDCRP clinical research investigators and staff located at each MTF and protocol investigators from relevant hospital departments. The protocol is designed to be modified to accommodate longitudinal observational study of specific emerging or re-emerging pathogens. Protocol modifications will describe methods for serial collection and clinical characterization of specimens and associated demographic, laboratory, clinical, and other data specific for the pathogen of interest and associated study population(s).

9.2 Key Words:

Provide up to 5 key words that identify the broad topic(s) of your study

Epidemiology, Immunology, Emerging Infectious Diseases

9.3 Background and Significance:

Include a literature review that describes in detail the rationale for conducting the study. Include descriptions of any preliminary studies and findings that led to the development of the protocol. The background section should clearly support the choice of study variables and explain the basis for the research questions and/or study hypotheses. This section establishes the relevance of the study and explains the applicability of its findings

The EPICC protocol is intended to serve as a foundation for modification to allow the observational, longitudinal study of specific emerging or re-emerging pathogens causing disease with pandemic potential within the MHS. Modifications responsive to specific diseases will be described to include a schedule for serial collection of clinical specimens and data and methods for recruiting, enrolling, and observational study within the desired MHS study population(s). In general, enrolled participants (eligible adults or children) who are known or suspected cases at MTFs as inpatients or in isolation settings will undergo serial collections of biological specimens and clinical and laboratory data at day of enrollment and during the duration of hospitalization or isolation, with follow-up specimen and data collection convalescent and long-term follow-up timepoints. The study design can also include clinical observational investigation in outpatients. The major focus of this protocol is to conduct clinical observational study in symptomatic individuals; however, there is also the opportunity to enroll and follow (potentially at a reduced specimen and data collection frequency relative to symptomatic participants) individuals who are asymptomatic but who are at high/some risk for exposure. Inclusion of an asymptomatic comparator cohort could provide insight on risk factors or biomarkers associated with symptomatic versus asymptomatic presentations. The protocol supports observational, longitudinal collection of clinical specimens and data with a goal of identifying host biomarkers that predict severity of illness, resistance to infection, treatment failure, etc. If volunteers receive an experimental treatment product under eIND, the data collected through the EPICC protocol may help inform about the safety and efficacy of that product.

In subsequent sections, relevant background information on acute respiratory infections (ARI) of pandemic potential is provided, followed by specific schedule of events and other details to allow observational study of an ongoing outbreak of the 2019 novel coronavirus (COVID-19).

Acute Respiratory Infections of Pandemic Potential: Epidemiology, Immunology and Clinical Characteristics

ARI Case Definitions

To characterize the epidemiology, immunology and clinical features of novel pathogens associated with severe ARI, case definitions appropriate to emerging infection scenarios will be utilized. These definitions will vary by the availability and result of laboratory-based diagnostic tests.

I. *For laboratory-confirmed cases*, the case definition includes the presence of laboratory-confirmed infection with an agent of severe ARI (e.g., SARS-CoV-2, influenza A/H7N9, MERS-CoV, SARS, etc.).

OR

II. For persons under investigation (PUI), the case definition includes:

1. The current CDC case definition for a PUI for a respiratory pathogen under study.
2. An unknown or pending laboratory confirmation of an agent of severe ARI.

III. For individuals who are asymptomatic and have had recent high-risk exposure to the pathogen of interest such that they have increased their risk for infection, 1 or more of the following criteria must be met:

1. Unprotected exposure (for e.g., < 14 days of illness onset, modified commensurate with evolving epidemiologic information) with confirmed or suspected cases of human infection with a novel respiratory pathogen. Close contact is typically defined as coming within about 6 feet (2 meters) of a confirmed or suspected case while the case was ill (beginning 1 day prior to illness onset and continuing until resolution of illness).
2. Unprotected exposure to infectious pathogens in a laboratory setting.
3. If exigencies warrant, then asymptomatic/high exposure risk individuals in isolation may be considered for enrollment.

Acute respiratory infections (ARI) are among the most common infectious diseases. Due to conditions and circumstances of military training and deployment (e.g., crowding, inadequate hygiene practices, physical stress, etc.), military personnel in congregate settings are at increased risk for outbreaks due to ARI. There are a number of emerging viral respiratory pathogens to which military forces may be exposed, that carry substantial importation risk, and that also have significant pandemic potential. Recent and current examples are described below.

1. Influenza A/H5N1 was first identified in the human population in Hong Kong in 1997. Since 2003, 16 countries have reported 668 confirmed cases and 393 deaths due to influenza A/H5N1 (as of 1 Nov 2014). The global case fatality rate for influenza A/H5N1 infection is 60%, and the estimates range widely (0% to 100%) by country. While the significant morbidity and mortality of influenza A/H5N1 infection is readily apparent, its occurrence among humans has been relatively infrequent to date. Circulation of H5N1 predominates in poultry, currently the main reservoir, in Asia and northeast Africa. Host-to-human (i.e., zoonotic transmission of A/H5N1) infections are unpredictable and “remain rare

despite frequent and widespread contact with infected poultry and contaminated environments" (1). The majority (71%) of cases of influenza A/H5N1 have had recent exposure to live birds or contaminated areas. Cases of influenza A/H5N1 to date have been relatively young (median age: 19 years) (2). Exposure to other cases of influenza A/H5N1 has not been a major risk factor to date, suggesting that the likelihood of human-to-human transmission of this particular strain remains low (R0< 1) (3). The severity of influenza A/H5N1 is dependent upon the strain associated with infection /disease. Among strains with low pathogenicity, generally mild, non-fatal illnesses have been observed, ranging from conjunctivitis to influenza-like illness (e.g., fever, cough, sore throat, muscle aches) to lower respiratory disease (pneumonia). Among strains with high pathogenicity, the disease spectrum has ranged from a milder febrile respiratory illness to severe disease (e.g., dyspnea, hypoxemia, pneumonia, acute respiratory failure) with multi-organ disease, sometimes accompanied by nausea, abdominal pain, diarrhea, vomiting and delirium. The high mortality rate (~60%) associated with pathogenic strains of influenza A/H5N1 has been reported from multiple studies (2).

2. Influenza A/H7N9 infection of humans was first identified in China in March 2013. Since then China has reported 453 confirmed cases and 175 deaths due to influenza A/H7N9 (WHO 2014). As with influenza A/H5N1, most of the cases had recent exposures to live poultry or had visited areas where live poultry was sold (75%) (3). By contrast to influenza A/H5N1, the host reservoir, main exposures, routes of transmission, and distribution and prevalence of influenza A/H7N9 among humans and animals are unknown. Influenza A/H7N9 does not appear to transmit easily from birds or the environment to humans. In addition, reported cases of Influenza A/H7N9 have not demonstrated high transmissibility between humans to date, suggesting that the risk of pandemic potential currently remains low (4). Though limited in its reach, influenza A/H7N9 infection has been associated with significant mortality: approximately 39% of cases resulted in a fatal outcome (4).
3. The coronavirus associated with the Middle Eastern Respiratory Syndrome (MERS-CoV) was first identified in Saudi Arabia in 2012 (5). Several outbreaks have since occurred, originating in Middle Eastern countries and spreading globally, albeit to a limited extent, by international travelers. Symptoms including fever, rigors, malaise, cough, myalgia, headache, shortness of breath, anorexia, diarrhea and abdominal pain were observed in MERS-CoV cases (6,7). As of October 2014, a total of 897 confirmed cases and 325 deaths (case fatality rate [CFR]: 36%) have been reported worldwide (WHO 2014). In 2013, two studies concluded that MERS-CoV did not yet exhibit pandemic potential, with an estimated basic reproduction number less than 1.0 (8, 9). Some research indicates that camels may act as the host reservoir for MERS-CoV, although evidence is inconclusive (10). Human-to-human transmission of MERS-CoV has been observed in healthcare settings, possibly due to greater infectiousness in the later stages of illness; however, evidence that may further elucidate risk factors for transmission is limited (6, 7).
4. The 2002-3 pandemic of Severe Acute Respiratory Syndrome (SARS), also due to a novel Coronavirus, originated in China's Guangdong Province and resulted in 8,422 probable cases and 916 deaths reported from 29 countries (mostly in mainland China, where 63% of cases and 38% of deaths occurred) (11). The global case fatality rate due to SARS was estimated to be 11% (11). Although the host reservoir remains unknown, some studies have found coronaviruses similar to the SARS coronavirus circulating in bat populations (12). Transmission in human populations occurs by direct contact with the infectious respiratory droplets and/or fomites via the eyes, nose, and mouth (13). The incubation period ranges from 1-14 days (mean 4-6 days) and transmissibility appears to increase with severity of illness (greatest at day 10) (14). Attack rates ranged widely by geographic region from 2.2 per 100,000 in Guangdong Province to 25.6 per 100,000 in Hong Kong (14). Risk factors for infection include occupation as a healthcare worker (21% of all probable cases), household contact with a probable case, older age, male sex and existing illnesses (14). Symptoms include fever, dry cough, dyspnea, headache and hypoxemia (14).
5. Since December 2019 through March 2020, the **2019 novel coronavirus (SARS-CoV-2) causing "coronavirus disease 2019" (COVID-19)** has spread from Wuhan, China to 64 countries and has led to more than 88,948 confirmed cases globally, including 80,174 confirmed cases in China and 8,774 confirmed cases outside of China (15). To date, there have been 2,915 deaths in China and 128 deaths outside of China. As of 03 March 2020, in the United States there are 60 confirmed cases (22 travel-related, 11 person-to-person spread, 27 under investigation) and a total of 6 deaths (16). Individuals with COVID-19 can present with fever, and cough, ground-glass opacity on chest computed tomography (CT), and lymphocytopenia (17). The case-fatality rate (CFR) appears to be highly variable with early reports of 8-15% in older adults infected in Hubei Province (CFR is increased in adults with comorbid conditions who develop respiratory symptoms) but likely no higher than 1-2% outside of Hubei (though data is limited and this will depend on accurate detection of those with mild disease) (18). Several risk factors, including age, history of smoking, maximum body temperature on hospital admission, respiratory failure, albumin, and C-reactive protein, appear associated with increased risk for progression to pneumonia among COVID-19 hospitalized patients (19). Risk factors of disease progression and magnitude of clinical severity are incompletely understood and require further study. SARS-CoV-2 is believed to be transmitted primarily via person-to-person, such as through close

contact (within about 6 feet) and exposure to respiratory droplets from an infected individual who coughs or sneezes (20). Evidence exists for transmission of SARS-CoV-2 via the oral-fecal route, as viral nucleotide was detected in rectal swabs and blood later in the course of infection when virus was not detected in oral swabs (21). On the population level, there is a poorly-defined risk of transmission via asymptomatic infection (22). The occurrence of asymptomatic infections has raised concerns about the rapid spread of transmission to vulnerable populations (21).

9.4 Objectives/Specific Aims/Research Questions:

Describe the purpose and objective(s) of the study, specific aims, and/or research questions/hypotheses

General Goals and Objectives:

1. To describe the natural history of clinical disease
2. To identify risk factors for infection and severe clinical course
3. To characterize the host immune response to infection
4. To evaluate the correlation between symptom severity, virologic/microbiologic characteristics, the host immune response and clinical course and outcome
5. To develop and/or operationally assess diagnostic and prognostic tools for the study of novel pathogens / emerging infectious diseases
6. To establish a clinical and laboratory database, and related specimen repository, for future studies of novel pathogens / emerging infectious diseases
7. To investigate efficacy of emergency Investigational New Drug (eIND) therapies that may be administered to enrolled study participants during the course of clinical care

For COVID-19 / SARS-CoV-2 infection, clinical and demographic data will be evaluated, along with laboratory findings as appropriate, to assess key endpoints of interest to include, but not limited to, the following:

- Incubation period
- Duration of illness / symptoms
- Duration of functional disability
- Duration of shedding from respiratory and gastrointestinal tract
- Incidence and duration of viremia
- Predictors of severe disease: clinical signs, symptoms, laboratory features; host factors, eg smoking, vaping, alcohol consumption, comorbid illness/conditions, occupational health factors influencing lung disease; host biomarkers; virologic (strain/virulence) attributes
- Supportive management required: O_2, HFNC, NIPPV, ventilation; hemodynamics
- Complications: e.g., bacterial pneumonia; bacteremia; AKI; liver injury; CNS involvement, etc.
- Outcomes: resolution of symptoms; return to pre-illness functional status; requirement for O_2; development / exacerbation of reactive airway disease; pulmonary function; resolution of medical complications; new Rx requirements, eg bronchodilators; other sequelae
- Development of immunity (i.e., correlates), kinetics, duration of antibody detection
- Relationship between acute illness (or pre-illness) HCoV titer and infection, outcome, virologic parameters
- Costs of care, costs of duty days lost

9.5 Study Design:

Describe study design in one to two sentences (e.g., prospective, use of existing records/data /specimens, observational, cross-sectional, interventional, randomized, placebo-controlled, cohort, etc.). Specify the phase – Phase I, II, III, or IV – for FDA-regulated investigational drug research

A prospective, longitudinal cohort study of novel pathogens / emerging infections with pandemic potential

9.6 Target Population:

Describe the population to whom the study findings will be generalized

Study findings will be generalized and applicable to all populations that travel to or reside in disease endemic areas or live within an area that has been exposed to infectious pathogens with pandemic potential; some of which include military personnel and the general public.

9.7 Benefit to the DoD:

State how this study will impact or be of benefit to the Department of Defense

Infectious diseases are a significant source of morbidity among military forces. Infectious disease outbreaks compromise force readiness and interfere with training programs. Strategies to characterize and ultimately to treat and prevent the major infectious disease threats to military personnel are urgently needed.

10.0

Study Procedures and Data management

10.1 Study Procedures:

Describe step-by-step how the study will be conducted from beginning to end

Recruitment and enrollment strategies will be dynamically responsive to the outbreak as the situation evolves. Study volunteers may be recruited from areas of the hospital where they present for care or in the inpatient and isolation settings. Informed consents and enrollments will be conducted by study staff included on the IRB approved protocol, including staff named on delegation log, and trained in study procedures. Clinical evaluations will be performed by the MTF healthcare team and may also be conducted by qualified study team members.
In addition to MTF enrollment, MHS beneficiaries who have been clinically tested for COVID-19 will be invited to participate in a limited schedule of assessments for EPICC, including completion of a series of online questionnaires and at-home capillary blood sample collection at three timepoints. See Appendix A for further details.
At the time of a participant's initial consultation at an MTF, study activities will consist of explanation of study goals and purpose, and obtaining informed consent (may include consultation with Legally Authorized Representative). The day of enrollment will be defined as Study Day 0. The team will subsequently conduct serial collection of data (demographics, vital signs, clinical findings, diagnostics and other laboratory results, etc.) through study participant interview and from medical record review from time of disease onset throughout study duration. Specific risk factors linked to pathogen exposure (eg., travel history to disease endemic regions, contact with sick individuals or known reservoirs of disease, etc.) will also be acquired from study volunteers, mainly through interview at Day 0. Medical records collected prior to day of enrollment will also be evaluated to ascertain medical history information pertinent to understanding baseline health and analysis of underlying risk factors associated with disease progression and other outcomes. Study team members will collect for research purposes biological specimens (e.g., nasopharyngeal and oropharyngeal swabs, rectal swabs, and blood) from enrolled participants who are laboratory-confirmed or suspected cases at time of enrollment, during the duration of hospitalization and/or isolation, with follow-up specimen collection up to approximately 1 year post-enrollment.

When possible, the study team will also obtain residual volumes of specimens collected for clinical care that would otherwise be discarded. Sample types include but are not limited to bronchoalveolar lavage, blood, urine, sputum, saliva, stool, cerebrospinal fluid, semen (adults only), or vomit.As available, these residual samples will be obtained from the time of onset of the disease under study. For active duty servicemembers, the study team will also request a pre-illness (up to 2 years prior to enrollment) serum sample from the DoD Serum Repository (DoDSR).

Some subjects may undergo clinical imaging studies as part of regular patient care or as part of this protocol using clinical imaging devices. For example, point-of-care portable ultrasound may be used to image areas such as the lungs, heart, and lower extremity veins, to explore the prognostic use of ultrasound in early detection of pneumonia and cardiovascular sequelae associated with severe disease. These imaging studies, when available, will be part of the collected subject data.

Study participants recruited and enrolled at MTFs who are ambulatory may also be evaluated via serial self-collection of samples such as nasopharyngeal swabs, oropharyngeal swabs, capillary blood samples, and/or other specimens appropriate for self-collection.

Recruitment of individuals who are asymptomatic at high/some risk of exposure may involve study team members approaching family or friends who escorted the sick individual to the hospital as well as healthcare providers who have potentially been exposed to known or suspected cases. Enrollees who are asymptomatic high/some risk will undergo serial collections of clinical and laboratory data as well as biological specimens (e.g., nasopharyngeal, oropharyngeal, and rectal swabs and blood) at enrollment, with follow-up specimen collection up to approximately 12 months post-enrollment. The study team may consider implementing a plan for asymptomatic study volunteers to conduct self-collection of clinical specimens (e.g. nasopharyngeal swabs, oropharyngeal swabs, rectal swabs, capillary blood smaples) through the follow-up period. Demographic, clinical, and other data as described above for symptomatic volunteers may also be acquired for asymptomatic high/some risk participants.

The Flu- PRO questionnaire (24-27) may be used to record the patient-reported outcome (PRO) on severity of clinical symptoms and the impact of clinical interventions on infection. A web link to the Flu-PRO questionnaire may be provided to participants to record the severity of ILI symptoms daily for 14 days from either the day of enrollment, or the date of onset of symptoms (for subjects who were asymptomatic at enrollment). Paper versions of the Flu-PRO questionnaire may be administered as needed.

In addition, an online questionnaire will be administered at enrollment and follow-up timepoints to collect data on medical history, comorbidities, behavioral risk factors, symptoms and recovery status.

If a participant is dually enrolled in a treatment protocol and scheduled to receive investigational products under an eIND, an additional sample may be collected in the first 48 hours after test article administration. If eligible, study participants may also be considered for enrollment concurrently in an interventional clinical trial evaluating efficacy of treatment regimens. The priority of collection of clinical specimens will be as follows: 1. specimens collected for clinical care; 2. specimens collected in support of an interventional clinical trial; and 3. specimens collected for observational study (i.e., EPICC protocol).

The study team may consider discontinuation of study participants who have a negative test result for the pathogen of interest (based on current standard of care diagnostics) and/or are diagnosed with another respiratory pathogen other than the one of interest and/or the disease of interest is highly unlikely based on the primary clinical team assessment. This approach may need to be taken to conserve study staff time and resources depending upon local epidemiologic circumstances, e.g. very high incidence and subject accrual.

Results obtained from research analyses will not be entered into the participant’s medical record. However, the study team will communicate relevant findings to the participant’s primary care provider overseeing care of the indicated infection of study. The study volunteer’s primary healthcare provider is likely to be a member of the protocol study team, and as such will be directly aware of study findings. If the primary healthcare provider is not a part of the protocol team, relevant findings from the study will be communicated to the healthcare provider verbally in a private setting to protect patient confidentiality or via a secure encrypted electronic communication.

Pregnant participants will be followed monthly until delivery to collect information on pregnancy outcome and health of the child with regards to the disease under study, to the extent practicable. A cord blood sample may be collected for research purposes following childbirth.

Specimens collected through this observational study will be evaluated in various laboratory assays to characterize the epidemiology, immunology, clinical characteristics, and other important outcomes of interest. Associated clinical and demographic data will also be evaluated, along with laboratory findings as appropriate, to assess key endpoints of interest as described in Section 9.4.

Schedule of Patient Evaluation and Data Collection

The table below shows the schedule of assessments for study participants enrolled in EPICC. Given the possibility of critical illness of the patient and the potential increased risk of exposure to medical personnel, assessments that cannot be completed due to patient care considertaions, indection control measures, or other logistical challenges will not constitute protocol deviations. In addition, the frequency of specimen and data collection may be reduced to accommodate logistics of acquiring specimens and data among study volunteers who are outpatients.
Ambulatory subjects who do not have restrictions of movement for infection control purposes (i.e., not advised by their care provider/local health authority to remain home in isolation) will be invited to return to the hospital for follow-up assessment and collection of specimens.

If an asymptomatic and/or COVID-19-negative subject develops symptoms or receives a positive test result for COVID-19, they will be asked to contact study staff to report their symptoms/test result and will restart the schedule of assessments from Day 0.

Inpatient MTF-Enrolled Subjects

Specimen/ Assessment	Enrollment (Day 0)	Days 3, 7 (± 1 day)	Once Weekly until discharge (± 2 days)	Day 14 if Discharged (± 3 days)	Day 28 (± 7 days)	6 Months (± 14 days)	12 Montl (± 28 day
Virology Sample: Nasal, Throat, and/or Oral specimen*	√	√	√	√			
Virology Sample: Rectal Swab	√	√	√		√		
Blood	√	√	√	√¶	√¶	√¶	√¶
Residual Clinical Samples #	√	√	√	√	√	√	√
Radiologic images **	√	√					
Flu-PRO Symptom Diary ***	√	√	√	√			
Online Questionnaire	√				√	√	√

* To detect and analyze virus in the nose, throat, and/or mouth, the specimen collection method employed may change to follow updated best practices as reported in the scientific literature. Example sample types are swabs (nasal, nasopharyngeal, oropharyngeal) and/or saliva that may be collected by study staff or self-collected by subjects.
¶ Blood collection after discharge may be done using a commercial at-home microsampling blood collection kit.
# Residual samples from clinically-indicated lab tests will be collected for research. Sample types may include sputum, saliva, urine, stool, blood, semen (adults only) or vomit. Residuals may be obtained from samples collected starting at the date of disease onset, which may be prior to Study Day 0.
** Lung, cardiac, and/or venous ultrasound may be conducted on hospitalized subjects on days 0, 1, and 3 as resources and local patient care policy allows. Findings from any additional radiologic images obtained during routine clinical care will be collected for research.
*** Subjects aged 7 and older who are experiencing symptoms will be asked to complete the Flu-PRO diary for 14 days.

Outpatient MTF-Enrolled Subjects

Specimen/ Data	Enrollment (Day 0)	Day 7 (± 1 day)	Day 14 (± 3 days)	Day 28 (± 7 days)	6 Months (± 14 days)	12 Months (± 28 days)
Virology Sample: Nasal, Throat, and/or Oral specimen*	√‡		√			
Blood¶	√‡	√	√	√	√	√
Flu-PRO Symptom Diary **	√	√	√			
Online Questionnaire	√			√	√	√
Residual clinical samples #	√	√	√	√	√	√

* To detect and analyze virus in the nose, throat, and/or mouth, the specimen collection method employed may change to follow updated best practices as reported in the scientific literature. Example sample types are swabs (nasal, nasopharyngeal, oropharyngeal) and/or saliva that may be collected by study staff or self-collected by subjects.
¶ Blood collection may be done using a commercial at-home microsampling blood collection kit.
# Residual samples from clinically-indicated lab tests will be collected for research. Sample types may include sputum, saliva, urine, stool, blood, semen (adults only) or vomit. Residuals may be obtained from samples collected starting at the date of disease onset, which may be prior to Study Day 0.
** Subjects aged 7 and older who are experiencing symptoms will be asked to complete the Flu-PRO diary for 14 days.
‡ There is a +3 day window for Day 0 research sample collection to allow for distribution of self-collection kits to participants whose samples could not be collected at enrollment.

Online Cohort: See Appendix A

Specimen/ Data		Day 28		

	Enrollment (Day 0)	(± 7 days)	6 Months (± 14 days)	12 Months (± 28 days)
Online Questionnaire	√	√	√	√
Self-collected blood¶		√	√	√

¶ All blood collection for this cohort will be done using a commercial at-home microsampling blood collection kit.

Considerations for Pediatric Participants

- Flu-PRO symptom diary may be completed by subjects aged **7 and older**.
- Pediatric subjects will not be asked to complete the online questionnaires.
- Self-collection of specimens is an option for subjects aged **8 and older**.
- Blood collection volume and frequency may be reduced to comply with safety limits. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.

Research Laboratory Methods

Clinical specimens collected through this observational study will be evaluated in a variety of laboratory assays developed by laboratory partners. Specific laboratory assays to study the virologic, immunologic, and other relevant aspects of infections are unlikely to be available at the time of specimen collection (i.e., during the outbreak) given the time required to establish assays specific for novel, emerging pathogens. Below is a general description of laboratory assay methods and endpoints that may be used; however, this does not represent an exhaustive list to include new methodologies that are later developed in response to assaying novel pathogens.

To characterize virologic correlates (e.g., viremia) of clinical symptoms and disease severity, serum levels of viral genomes can be assessed by real time-PCR at various time points. Serum concentration of viral antigen can be evaluated by enzyme-linked immunosorbent assay (ELISA). These same assays may be utilized in the longitudinal detection of virus in body fluids (e.g., nasal mucosa, sputum, urine, stool, etc.) throughout the course of evaluation/illness.

In addition, serum may be assayed for concentrations of virus-specific IgM and IgG in the acute and convalescent stages of evaluation/illness. Cytokine profiles can be conducted via multiplex assay to describe the pro- versus anti-inflammatory cytokine responses of study volunteers throughout the course of illness. PBMCs may be collected to investigate cell-mediated immune responses in the acute and convalescent phases of illness.

Novel biomarkers, including transcriptomic and proteomic signatures, are promising for diagnosis and prognosis. Collection of appropriate specimens from individuals with well-characterized illnesses is required to inform assay development. Immune factors have been used as biomarkers for predicting clinical course and outcome, as well as the severity of clinical symptoms. For example, higher levels of cytokines IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNFα were observed in patients with SARS-CoV-2 infection requiring ICU-level care compared to those receiving non-ICU care (21). Host specific responses may be evaluated using real-time PCR and other molecular methods to identify biomarkers, transcriptomic signatures, or other lab-based endpoints indicative of severity of clinical course of disease progression and other risk factors associated with clinical outcomes, etc. In addition, evaluation of host transcriptomic responses among symptomatic versus asymptomatic, high risk exposure individuals may identify an early warning means for identifying asymptomatic people who eventually develop disease. RNA sequencing of blood specimens may also be conducted to assess viral transcription signatures as biomarkers for clinical outcomes. Blood specimens may also be used in phosphoproteomic assessments by measuring the proportion of host immune system proteins that are phosphorylated to evaluate novel biomarkers associated with activation of protein signaling pathways in response to infection.

10.2 Data Collection:

Describe all the data variables, information to be collected, the source of the data, how the data will be operationally measured, and approvals needed for use of information from DoD databases

Diagnostic Tests and Laboratory Analysis to be Obtained:

As available, laboratory data elements, such as but not limited to hematologic, biochemical, and other clinical parameters; clinical images and associated reports; and clinical diagnosis of the pathogen of interest (SARS-CoV-2) and

/or alternate or co-infective pathogens, will be abstracted from the medical record of study volunteers on the day of enrollment (Study Day 0), throughout the period of hospitalization (inpatients) and through duration of follow-up. In addition, laboratory and diagnostic data available in medical records prior to Day 0 will be abstracted for study of baseline health, existing co-morbidities, etc.

Data Elements

Case report forms (CRFs) for this protocol have been adapted from those of the International Severe Acute Respiratory Infection Consortium (ISARIC).

There are five main components of the data collection forms, encompassing study participant information at:

1. The time of enrollment (at time of presentation at MTF, hospital admission, or being placed in isolation),
2. Period prior to Day 0, as appropriate, to assess recent medical history,
3. Medical encounter data (inpatient or outpatient),
4. The time of discharge or death
5. Follow-up period (following hospital discharge or being released from isolation setting, or otherwise as an out-patient)

Major data variable categories to be collected include, but are not limited to the following:

- Day of enrollment
    - Demographics (Residence, Occupation, Military Status)
    - Signs and Symptoms at onset of illness and presentation for care
    - Epidemiological Risk Factors and Exposures
    - Pre-admission Medications
    - Review of recent medical history via medical record review
    - Flu-PRO symptom severity questionnaire
- Hospitalization or Isolation Period
    - Daily Observations (i.e., vital signs) and Treatments (i.e., receipt of IV fluids)
    - Daily Laboratory Results
    - Medications and Blood Products
    - Microbiology Results
    - Critical Care
    - Severe Symptoms and Complications
    - Flu-PRO symptom severity questionnaire
- Outcome
    - Final Diagnosis
    - Patient Status at Outcome
    - Follow-up period

Data Collection

Study data will be copied onto electronic CRFs at the study site. These transcriptions will occur on an ongoing basis during the study in accordance with Good Clinical Practice (GCP) guidelines. Data entered on CRFs, as well as corrections made, shall be performed by authorized individuals. This study will make use of electronic case report forms (eCRFs) using tablets with the Research Electronic Data Capture (REDCap). For cases where a tablet cannot be used because of infection control concerns or logistical issues, authorized personnel will capture CRF information using a paper based version and later electronically record results into REDCap. All data transmitted to and from the application is done using a secure, encrypted transmission (SSL/HTTPS). For increased security, the application additionally verifies the SSL certificate of the REDCap server that it is communicating with in order to validate the server’s identity. The REDCap mobile application employs encryption-at-rest on the mobile device’s hard drive, so all data and information stored on the device is protected from unauthorized users. REDCap has built-in safeguards such as username and PIN, and remote lockout. When resources are available, data from clinical devices including de-identified ultrasound or radiograph images, continuous vital signs, and/or cardiac telemetry will be locally stored on password-protected devices, and if possible, transmitted wirelessly to a secure Cloud backup. Henry Jackson Foundation (HJF) headquarters IT will host deidentified study data on HJF HQ secured servers. HJF is responsible for the purchase, setup, maintenance, and service of any its servers and technology.

The supervising principal investigator, or designated associate investigator, will be responsible for regular maintenance and review of CRFs for accuracy. Access to study files (e.g., CRFs) is restricted to care providers and research team members given specific tasks to perform. The files are kept in a secured location in each study site. Some participant-completed forms such as questionnaires may be completed through the web-based program. These will be transmitted directly into the study database. Research labs will transmit their testing data to the DCC using standard procedures for data handling and transmission.

Data Management/Quality Control

During the study, the investigator will review study data from their site, including medical records, records detailing the progress of the study for each volunteer, laboratory reports, CRFs, documentation of informed consent and authorization forms for each study volunteer, correspondence with the IRB, adverse event (AE) reports and information regarding volunteer discontinuation of the study. All required study data will be clearly and accurately recorded on source documents or CRFs by either the participant themselves or authorized study personnel. Only designated study personnel shall record or change data on a CRF. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded on the CRFs. Original observations entered directly onto the CRFs are defined as source data. PHI will be removed from clinical devices, such as those used for radiology, and associated data will be labeled with the study participant's ID. For each pivotal analysis (e.g., for a sub-study, manuscript, or presentation), the 'snapshot' (or 'data freeze') database will be made and archived in the study data repository. After the study analysis ends, the database will be locked.

10.3 At any point in the study, will you request, use, or access PII from the Military Health System (MHS)?

● Yes ○ No

10.4 Have you consulted with an MHS data expert to determine the data elements to be extracted or the information system(s) to access?

Consulting with a data expert often saves time later in the compliance process because the data expert can advise on the data available in the numerous MHS information systems, the quality of that data and the methods for encrypting and collapsing data. To schedule a consult with an MHS data expert, send an email to: (**dha.ncr.pcl.mbx.privacyboard@mail.mil**)

● Yes, then complete the questions below according to the data consult

○ No, then complete the questions below according to the best of your knowledge (NOTE: It is highly recommended that you work with an MHS data expert)

10.5 Indicate whether you plan to receive a data extract from the MHS or plan to access an information system directly to create a data set:

A data extract is when the MHS or a contractor provides the data set directly to the researcher. When receiving a data set through data extract, the researcher may indicate whether the data elements should be provided as is, encrypted or collapsed. In contrast to a data extract, access to an information system means that the researcher may directly access an MHS information system and create a data set for the research study

☐ Data Extract

☑ Access

10.6 Do you intend to use only de-identified data from the MHS in your research study?

There are different two methods for de-identifying data pursuant to HIPAA:
1) Safe Harbor Method: Removing all of the identifiers listed in Table 1 below, provided that the researcher does not have actual knowledge that the remaining data can be used alone or in combination with other information to identify the individual who is the subject of the information
2) Statistical Method: An expert, with appropriate knowledge of and experience with generally accepted statistical and scientific principles and methods for rendering information not individually identifiable, determines that the data is not individually identifiable

○ Yes ● No

10.7 If your research study requires access to an MHS information system, please indicate the system to obtain data:

If you do not know which system(s) contain the data elements you need, refer to the Guide for DoD Researchers on Using MHS Data or seek guidance from an MHS data expert:

PHI Systems:

MHS Information System	Requesting Data

:AHLTA	:Yes
:CHCS	:Yes
:ESSENTRIS	:Yes
:MDR	:Yes

PII-Only Systems:

MHS Information System	Requesting Data	
No records have been added		

De-Identified Data & Other Systems:

Information System	Requesting Data
Expense Assignment System	
MHS Genesis; JLV (accessed via AHLTA or MHS Genesis)	:Yes
DMSS, DRSi	:Yes

10.8 Do you intend to merge or otherwise associate the requested data with data from any sources outside of the MHS, including other DoD systems that are not part of the MHS?

● Yes, will merge data
○ No, will not merge data

10.9 Indicate the categories of data that you will request from MHS systems or MHS health care providers about research participants or relatives, employers, or household members of the research participants.

Data Element(s)	MHS	Non-MHS Systems
1. Names	☑	☐
2. Postal address with only town, city, state and zip code	☑	☐
3. Postal address with all geographic subdivisions smaller than a state, including street address, city, county, precinct, zip code and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly available data from the Bureau of Census: 1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people; and 2) the initial three digits of a zip code for all such geographic units containing	☑	☐

20,000 or fewer people is changed to 000		
4. Dates including all elements (except year) directly related to an individual, including birth date, admission date, discharge date, and date of death	☑	☐
5. Ages over 89 and all elements of dates (including year) indicative of such age, unless you will only request a single category of “age 90 or older”	☑	☐
6. Telephone numbers	☑	☐
7. Fax numbers	☑	☐
8. Electronic mail addresses	☑	☐
9. Social Security numbers (SSNs)	☑	☐
10. Medical record numbers	☑	☐
11. Health plan beneficiary numbers	☑	☐
12. Account numbers	☐	☐
13. Certificate/license numbers	☐	☐
14. Vehicle identifiers and serial numbers, including license plate numbers	☐	☐
15. Device identifiers and serial numbers	☐	☐
16. Web Universal Resource Locators (URLs)	☐	☐
17. Internet Protocol (IP) address numbers	☐	☐
18. Biometric identifiers, including finger and voice prints	☐	☐
19. Full-face photographic images and any comparable images	☐	☐

20. Any other unique identifying number, characteristic, or code (DEERs ID, EDIPN, Rank)	☑	☐

If you are obtaining SSNs, provide a justification as to why and explain why a substitute cannot be used

We request to access potential subjects' medical records in order to evaluate them for recruitment for this protocol. PHI to be screened include (name, date of birth, social security number and health beneficiary numbers). These risks are no more than minimal because they are not greater than those ordinarily encountered by the patient during a medical visit.

Study investigators and personnel will review potential subjects' medical history to assess protocol eligibility. If the person consents to participate in the research study, the PHI will be stored in accordance with the protocol.

10.10 Is it possible that the data will become identifiable because of triangulation, a small cell size, or any unique data element(s)?

Triangulation means using different data elements that are not themselves identifiable but that when combined can be used to identify an individual. For example, triangulation would be using rank and race together to determine the identity of an individual with a particular health condition
Small cell size means that there are only a small number of eligible individuals that satisfy the category description. Guidance for acceptable cell size is available from the Centers for Medicare and Medicaid Services. For example, the rank category of four star generals with a particular diagnosis may be less than 30 so the rank category may need to be expanded to include lower ranks
A unique data element includes any unique features that are not explicitly enumerated in the categories of data in rows 1 – 19 of Table 1 above, but that could be used to identify an individual. Examples of unique data elements include: 1) a unique number, such as a medical record number or EDIPN; 2) a unique code, such as a diagnosis code or a bar code on an electronic health record; and 3) any unique characteristic, such as the rank of general or admiral, or a race or gender combined with another unique characteristic

● Yes, there is a reasonable possibility the data will become identifiable
○ No, there is no reasonable possibility the data will become identifiable

10.11 HIPAA Privacy Rule and Use of Protected Health Information in Research:

○ N/A – will not use or disclose protected health information (PHI)
○ HIPAA Authorization will be obtained
○ Use of a limited data set where a data use agreement will be obtained
● Waiver/alteration of HIPAA Authorization is being requested

10.12 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for this Study:

Include in this section the plan for acquiring data (both electronic and hard copy), access during the study, data/specimen storage and length of time stored, shipment/transmission, and the plan for storage and final disposition at the conclusion of the study. Describe any data agreements in place for accessing data within and/or outside of your institution (e.g., Data Sharing Agreement, Data Use Agreement, Business Agreements, etc.)

Human Biological Specimens

1. Intended Use of the Samples/Specimens/Data

Specimens will be processed and handled as appropriate following applicable MTF enhanced precaution and Infection Control SOPs. Methods for specimen collection, handling, and processing are described in their respective SOPs. Details of specimen storage, maintenance, and QA/QC are described in their respective SOPs.

Samples and data collected under this protocol will be analyzed to study the pathogenesis of disease and the host immune response to infection. Genetic testing on human specimens
will be conducted to evaluate the host (subject's) immune response to SARS-CoV-2 infection and its association with clinical outcomes. Additional genetic testing will examine viral genetic markers and transcription signatures as predictors of infection severity, clinical course, and outcomes. Genetic testing results will not be provided to participants.

This is a purely observational study. Any experimental treatments will be done under other protocols for which separate IRB review and approval will be obtained.

2. How Samples/Specimens/Data Will Be Stored

Access to research samples will be limited using locked facilities and monitored freezers. Samples and data will be stored using codes assigned by the investigators or their designee(s). Data will be kept in password-protected computers. Only investigators or their designee(s) will have access to the samples and data.

3. How Samples/Specimens/Data Will Be Stored & Tracked

Samples from individuals found infected with a novel pathogen / emerging infectious disease of interest will be archived and stored at an appropriate biosafety level facility.

Samples acquired as part of the protocol will be tracked using appropriate software, such as Freezer Works, a sample tracking, biorepository management and freezer inventory program.

4. What Will Happen to the Samples/Specimens/Data at the Completion of the Protocol?

In the future, other investigators may wish to study these samples and/or data. IRB approval will be sought prior to any sharing of samples/data. Any clinical information associated with the sample, with or without patient identifiers, will be shared after IRB approval has been obtained.Upon study closure, all samples and data will be de-identified in accordance with the DHA-approved Data De-Identification Plan.

5. What Circumstances Would Prompt the PI to Report to the IRB Loss or Destruction of Samples/Specimens/Data

Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB.

6. Storage and Tracking of Samples/Specimens/Data

Research use of data collected through this protocol will be coded and will not contain identifiers. These data will be coded with access maintained by a custodian not affiliated with the study team per IDCRP policy. Much of the subsequent research using the coded materials collected under the auspices of this study will be accomplishable through approved exempt or non-human use sub-studies. All research using the materials collected under this protocol will be conducted in accordance with an IRB-reviewed protocol. Data and specimens released for research use will be labeled by PIN only; data will be shared without any identifiers or links to individuals. The DCC custodian, the DCC Chief, will not share identifying information with investigators without explicit IRB approval.

A Defense Health Agency (DHA) Data Sharing Agreement will be implemented as needed to access MHS data. Appropriate data sharing agreements and other legal agreements will be implemented prior to sharing of data and specimens with collaborators.

10.13 Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for Future Research:

If the study involves collecting, storing, or banking human specimens, data, or documents (either by the Investigator or through an established repository) for FUTURE research, address. How the specimens /data will be used, where and how data/specimens will be stored (including shipping procedures, storage plan, etc.), whether and how consent will be obtained, procedures that will fulfill subjects' request as stated in the consent, whether subjects may withdraw their data/specimens from storage, whether and how subjects may be recontacted for future research and given the option to decline, whether there will be genetic testing on the specimens, who will have access to the data/specimens, and the linkage, the length of time that data/specimens will be stored and conditions under which data/specimens will be destroyed

Establishment of a specimen repository for future clinical and laboratory investigation for future emerging infectious disease research:

The study aims to build a repository of biological specimens (e.g. whole blood, serum, and PBMCs, as well as nasopharyngeal fluid, sputum, bronchioalveolar lavage fluid, oropharyngeal fluid, saliva, urine, semen, and rectal swab specimens, etc.) from an observational, longitudinal study of emerging infectious diseases. Specimens may be stored in an appropriate biosafety level facility at a protocol-approved biorepository. These specimen repositories will be linked to an extensive de-identified clinical database.

In the future, this repository might be used in the identification and validation of biomarkers for severe infection, identification and molecular characterization of novel pathogens, development and evaluation of new diagnostic platforms, and/or evaluation of host factors that may be associated with susceptibility and outcome. Coupled with detailed clinical history and risk factor information gleaned through electronic medical record review, this repository will ultimately be an invaluable resource for future investigations of emerging infectious diseases in clinical as well as laboratory settings. Moreover, the infrastructure developed through these activities will serve as a broad-based platform for a diverse portfolio of relevant sub-studies into emerging infectious diseases and strategies for its control and prevention.

Study Time Line

This natural history core protocol will be open indefinitely, although sites may close once an epidemic has passed.

11.0 Statistical/Data Analysis Plan

11.1 Statistical Considerations:

List the statistical methods to be used to address the primary and secondary objectives, specific aims, and/or research hypotheses. Explain how missing data and outliers will be handled in the analysis. The analysis plan should be consistent with the study objectives. Include any sub-group analyses (e.g., gender or age group). Specify statistical methods and variables for each analysis. Describe how confounding variables will be controlled in the data analysis

Statistical Considerations

This is an enhanced surveillance protocol using clinical observations and laboratory testing, supplemented by additional lab testing for research purposes. The frequency and expansion of such outbreaks is unknown, and hence this protocol will be activated on contingencies in many cases. Data from a given outbreak will be compiled into a dataset for descriptive analyses. Risk factors will be identified retrospectively using odds ratios. Comparison between similar outbreaks may be performed if sufficient data allows. It is recognized that a proportion (i.e., not all) of eligible subjects will consent to participate in this study. As such, the limitations and generalizability of the study findings will be acknowledged in associated reports and manuscripts.

11.2 Sample Size Estimation:

As determined by exigencies of the outbreak, and resource availability. Please see Appendix A for sample size considerations for the online cohort.

11.3 Data Analysis Plan:

Data Analysis Plan

Student’s t test (or Wilcoxon rank-sum test if appropriate) on continuous variables and Pearson’s chi-square tests (or Fisher’s exact test if appropriate) for categorical variable to summarize key outcomes of interest. Multivariate regression techniques will be used to assess relationships between key variables adjusted for potential confounders.

A data analysis plan will be created to meet the following reporting requirements:

- During an outbreak, descriptive clinical and laboratory data will be summarized per patient on a regular basis as appropriate for all investigators, involved hospitals, the respective service's public health agencies, AFHSB, and DHA leadership as a part of the epidemiologic reporting requirements. It is recognized this data may not be fully cleaned. Aggregate data report will be prepared monthly.
- At conclusion of an outbreak, a summary report per outbreak will be prepared once all data is cleaned. This will take an estimated 90 days.

With respect to the analysis of the study data, the following objectives will be addressed, and more fully articulated, in a statistical analysis plan:

1) Describe natural history of clinical disease.

Data items related to disease duration include duration of fever and other predominant symptoms, and days from onset of symptoms until hospital discharge/return to normal activity. Each variable will be summarized by mean, standard deviation, median, inter-quartile range, minimum and maximum overall and also for each group within several groupings: age categories, adult vs. minor, gender, different race groups, index cases vs. secondary cases, military rank and/or nature of duties for those with active duty military duty status. We will also use symptom severity data from the Flu-PRO questionnaire to explore the extent to which ARI symptom severity scores differs across the cohort.

Adverse binary outcomes where the time to event may also be of interest: These include requirement for ICU admission, development of any super infection, development of oxygen requirement, mortality, etc. For each of these, we will first give a simple summary of the number and percent experiencing each outcome. We will also summarize by different subgroups as above.

2) Identify risk factors for infection and severe clinical course.

One set of analyses will treat laboratory-confirmed diagnosis as the outcome in logistic regression models, with the following covariates: age, sex, race, military rank, service branch, type of exposure, use of personal protective equipment, etc. Similar logistic regression models will be developed to determine predictors of severe clinical course, such as mortality.

Another set of analyses will treat illness duration as a quantitative endpoint to be used as the outcome variable in multiple linear regressions. The key endpoint will be the total symptom score considered at three different time points: at symptom onset, at 48 hours post symptom onset, and at its maximum value whenever that occurs. Other potential confounders to be included as covariates are: age, sex, race, and number of days since presumed exposure.
Complementary methods of subject recruitment and data collection within the study will address the following specific aims:

1. Among COVID-19 confirmed cases, compare risk factors (comorbidities, lifestyle factors, medications, exposure opportunities, general symptoms) for:
    1. Those who get hospitalized vs. those who do not get hospitalized
    2. Those who get hospitalized with a ventilator vs. those who do not get hospitalized and those hospitalized but not placed on a ventilator
    3. Those who get hospitalized in the ICU vs. those who do not get hospitalized and those hospitalized but not in the ICU
    4. Those who go on to die vs. those who do not die

1. Compare risk factors (comorbidities, lifestyle factors, medications, exposure opportunities, general symptoms) for infection among the total COVID-19 tested study population of COVID-19 confirmed cases (test positive) vs. confirmed non-cases (test negative).
2. Compare symptoms between various groups:
    1. Among the total COVID_19 tested study population, COVID-19 confirmed cases vs. confirmed non-cases
    2. Among COVID-19 confirmed cases for those:
        1. who get hospitalized vs. those who do not get hospitalized
        2. who get hospitalized in the ICU vs. those who are hospitalized, but not in the ICU
        3. who go on to die vs. those who do not die
3. Among the COVID-19-confirmed cases and an appropriate and hypothesis-specific randomly selected comparison group, using medical record data, identify comorbidities and medications that are associated with reduced occurrence of COVID-19 symptoms as well as with becoming infected with COVID-19.

The proposed analytic strategy will be to report crude (non-adjusted), age and sex-adjusted, and multivariable adjusted probability of hospitalization in those with and without the exposures of interest.

1. Calculate crude (non-adjusted) likelihood of hospitalization in demographic sub-populations of interest (e.g. by age strata, sex, and race/ethnicity)

2. Determine the age-specific prevalence of the major comorbidities (obesity, hypertension, asthma) separately by sex and race/ethnicity
3. The primary model will be logistic regression, calculating adjusted predicted prevalence of hospitalization for those with each comorbidity alone and comorbidity clusters (since comorbidities are correlated)
    1. A priori, evaluate whether risk associated with each comorbidity is similar for men and women and for African Americans vs others (interaction by sex; interaction by race).

Conduct separate models for the active duty population and the beneficiary populations

3) Characterize host immune response.

We will examine the relationship between factors, such as the immune response (e.g., serum antibody concentration) and pathogen infection kinetics, and disease progression, symptom severity, and clinical outcomes of interest. We will look at both cross-sectional relationships (i.e., among people) and within person relationships. For cross-sectional relationships, we will start by plotting the total symptom severity score against the immune response, separately for each of the four blood samples. We anticipate a monotonic relationship, but it is possible for a U-shaped relationship in some cases, i.e., illness severity is greater if there is a too small immune response or a too large one. We will develop regression models with the outcome of the symptom severity or clinical course on the chosen function of immune response to see if there is a statistically significant relationship. We will add covariates to control for possible confounders, such as age, race, sex, military status, etc. and others mentioned above.

4) Evaluate correlations between symptom severity, virologic/microbiologic characteristics, host immune response and clinical course/outcome.

We will consider two groups: subjects with versus without a severe clinical course, such as mortality. We will compare the proportions with a significant serum antibody ‘response’ between the two groups by contingency table chi-square tests. We anticipate that the categorization of immune response will be binary (i.e., proportion of individuals exceeding a given concentration of serum antibody), in which case we will be comparing a binomial probability between the two groups. These analyses will form the backbone for more elaborate multivariate analyses.

5) Develop diagnostic and prognostic tools.

Data and specimens from this protocol may be used in the development of diagnostic and prognostic assays for novel pathogens. For example, the collection of blood and other body fluids from individuals with laboratory-confirmed infection may aid in the development of novel assays with higher sensitivity and specificity. Additionally, the collection of longitudinal clinical characteristics in addition to clinical laboratory data may aid in the development of prediction models to determine risk factors for severe clinical course, such as mortality.

12.0

Participant Information

12.1 Subject Population:

As this is an observational study, subjects of all ages will be enrolled. Military Treatment Facilities see a diverse population including all genders and races, and a wide range of ages. This study will examine the epidemiology of novel pathogens / emerging infectious diseases, seek to identify risk factors for severe disease and elucidate mechanisms of host immunity to these pathogens.

Given the possibility of MTFs being used as surge capacity to support the care of individuals affected by an infectious disease outbreak, this study also allows for enrollment of anyone meeting the inclusion criteria and obtaining care at a participating MTF. Males and females of all ages who are symptomatic or asymptomatic individuals (at high/some risk of exposure) meeting inclusion criteria are eligible for participation.

Participation in the online-only study group is limited to adults only.

12.2 Age Range:

☑ 0-17
☑ 18-24

☑ 25-34
☑ 35-44
☑ 45-54
☑ 55-64
☑ 65-74
☑ 75+

12.3 Gender:

☑ Male
☑ Female

12.4 Special categories:

☑ Minors /Children - “You must also consider the requirements of 45 CFR 46 Subpart D and DoDI 3216.02, Enclosure 3, paragraph 7.d.”
☐ Students
☐ Employees - Civilian - “You must also consider the requirements of DoDI 3216.02, paragraph 7.e.”
☐ Employees - Contractor
☐ Resident/trainee
☐ Cadets /Midshipmen - “You must also consider the requirements of DoDI 3216.02, Enclosure 3, paragraphs 7.e. and 12.”
☑ Active Duty Military Personnel - “You must also consider the requirements of DoDI 3216.02, Enclosure 3, paragraph 7.e.”
☐ Wounded Warriors - “Depending on your intended subjects’ status, you may also need to consider the requirements of DoDI 3216.02, Enclosure 3, paragraph 7.e.”
☐ Economically Disadvantaged Persons - “You must also consider the requirements of 32 CFR 219.111 (b).”
☐ Educationally Disadvantaged Persons - “You must also consider the requirements of 32 CFR 219.111 (b).”
☐ Physically Challenged (Physical challenges include visual and/or auditory impairment)
☑ Persons with Impaired Decisional Capacity - “You must also consider the requirements of 10 USC 980.”
☐ Prisoners - “You must also consider the requirements of 45 CFR 46 Subpart C and DoDI 3216.02, Enclosure 3, paragraphs 7.b. and 7.c.”
☑ Pregnant Women, Fetuses, and Neonates
☐ Non-English Speakers
☐ International Research involving Foreign Nationals - Headquarters Review is necessary

12.5 Inclusion Criteria:

Order Number	Criteria
1	Inclusion Criteria: Individuals of any age who are eligible for care at an MTF and meet at least one of the following criteria: 1. **Laboratory-confirmed presence of the pathogen of interest** (e.g., SARS-CoV-2, influenza A /H7N9, etc.) ***OR*** 2. **Meet criteria for testing for the pathogen of interest, as identified per current CDC guidelines** at **https://www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html**
	Additional Inclusion Criteria for **Online Enrollment:**

2	1. An MHS Beneficiary who has been clinically tested for the pathogen of interest 2. Able to receive email communications and respond to web-based questionnaires 3. ≥18 years of age

12.6 Exclusion Criteria:

Order Number	Criteria
1	Individuals who decline participation in the study
2	Individuals who the study investigators believe are unable to comply with the requirements of the study

13.0

Recruitment and Consent

13.1 Identification and Selection of Subjects:

Given the dynamic nature of novel pathogens/emerging infectious diseases, there is no pre-defined target enrollment for the protocol. The main objective of this protocol is to recruit and enroll a representative proportion of cases of emerging infectious diseases to characterize the epidemiology and clinical characteristics of disease. Site study team members will engage with relevant clinical staff to identify eligible patients in inpatient wards, intensive care units, acute care clinics, and emergency departments. Participants will be identified through screening of medical records to identify individuals with the recent exposures and clinical manifestations of interest, and will be approached following the initial medical evaluation. The study will be described to patients, and their eligibility and willingness to participate will be assessed. For those with confirmed or suspected infection, room access may be limited and consent will be performed by study staff members with patient access.

Individuals presenting for care at MTFs, such as at clinics or in the inpatient and isolation setting, will be recruited and enrolled and will participate in serial collection of biological specimens over a 12-month period, with more intensive (i.e., daily, weekly) data collection occurring during the inpatient or isolation period. Information on current and prior medical conditions and hospitalizations will be collected via medical chart review and/or MDR data extractions. Information on specific epidemiologic risk factors during or related to deployment (e.g., travel history, zoonotic exposures, etc.) will be ascertained through participant interview or review of available medical records (e.g., AHLTA-T, ALTHA, CHCS, Essentris, MHS Genesis, JLV (accessed via AHLTA or MHS Genesis), MDR, DMSS, and/or DRSi). Study participants evaluated at MTFs and who are either 1. ambulatory cases advised to return home and remain home in isolation or 2. classified as asymptomatic individuals with high/some risk for exposure may also be evaluated for follow up through a study design including serial self-collection of nasal swabs, oropharyngeal swabs, rectal swabs, or other specimens appropriate for self-collection. A similar schedule of data collection and methods used for enrolled inpatients will be applied for ambulatory cases (non-hospitalized) and asymptomatic individuals as feasible.

Details on the recruitment, consent, and enrollment of subjects participating through the MHS-wide online process are included in Appendix A.

13.2 Recruitment Process:

Recruitment of study volunteers at the MTFs will rely heavily on the direct interface of healthcare providers with potential eligible participants. The study team will develop an MTF communications plan, outlined in the protocol’s Manual of Procedures, that will be implemented by local site teams to facilitate identification of potential eligible participants. Study volunteers may be recruited from clinics and inpatient or isolation settings. In addition, recruitment of individuals who are asymptomatic at high/some risk of exposure may involve study team members approaching family or friends who escorted the sick individual to the hospital. Another group of individuals who may be approached for recruitment as asymptomatic high/some risk participants, are healthcare providers who are in close contact with and potentially exposed to known cases. Adults and children are eligible for participation.

The study team will use multiple means of communicating study awareness to enhance recruitment efforts, including periodic briefings delivered by the site PI's and their study teams to their critical care and infectious disease staff and inpatient nursing staff. Information notices with points of contacts will be posted in clinical sites such as inpatient units, acute care clinics, and several points in the emergency room. Research team members who are Active Duty military personnel will not wear visible signs of their rank during recruitment. Site PI's or their authorized designees will conduct daily queries of MTF electronic health heath records (including infectious disease reportables list, DRSi, and searching in CHCS for patients with coronavirus lab code) to identify individuals who tested positive for disease of interest and will review daily admissions into the ICU, general pediatric and internal medicine wards with their study personnel to identify new inpatients. Information from infection control, preventive medicine and infectious disease departments may be obtained to find patients who may be managed / followed outside of the MTF.

J6 Data: Staff at the J6 CarePoint MHS Information Portal will provide subject identifiers (including names, Active Duty status, DoD Identifier, DOB, Email addresses, Date of Initial COVID-19 Respiratory Swab PCR Test, and all available COVID-19 Respiratory Swab PCR Test results) of AD members and beneficiaries who have been tested for COVID-19 to the approved research team using DoD SAFE. These data will be used to contact subjects for potential enrollment in the study and to contact subjects while enrolled. The original data file is retained only until a QC check determines the data have been uploaded successfully to REDCap, after which the file is destroyed. At study end, all PHI and PII shall be de-identified per the De-identification Plan.

Depending on age, severity of infection, and MTF requirements, study staff will determine if a potential subject is capable and/or allowed to provide a signed consent, verbal consent, or if an LAR is required according to IRB, HIPAA, and local MTF requirements.

13.3 Compensation for Participation:

In accordance with 24 USC 30:

MTF-enrolled subjects will be provided compensation in the form of a gift card. Subjects enrolled in the online-only group will receive compensation in the form of an electronic gift code sent via email.

Subjects will receive:

- $50 for each outpatient venipuncture
- $10 for each self-collected sample completed at home
- $10 for completion of each online questionnaire
- $10 for completion of 14-day Flu-PRO symptom diary

13.4 Eligibility Assessment Process:

As part of medical record review, the team will assess the eligibility of potential subjects. Individuals identified as having known risk factors for an emerging infectious disease (e.g., recent travel to a disease-endemic region, or close contact with a known or suspected infected individual or a returning traveler) who present to a participating MTF for medical care (e.g., febrile illness) or are placed into isolation are eligible for study participation. Additionally, asymptomatic high/some risk for exposure individuals may be enrolled at MTFs. Given the possibility of MTFs being used as surge capacity to support the care of individuals affected by an infectious disease outbreak, this study allows for enrollment of anyone meeting the inclusion criteria and obtaining care at a participating MTF. Eligibility will be assessed and a final determination of subject eligibility will be made during the consent process. Please refer to section 12.5 for the inclusion criteria.

13.5 Consent Process:

Are you requesting a waiver or alteration of informed consent?

● Yes ○ No

What type?

☑ Waiver of documentation of informed consent
☐ Waiver or alteration of informed consent

Please explain why you qualify for a waiver or alteration of informed consent:

Due to the contagious nature of the emergent infectious diseases being studied under this protocol, study staff may not always be allowed work near the potential and/or active study subjects or handle any items that they may have come in contact with per local MTF infection control policies. Therefore, obtaining signed ICDs and HIPAA Authorizations for subjects enrolled in this study may not always be feasible or safe for study staff. The study team is requesting a waiver of documentation of informed consent to allow for verbal subject/LAR informed consent, an Alteration of Authorization of HIPAA to allow for verbal subject/LAR HIPAA authorization, as well as a partial Waiver of HIPAA Authorization to identify eligible potential subjects and make initial contact with individuals to notify them regarding the study. These will be used as appropriate based on the circumstances of the disease being studied and MTF requirements. This study falls under the following criteria noted in 32 CFR 219.117: “That the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context."

Please explain the consent process:

ICD and Assent Regulatory Requirements

The Informed Consent Document (ICD) and Assent Form, including a HIPAA authorization, will be reviewed and approved by the IRB prior to initiation of the study. The ICD will contain a full explanation of the possible advantages and risks of study participation. The ICD will be compliant with applicable DoD regulations, 32 CFR 219, and the Belmont Principles, and the Health Insurance Portability and Accountability Act (HIPAA) Authorization. Informed consent includes the principle that it is critical the participant be informed about the principal potential risks and benefits. This information will allow the participant to make a personal risk versus benefit decision and understand the following general principles:

- Participation is entirely voluntary,
- Participants may withdraw from participation at any time,
- Refusal to participate involves no penalty; and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

An ICD or Assent Form (with parental permission) will be signed by the participant before any study-related procedures are initiated for each participant. The investigators or their designees will present the protocol in lay terms to the participants. If access is limited, an authorized member of the study team accompanied by a witness may conduct the informed consent process. Questions on the nature of the protocol, the means by which it is to be accomplished, and the risks to the participants will then be solicited. Any question that cannot be answered will be referred to the principal investigator (PI). All participants will be given the opportunity to ask questions. No participant should grant consent until questions have been answered to his/her satisfaction. Documented parental permission will be received prior to obtaining assent from a minor. If rules allow, a copy should be provided to the volunteer or their guardian.

Minors:

An assent form (with parental permission) will be used for children aged 7-17. The ICD and Assent Form (with parental permission) indicate that by signature, the participant permits access to relevant medical records by the study investigators and regulatory authorities. Documented parental permission is required prior to assent by a minor using the ICD. A Waiver of Documentation of Assent will be used for children 6 and under, documenting that the study was explained to the child in age-appropriate terms and that the child has either expressed verbal assent or does not have the capacity to do so.

For adults who are incapacitated by the illness:

Consent may be obtained by a Legally Authorized Representative (LAR). The patients should be consented themselves at such time that they are able to provide consent.

For Hospital Staff who are Active Duty Military:

Hospital staff who are active duty military, and therefore eligible for care at a military treatment facility, may be approached for consenting to the study due to their potential exposure to coronavirus. If hospital staff who are active duty military are approached for participation in the study, supervisors will not be present at any human subject recruitment sessions or during the consent process in which hospital staff who are active duty military under their supervision are afforded the opportunity to participate as human subjects. Supervisors (e.g., military and civilian supervisors or anyone in the supervisory structure) will not influence the decisions of their subordinates regarding their participation as subjects in this study.

Verbal Informed Consent:

Due to the contagious nature of the emergent infectious diseases being studied under this protocol, study staff may not always be allowed work near the potential and/or active study subjects or handle any items that they may have come in contact with per local MTF infection control policies. Therefore, obtaining signed ICDs and HIPAA Authorizations for subjects enrolled in this study may not always be feasible or safe for study staff.

The study team is requesting a waiver of documentation of informed consent, an Alteration of HIPAA Authorization, as well as a partial Waiver of HIPAA Authorization to use as appropriate based on the circumstances of the disease being studied and MTF requirements. When possible, the subject will provide signed ICD and HIPAA authorization. If the subject is unable to provide signed authorizations, the Alteration of HIPAA Authorization will be utilized, allowing the subject/LAR to provide verbal, rather than written, HIPAA Authorization. If study personnel are unable to work near the potential and/or active subject for safety reasons, the study personnel will contact the patient according to the requirements of the MTF. Options may include but are not limited to speaking with the patient from a safe distance in person, contacting the patient by phone in person with a barrier in between the potential and/or active subject and the study personnel, contacting the patient by phone from a separate location, or having a study physician trained in infectious disease safety procedures meet with the potential and/or active subject in close proximity (if allowed by the MTF).

Regardless of the method of delivery, the potential and/or active subject would be thoroughly briefed on the study's Informed Consent and HIPAA information. They would also be provided with an opportunity to ask any questions and an unsigned copy of the informed consent if permissible by the MTF. In turn, study staff will thoroughly document the informed consent process and the subject's verbal consent in the study chart. The study personnel will sign and file the ICD. The study personnel will also include a note in the study chart summarizing the informed consent process.

13.6 DoDI 3216.02 requires an ombudsman to be present during recruitment briefings when research involves greater than minimal risk and recruitment of Service members occurs in a group setting. If applicable, you may nominate an individual to serve as the ombudsman.

● N/A
○ Propose ombudsman

13.7 Withdrawal from Study Participation:

Explain the process for withdrawal and specify whether or not the subjects will be given the opportunity to withdraw their data their data/specimens in the event they wish to withdraw from the study

A subject may voluntarily end participation in the study at any time. If a subject withdraws, the investigator will make a reasonable effort to determine the reason for the subject's withdrawal from the study. Telephone calls, registered letters, and/or e-mail correspondence will be considered reasonable effort. Data and specimens collected up to the time of withdrawal will be retained and de-identified in accordance with IRB, HIPAA, and Safe Harbor requirements.

Subjects may be discontinued by the study team if there is a determination by the on-site physician that the volunteer may be unable to comply with the requirements of the study, or in the interest of the military mission.

The study team may consider discontinuation of study participants who have a negative test result for the pathogen of interest (based on current standard of care diagnostics) *and/or* are diagnosed with another respiratory pathogen other than the one of interest *and/or* the disease of interest is highly unlikely based on the primary clinical team assessment. This approach may need to be taken to conserve study staff time and resources depending upon local epidemiologic circumstances, e.g. very high incidence and subject accrual.

14.0
Risks and Benefits

14.1
Risks of Harm:

Identify all research-related risks of harm to which the subject will be exposed for each research procedure or intervention as a result of participation in this study. Consider the risks of breach of confidentiality, psychological, legal, social, and economic risks as well as physical risks. Do not describe risks from standard care procedures; only describe risks from procedures done for research purposes

Risks associated with obtaining specimens:

- Obtaining specimens could be associated with discomfort from a needle stick, swab, or other procedure, and occasionally associated with bleeding, bruising, local or systemic infection.
- Genetic testing may reveal sensitive information that subjects may not want disclosed, and may cause some emotional distress for the participant.

Risks associated with privacy:

- Despite meticulous precautions, breaches of confidential information (including genetic information) do occasionally occur, although the chances of such a breach are unlikely.

Expected Adverse Events from Research Risks and Reporting

a. Rare (Event Rate < 1%)
Secondary infection and syncope from vasovagal reactions occur from blood collection in rare cases. Risks of nasopharyngeal swab, rectal swab, cheek swab could include bleeding or mucous membrane irritation.

b. Less Likely (1% ≤ Event Rate < 5%)
No known risks

c. Likely (5% ≤ Event Rate < 10%)
Bruising and bleeding may occur as a result of blood collection procedures.

d. More likely (Event Rate ≥10%)
No known risks

14.2
Measures to Minimize Risks of Harm (Precautions, safeguards):

For each research procedure or intervention, describe all measures to minimize and/or eliminate risk of harms to subjects and study personnel

Participation in this protocol will impose minimal risks to the participant. As feasible, the study team will coordinate the collection of research purpose blood draws during timing of blood collection for clinical care to minimize discomfort to the patient. Blood volume collection per visit will be approximately 25 mL total. Total blood volumes collected over the 12 month duration of study will not exceed limitations for adults and children outlined in the CFR §46.110: Expedited review procedures for certain kinds of research involving no more than minimal risk, and for minor changes in approved research. Blood draw volumes will be reduced for pediatric subjects, small adults, and pregnant women in accordance with minimal risk and safety guidelines, and as needed per guidance from the primary care provider overseeing care of enrolled patients.

Staff safety in sample collection and handling is a concern that will be closely monitored by the Infection Control staff at the facility. Risk to staff is mitigated by minimizing an additional phlebotomy beyond that being done for clinical care. Specimen collection should be harmonized with the clinician’s requirements, and missed samples will not be re-drawn.

Unanticipated problems involving risk to subjects or others (UPIRTSO) may also occur that are not related to a described harm (adverse event) to study subject, rather a potential harm (such as loss of accountability of PII/PHI) or risks to others including medical staff. Specifically in this study is a concern that collection of infection fluids may cause harm to study personnel. Collection of samples for this study must only be conducted by individuals trained and authorized to collect and handle serious biohazardous specimens wearing proper personal protective equipment (PPE). PPE training will be conducted and documented for all personnel who may interact directly with patients and/or samples in order to mitigate any UPIRTSO related to accidental exposure of study staff and other individuals in the facility.

14.3
Confidentiality Protections (for research records, data and/or specimens):

Describe in detail the plan to maintain confidentiality of the research data, specimens, and records throughout the study and at its conclusion (e.g., destruction, long term storage, or banking). Explain the plan for securing the data (e.g., use of passwords, encryption, secure servers, firewalls, and other appropriate methods). If data will be shared electronically with other team members/collaborators outside the institution, describe the method of transmission and safeguards to maintain confidentiality. Explain whether this study may collect information that State or Federal law requires to be reported to other officials or ethically requires action, e.g., child or spouse abuse

A certificate of confidentiality will not be used in this protocol.

The only electronic links established between the participant name, SSN, and the Study ID number will be in the data management system managed by DCC and on a tracking spreadsheet used for scheduling purposes, which will both require a password to access and will only be accessed by authorized study personnel. During the study, source documents will be held in a secured cabinet with restricted access. Team members with access to the documents are counseled on the importance of confidentiality of participant medical records. All staff involved in this study have completed the required human subjects protection and ethics training. In all electronic records for data analysis, the participant is not referenced by name, but only Study ID number. This method is designed to protect the privacy of study participant medical information. All CRFs will be held at the individual study sites in the research spaces in secured cabinets with restricted access.

The only hard copy link between participant identifiers and Study ID will be on the Registry Form and Contact Information Form. This will be kept in a secure location separate from source document files. Any copies faxed to DCC personnel acting as a backup Registrar will be shredded after registration is complete.

In order to determine whether an individual has been enrolled previously at another study site, study staff at all active sites will have the ability to query DCC's data management system to locate an individual. The data management system is secure and can only be accessed via authorized individuals using a password.

What precautions will you take to protect the confidentiality of research source documents (Case Report Forms, questionnaires, etc.), the research data file, and the master code (if any)?

The only hard copy link established between the participant's name, SSN, and the study code number will be held in a secured cabinet with access restricted to research team members with a need to use the Registry Form or Contact Information Form. Team members with access to CRFs are counseled on the importance of confidentiality of participant medical records. In all electronic records for data analysis, the participant is not referenced by name, but only study code number.

The only electronic link will be stored on a secure password protected server. The file is only accessible directly to the DCC data custodian. The designated CRC at each site and two backups at DCC will also have access via DCC's data management system.

Will research data including Identifiable Protected Health Information be sent outside of Department of Defense facilities?

The study team may share coded data with collaborators at non-DoD facilities, and may work with participating MTFs to assist with appropriate public health data reporting requirements.

14.4
Potential Benefits:

Describe any real and potential benefits of the research to the subject and any potential benefits to a specific community or society

If the individuals in the research are considered experimental subjects (per 10 USC 980), and they cannot provide their own consent, the protocol must describe the intent to directly benefit all subjects

This is an observational study that does not test interventions that would offer direct benefit to study volunteers.

Study volunteers may indirectly benefit from enhanced clinical testing, physical examination, radiographic studies, and longitudinal follow up, or improved access to related trials of therapeutics.

14.5
Privacy for Subjects:

Describe the measures to protect subject's privacy during recruitment, the consent process, and all research activities, etc.

When possible, all means necessary will be taken to ensure participant privacy. This may entail conducting study procedures in a private area and keeping study documents coded. Non-coded study documents will be kept in a locked drawer and/or locked file in a locked room. Subjects will disclose information at their consent, if subjects choose not to disclose information, the study team will respect subject privacy and not inquire further.

14.6
Incidental or Unexpected Findings:

Describe the plan to address incidental findings and unexpected findings about individuals from screening to the end of the subject's participation in the research. In cases where the subject could possibly benefit medically or otherwise from the information, state whether or not the results of screening, research participation, research tests, etc., will be shared with subjects or their primary care provider. State whether the researcher is obligated or mandated to report results to appropriate military or civilian authorities and explain the potential impact on the subject

The study team will share relevant findings from research labs/imaging with the patient's healthcare provider(s).

Aspects of this study may include epidemiologic reporting to AFHSB and public health authorities at the respective services, state and local health departments, and CDC. That data transfer is not subject to Data Use Agreement or HIPAA requirements.

15.0
Study Monitoring

15.1 Data Monitoring Plan:

Describe the plan to monitor the data to verify that data are collected and analyzed as specified in the protocol. Include who will conduct the monitoring, what will be monitored and the frequency of monitoring

The supervising principal investigator, or designated associate investigator, will be responsible for regular maintenance and review of CRFs for accuracy. Access to study files (e.g., CRFs) is restricted to care providers and research team members given specific tasks to perform. The files are kept in a secured location in each study site. Some participant-completed forms such as questionnaires may be completed through the web-based program. These will be transmitted directly into the study database. Research labs will transmit their testing data to the DCC using standard procedures for data handling and transmission.

During the study, the investigator will review study data from their site, including medical records, records detailing the progress of the study for each volunteer, laboratory reports, CRFs, signed ICD and authorization forms for each study volunteer, correspondence with the IRB, adverse event (AE) reports and information regarding volunteer discontinuation of the study. All required study data will be clearly and accurately recorded on source documents or CRFs by either the participant themselves (questionnaires) or authorized study personnel. Only designated study personnel shall record or change data on a CRF. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded on the CRFs. Original observations entered directly onto the CRFs are defined as source data. A Quality Management Plan (QMP) will be used for this study. The QMP will include a QC subject data review checklist, a QC Subject data review checklist, as well as automatic queries from the electronic database based on expected parameters for data entry, noting possible inconsistencies or omissions. The QC subject data review checklist will be completed at each site on 100% of subject's study files. This will be completed by another member of the study team who did not complete the original source document or CRF. A QA subject data review checklist will also be used on a small subset of study records at each site to ensure that quality management processes are being conducted appropriately for the study. QA review will be conducted by an individual on the study who is not involved in the day-to-day activities of the study.

Responsibilities of the Data Coordination Center (DCC)

1. Provides review of protocol, CRFs, data security plan and data management SOPs
2. Complies with protocol, SOPs, and all federal and local regulations and policies
3. Develops clinical database
4. Entry and quality control of CRF based study data
5. Receipt and management of specimen tracking and laboratory data
6. Provides study database to IDCRP Program Coordination Center (PCC)
7. Locks final study database

15.2 Safety Monitoring Plan:

Describe the plan to monitor the data to ensure the safety of subjects

Participation in this protocol will impose minimal risks to the participant. Staff safety in sample collection and handling is a concern that will be closely monitored by the Infection Control staff at the facility. Risk to staff is mitigated by minimizing an additional phlebotomy beyond that being done for clinical care. Specimen collection must be harmonized with the clinician's requirements, and missed samples will not be re-drawn. Proper safety training, following hospital Infection Control requirements, of study team members who come in contact with study participants will be implemented to mitigate disease transmission risk.

Termination of study participation will take place through 1) an expressed desire by the volunteer to terminate participation, 2) a determination by the on-site physician that the volunteer risks his/her health by further participation in the study, or 3) in the interest of the military mission.

15.3 Does your study require independent data and safety monitoring?

○ Yes ● No

16.0 Reportable Events

16.1 Reportable Events:

Consult with the research office at your institution to ensure requirements are met

- Describe plans for reporting expected adverse events. Identify what the expected adverse events will be for this study, describe the likelihood (frequency, severity, reversibility, short term management and any long term implications of each expected event)
- Describe plans for reporting unexpected adverse events and unanticipated problems. Address how unexpected adverse events will be identified, who will report, how often adverse events and unanticipated problems will be reviewed to determine if any changes to the research protocol or consent form are needed and the scale that will be used to grade the severity of the adverse event

Reporting Protocol Deviations

The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or study-specific procedure. Investigators will report protocol deviations to the USU IRB within 2 business days that:

- relate to participant safety
- relate to the informed consent process
- are any other protocol deviations which, in the opinion of the PI, should be promptly reported to the IRB.

A log of all deviations will be reported annually in the continuing review report to the IRB and in the Final Study Report.

Reportable events will also be submitted to reporting site's regulatory authorities as required.

Reporting Adverse Events and Serious Adverse Events

In this observational, no-greater-than-minimal risk study, the reporting of adverse events is limited to an analysis at the time of annual reporting. Prompt reporting is required for any related serious adverse event or unanticipated problems involving risk to subject or others (UPIRTSO).

An adverse event is defined as any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject’s participation in the research, whether or not considered related to the research.

AEs that meet all of the following criteria will be recorded by the study team and reported to the IRB at the annual Continuing Review:

- At least possibly related to study procedures
- Unexpected (i.e. not included in the list of known risks in section 14)
- Moderate or greater severity

Additional AEs may be reported if the determined by the study site investigator to be of sufficient significance to require reporting to the IRB.

AEs are recorded and inspected by the supervising physician investigator to determine severity and relatedness to participation (definite, probable, possible, or unlikely). The physician investigator is responsible for directly managing or coordinating management of adverse events.

Reporting unanticipated problems involving risk to subjects or others

Unanticipated problems involving risk to subjects or others (UPIRTSO) may also occur that are not related to a described harm (adverse event) to study subject, rather a potential harm (such as loss of accountability of PII /PHI) or risks to others including medical staff. Specifically in this study is a concern that collection of infection fluids may cause harm to study personnel. Collection of samples for this study must only be conducted by individuals trained and authorized to collect and handle serious biohazardous specimens wearing proper personal protective equipment. Any sample collection for research purposes (as compared to clinical care) that results in an exposure to clinical staff is considered a UPIRTSO.

All UPIRTSOs will be collected and reported to the IRB within 24 hours of the PI recognition of the problem.

17.0

Equipment/non-FDA Regulated Devices

17.1 Does the study involve the use of any unique non-medical devices/equipment?

○ Yes ● No

18.0 FDA-Regulated Products

18.1 Will any drugs , dietary supplements, biologics, or devices be utilized in this study?

☐ Drugs
☐ Dietary Supplements
☐ Biologics
☐ Devices
☑ N/A

18.5 Sponsor (organization/institution/company):

☐ N/A

If applicable, provide sponsor contact information:

Infectious Diseases Clinical Research Program
Department of Preventive Medicine and Biometrics
Uniformed Services University of the Health Sciences
11300 Rockville Pike, Suite 1211
Rockville, MD 20852

19.0 Research Registration Requirements

19.1 ClinicalTrials.gov Registration:

● Registration is not required
○ Registration pending
○ Registration complete

19.2 Defense Technical Information Center Registration (Optional):

● Registration is not required
○ Registration pending
○ Registration complete

20.0 References and Glossary

20.1 References:

1. Van Kerkhove MD, Mumford E, Mounts AW, Bresee J, Ly S, Bridges CB, et al. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. PloS one. 2011;6 (1):e14582. Epub 2011/02/02. doi: 10.1371/journal.pone.0014582. PubMed PMID: 21283678; PubMed Central PMCID: PMC3025925.

2. WHO. Weekly Epidemiological Record: Update on human cases of highly pathogenic avian influenza A (H5N1) virus infection, 2011. Geneva, Switzerland: World Health Organization, 2012 30 Mar. Report No.
3. Cowling BJ, Jin L, Lau EH, Liao Q, Wu P, Jiang H, et al. Comparative epidemiology of human infections with avian influenza A H7N9 and H5N1 viruses in China: a population-based study of laboratory- confirmed cases. Lancet. 2013;382 (9887):129-37. Epub 2013/06/28. doi: 10.1016 /s0140-6736 (13) 61171-x. PubMed PMID: 23803488; PubMed Central PMCID: PMC3777567.
4. WHO. WHO Risk Assessment: Human infections with avian influenza A (H7N9) virus. Geneva, Switzerland: World Health Organization, 2014 Oct 2. Report No.
5. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England journal of medicine. 2012;367 (19):1814-20. Epub 2012/10/19. doi: 10.1056/NEJMoa1211721. PubMed PMID: 23075143.
6. Assiri A, McGeer A, Perl TM, Price CS, Al Rabeeah AA, Cummings DA, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. The New England journal of medicine. 2013;369 (5):407-16. Epub 2013/06/21. doi: 10.1056/NEJMoa1306742. PubMed PMID: 23782161; PubMed Central PMCID: PMC4029105.
7. Memish ZA, Zumla AI, Al-Hakeem RF, Al-Rabeeah AA, Stephens GM. Family cluster of Middle East respiratory syndrome coronavirus infections. The New England journal of medicine. 2013;368 (26):2487- 94. Epub 2013/05/31. doi: 10.1056/NEJMoa1303729. PubMed PMID: 23718156.
8. Breban R, Riou J, Fontanet A. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. Lancet. 2013;382 (9893):694-9. Epub 2013/07/09. doi: 10.1016/s0140-6736 (13)61492-0. PubMed PMID: 23831141.
9. Cauchemez S, Fraser C, Van Kerkhove MD, Donnelly CA, Riley S, Rambaut A, et al. Middle East respiratory syndrome coronavirus: quantification of the extent of the epidemic, surveillance biases, and transmissibility. The Lancet Infectious diseases. 2014;14 (1):50-6. Epub 2013/11/19. doi: 10.1016/s1473-3099 (13)70304-9. PubMed PMID: 24239323; PubMed Central PMCID: PMC3895322.
10. Hemida MG, Chu DK, Poon LL, Perera RA, Alhammadi MA, Ng HY, et al. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis. 2014;20 (7):1231-4. Epub 2014/06/26. doi: 10.3201/eid2007.140571. PubMed PMID: 24964193; PubMed Central PMCID: PMC4073860.
11. WHO. Summary of probably SARS cases with onset of illness from 1 November 2002 to 31 July 2003 Switzerland, Geneva: World Health Organization; 2003 [cited 2014 Oct 31]. Available from: http://www. who.int/csr/sars/country/table2004_04_21/en/.
12. Ge X-Y, Li J-L, Yang X-L, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013;503 (7477):535-8. doi: 10.1038/nature12711.
13. WHO. Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). Geneva, Switzerland: World Health Organization, Response DoCDSa; 2003.
14. Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. The New England journal of medicine. 2003;348 (20):1967-76. Epub 2003/04/12. doi: 10.1056/NEJMoa030747. PubMed PMID: 12690091.
15. WHO. Coronavirus Disease Situation Report, 2020. Geneva, Switzerland: World Health Organization, 2020 Mar 2. Report No 42. Available from: https://www.who.int/docs/default-source /coronaviruse/20200302-sitrep-42-covid-19.pdf?sfvrsn=d863e045_2
16. CDC. Coronavirus Disease 2019 (COVID-19) in the U.S. https://www.cdc.gov/coronavirus/2019-ncov/cases-in-us.html. Accessed 03 March 2020
17. Wei-jie G., Zheng-yi N., Yu H., Wen-hua L., Chun-quan O., Jian-xing H., et al. Clinical Characteristics of Coronavirus Disease 2019 in China. 2020. Epub 2020/02/28. The New England Journal of Medicine. doi: 10.1056/NEJMoa2002032
18. del Rio C, Malani PN. COVID-19—New Insights on a Rapidly Changing Epidemic. JAMA. Published online February 28, 2020. doi:10.1001/jama.2020.3072
19. Liu W, Tao ZW, Lei W, Ming-Li Y, Kui L, Ling Z, Shuang W, Yan D, Jing L, Liu HG, Ming Y, Yi H. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chin Med J (Engl). 2020 Feb 28. doi: 10.1097/CM9.0000000000000775.
20. CDC. How COVID-19 Spreads. 2020. https://www.cdc.gov/coronavirus/2019-ncov/about /transmission.html
21. Wei Zhang, Rong-Hui Du, Bei Li, Xiao-Shuang Zheng, Xing-Lou Yang, Ben Hu, Yan-Yi Wang, Geng-Fu Xiao, Bing Yan, Zheng-Li Shi & Peng Zhou (2020) Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes, Emerging Microbes & Infections, 9:1, 386-389, DOI: 10.1080/22221751.2020.1729071
22. Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirglmaier K, Zange S, Wolfel R, Hoelscher M. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N Engl J Med. 2020. Epub 2020/02/01. doi: 10.1056/NEJMc2001468.
23. Chan JF Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet (London, England). 2020. Epub 2020/01/28. doi: 10.1016/s0140-6736 (20) 30154- 9

24. Powers JH, Bacci ED, Leidy NK, Poon JL, Stringer S, Memoli MJ, Han A, Fairchok MP, Coles C, Owens J, Chen WJ, Arnold JC, Danaher PJ, Lalani T, Burgess TH, Millar EV, Ridore M, Hernández A, Rodríguez-Zulueta P, Ortega-Gallegos H, Galindo-Fraga A, Ruiz-Palacios GM, Pett S, Fischer W, Gillor D, Moreno Macias L, DuVal A, Rothman R, Dugas A, Guerrero M. Performance of the inFLUenza Patient-Reported Outcome (FLU-PRO) diary in patients with influenza-like illness (ILI). PLoS One. 2018 Mar 22; 13(3):e0194180.
25. Coles C, Millar EV, Burgess T, Ottolini MG.The Acute Respiratory Infection Consortium: A Multi-Site, Multi-Disciplinary Clinical Research Network in the Department of Defense. Mil Med. 2019 Nov 1;184 (Supplement 2):44-50. doi: 10.1093/milmed/usz174.
26. Han A, Poon JL, Powers JH, Leidy NK, Yu R, Memoli MJ. Using the Influenza Patient-reported Outcome (FLU-PRO) diary to evaluate symptoms of influenza viral infection in a healthy human challenge model. BMC Infect Dis. 2018 Jul 28;18(1):353. doi: 10.1186/s12879-018-3220-8.
27. Yu J, Powers JH, Vallo D, Falloon J. Evaluation of Efficacy Endpoints for a Phase IIb Study of a Respiratory Syncytial Virus Vaccine in Older Adults Using Patient-Reported Outcomes With Laboratory Confirmation. Value Health. 2020 Feb;23(2):227-235. doi: 10.1016/j.jval.2019.09.2747.

20.2 Abbreviations and Acronyms:

CoV - Coronavirus

AE - Adverse Event

AFHSB - Armed Forces Health Surveillance Branch

AHLTA - Armed Forces Health Longitudinal Technology Application

AIDS/HIV - Acquire Immunodeficiency Syndrome/Human Immunodeficiency Virus

APTT - Activated Partial Thromboplastin Time

ARI - Acute Respiratory Infection

ALT/SGPT - Alanine Aminotransferase/Serum Glutamic Pyruvic Transaminase

AST/SGOT - Aspartate Aminotransferase/Serum Glutamic Oxaloacetic Transaminase

BSL - Biosafety Level

CDC - Centers for Disease Control and Prevention

COVID-19 - Coronavirus Disease 2019–

CRC - Clinical Research Coordinator

CRF - Case Report Form

DARPA - Defense Advanced Research Projects Agency

DCC - Data Coordination Center

DEERS - Defense Enrollment Eligibility Reporting System

DHA - Defense Health Agency

DoD - Department of Defense

DoDSR - DoD Serum Repository

eIND - Emergency Investigational New Drug

EVD - Ebola Virus Disease

FDA - Food and Drug Administration

GCP - Good Clinical Practice

GEIS - Global Emerging Infections Surveillance and Response System

HIPAA - Health Insurance Portability and Accountability Act

HRPP - Human Research Protection Program

ICD - Informed Consent Document

ICU - Intensive Care Unit

ICH - International Conference on Harmonization

IDCRP - Infectious Disease Clinical Research Program

IFN - Interferon

Ig - Immunoglobulin

IL - Interleukin

IND - Investigational New Drug

IRB - Institutonal Review Board

ISARIC - International Severe Acute Respiratory Infection Consortium

MCP - Macrophage Chemo-attractant Protein

MDR – Military Health System Data Repository

MERS-CoV - Middle East Respiratory Syndrome-Coronavirus

MHRP - Military HIV Research Program

MIP - Macrophage Inflammatory Protein

MTF - Military Treatment Facility

NIAID - National Institute of Allergy and Infectious Diseases

NIH - National Institutes of Health

NMCP - Naval Medical Center Portsmouth

NMCSD - Naval Medical Center San Diego

OCRPRO - Office of Clinical Research Policy and Regulatory Operations

OHRP - Office for Human Research Protections

OUA - Operation United Assistance

PBMC - Peripheral Blood Mononuclear Cell

PCC - Program Coordination Center

PCR - Polymerase Chain Reaction

PHD - Predicting Health and Disease

PI - Principal Investigator

PHI - Protected Health Information

PII - Personally Identifiable Information

PPE - Personal Protective Equipment

PTT - Partial Thromboplastin Time

PUI - Person under Investigation

RCT - Randomized Controlled Trial

RNA - Ribonucleic Acid

SAE - Serious Adverse Event

SARI - Secure Acute Respiratory Infection

SARS - Severe Acute Respiratory Syndrome

SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2

SOP - Standard Operating Procedure

SPL - Specimen Processing Laboratory

SSN - Social Security Number

Study ID - Study Identification Number

TNF - Tumor Necrosis Factor

UPIRTSO - Unanticipated Problems Involving Risk to Subjects or Others

US - United States

USU - Uniformed Services University of the Health Sciences

WHO - World Health Organization

1.0 General Information

Title: Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC)
Protocol Number: IDCRP-085
Version/Date: Version 9.0, 01-JUN-2020

9. Study Details

9.1 Abstract/ Summary:

The overarching objective of the Epidemiology, Immunology and Clinical Characteristics of Emerging Infectious Diseases with Pandemic Potential (EPICC) protocol is to provide the Military Health System (MHS) with a contingency protocol intended for activation at Military Treatment Facilities (MTFs) during outbreaks to allow clinical investigation of severe or potentially severe acute infections with pathogens of concern to public health and U.S. military medical readiness. The protocol outlines a systematic approach to collection of clinical specimens and data by adapting standard procedures for conducting clinical characterizations of severe emerging infections as published by the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC) network (ISARIC/WHO Clinical Characterization Protocol for Severe Emerging Infections, version 3.1).

The EPICC protocol can be conducted as a multi-site study at participating MTFs, and overall protocol oversight will be coordinated centrally by the Uniformed Services University's Infectious Disease Clinical Research Program (IDCRP). EPICC activities will be executed by clinical study teams comprising existing IDCRP clinical research investigators and staff located at each MTF and protocol investigators from relevant hospital departments. MHS-wide enrollment for online survey participation will be coordinated by the IDCRP Program Coordinating Center (PCC).

The protocol is designed to be modified to accommodate longitudinal observational study of specific emerging or re-emerging pathogens. Protocol modifications will describe methods for serial collection and clinical characterization of specimens and associated demographic, laboratory, clinical, and other data specific for the pathogen of interest and associated study population(s).

9.3 Background and Significance:

The EPICC protocol is intended to serve as a foundation for modification to allow the observational, longitudinal study of specific emerging or re-emerging pathogens causing disease with pandemic potential within the MHS. Modifications responsive to specific diseases will be described to include a schedule for serial collection of clinical specimens and data and methods for recruiting, enrolling, and observational study within the desired MHS study population(s). In general, enrolled participants (eligible adults or children) who are known cases or Persons Under Investigation (PUIs) at MTFs as inpatients or in isolation settings will undergo serial collections of biological specimens and clinical and laboratory data at day of enrollment and during the duration of hospitalization or isolation, with follow-up specimen and data collection at convalescent and long-term follow up timepoints. The study design can also include clinical observational investigation in outpatients. The major focus of this protocol is to conduct clinical observational study in symptomatic individuals; however, there is also the opportunity to enroll and follow (potentially at a reduced specimen and data collection frequency relative to symptomatic participants) individuals who are asymptomatic but at increased risk for exposure. Inclusion of an asymptomatic comparator cohort could provide insight on risk factors or biomarkers associated with symptomatic versus asymptomatic presentations. The protocol supports observational, longitudinal collection of clinical specimens and data with a goal of identifying host biomarkers that predict severity of illness, resistance to infection, treatment failure, etc. If volunteers receive an experimental treatment product under eIND, the data collected through the EPICC protocol may help inform about the safety and efficacy of that product.

In subsequent sections, relevant background information on acute respiratory infections (ARI) of pandemic potential is provided, followed by specific schedule of events and other details to allow observational study of an ongoing outbreak of the 2019 novel coronavirus (COVID-19).

Acute Respiratory Infections of Pandemic Potential: Epidemiology, Immunology and Clinical Characteristics

ARI Case Definitions

To characterize the epidemiology, immunology and clinical features of novel pathogens associated with severe ARI, case definitions appropriate to emerging infection scenarios will be utilized. These definitions will vary by the availability and result of laboratory-based diagnostic tests.

I. *For laboratory-confirmed cases*, the case definition includes the presence of laboratory-confirmed infection with an agent of severe ARI (e.g., SARS-CoV-2, influenza A/H7N9, MERS-CoV, SARS, etc.).

OR

II. For persons under investigation (PUI), the case definition includes:

1. The current CDC case definition for a PUI for a respiratory pathogen under study.
2. An unknown or pending laboratory confirmation of an agent of severe ARI.

III. For individuals who are asymptomatic and have had recent high-risk exposure to the pathogen of interest such that they have increased their risk for infection, 1 or more of the following criteria must be met:

1. Unprotected exposure (for e.g., < 14 days of illness onset, modified commensurate with evolving epidemiologic information) with confirmed or suspected cases of human infection with a novel respiratory pathogen. Close contact is typically defined as coming within about 6 feet (2 meters) of a confirmed or suspected case while the case was ill (beginning 1 day prior to illness onset and continuing until resolution of illness).
2. Unprotected exposure to infectious pathogens in a laboratory setting.
3. If exigencies warrant, then asymptomatic/high exposure risk individuals in isolation may be considered for enrollment.

Acute respiratory infections (ARI) are among the most common infectious diseases. Due to conditions and circumstances of military training and deployment (e.g., crowding, inadequate hygiene practices, physical stress, etc.), military personnel in congregate settings are at increased risk for outbreaks due to ARI. There are a number of emerging viral respiratory pathogens to which military forces may be exposed, that carry substantial importation risk, and that also have significant pandemic potential. Recent and current examples are described below:

1. Influenza A/H5N1 was first identified in the human population in Hong Kong in 1997. Since 2003, 16 countries have reported 668 confirmed cases and 393 deaths due to influenza A/H5N1 (as of 1 Nov 2014). The global case fatality rate for influenza A/H5N1 infection is 60%, and the estimates range

widely (0% to 100%) by country. While the significant morbidity and mortality of influenza A/H5N1 infection is readily apparent, its occurrence among humans has been relatively infrequent to date. Circulation of H5N1 predominates in poultry, currently the main reservoir, in Asia and northeast Africa. Host-to-human (i.e., zoonotic transmission of A/H5N1) infections are unpredictable and “remain rare despite frequent and widespread contact with infected poultry and contaminated environments” (1). The majority (71%) of cases of influenza A/H5N1 have had recent exposure to live birds or contaminated areas. Cases of influenza A/H5N1 to date have been relatively young (median age: 19 years) (2). Exposure to other cases of influenza A/H5N1 has not been a major risk factor to date, suggesting that the likelihood of human-to-human transmission of this particular strain remains low (R0< 1) (3). The severity of influenza A/H5N1 is dependent upon the strain associated with infection/disease. Among strains with low pathogenicity, generally mild, non-fatal illnesses have been observed, ranging from conjunctivitis to influenza-like illness (e.g., fever, cough, sore throat, muscle aches) to lower respiratory disease (pneumonia). Among strains with high pathogenicity, the disease spectrum has ranged from a milder febrile respiratory illness to severe disease (e.g., dyspnea, hypoxemia, pneumonia, acute respiratory failure) with multi-organ disease, sometimes accompanied by nausea, abdominal pain, diarrhea, vomiting and delirium. The high mortality rate (~60%) associated with pathogenic strains of influenza A/H5N1 has been reported from multiple studies (2).

2. Influenza A/H7N9 infection of humans was first identified in China in March 2013. Since then China has reported 453 confirmed cases and 175 deaths due to influenza A/H7N9 (WHO 2014). As with influenza A/H5N1, most of the cases had recent exposures to live poultry or had visited areas where live poultry was sold (75%) (3). By contrast to influenza A/H5N1, the host reservoir, main exposures, routes of transmission, and distribution and prevalence of influenza A/H7N9 among humans and animals are unknown. Influenza A/H7N9 does not appear to transmit easily from birds or the environment to humans. In addition, reported cases of Influenza A/H7N9 have not demonstrated high transmissibility between humans to date, suggesting that the risk of pandemic potential currently remains low (4). Though limited in its reach, influenza A/H7N9 infection has been associated with significant mortality: approximately 39% of cases resulted in a fatal outcome (4).

The coronavirus associated with the Middle Eastern Respiratory Syndrome (MERS-CoV) was first identified in Saudi Arabia in 2012 (5). Several outbreaks have since occurred, originating in Middle Eastern countries and spreading globally, albeit to a limited extent, by international travelers. Symptoms including fever, rigors, malaise, cough, myalgia, headache, shortness of breath, anorexia, diarrhea and abdominal pain were observed in MERS-CoV cases (6,7). As of October 2014, a total of 897 confirmed cases and 325 deaths (case fatality rate [CFR]: 36%) have been reported worldwide (WHO 2014). In 2013, two studies concluded that MERS-CoV did not yet exhibit pandemic potential, with an estimated basic reproduction number less than 1.0 (8, 9). Some research indicates that camels may act as the host reservoir for MERS-CoV, although evidence is inconclusive (10). Human-to-human transmission of MERS-CoV has been observed in healthcare settings, possibly due to greater infectiousness in the later stages of illness; however, evidence that may further elucidate risk factors for transmission is limited (6, 7).

3. The 2002-3 pandemic of Severe Acute Respiratory Syndrome (SARS), also due to a novel Coronavirus, originated in China’s Guangdong Province and resulted in 8,422 probable cases and 916 deaths reported from 29 countries (mostly in mainland China, where 63% of cases and 38% of deaths occurred) (11). The global case fatality rate due to SARS was estimated to be 11% (11). Although the host reservoir remains unknown, some studies have found coronaviruses similar to the SARS coronavirus circulating in bat populations (12). Transmission in human populations occurs by direct contact with the infectious respiratory droplets and/or fomites via the eyes, nose, and mouth (13). The incubation period ranges from 1-14 days (mean 4-6 days) and transmissibility appears to increase with

severity of illness (greatest at day 10) (14). Attack rates ranged widely by geographic region from 2.2 per 100,000 in Guangdong Province to 25.6 per 100,000 in Hong Kong (14). Risk factors for infection include occupation as a healthcare worker (21% of all probable cases), household contact with a probable case, older age, male sex and existing illnesses (14). Symptoms include fever, dry cough, dyspnea, headache and hypoxemia (14).

4. Since December 2019 through March 2020, the **2019 novel coronavirus (SARS-CoV-2) causing “coronavirus disease 2019” (COVID-19)** has spread from Wuhan, China to 64 countries and has led to more than 88,948 confirmed cases globally, including 80,174 confirmed cases in China and 8,774 confirmed cases outside of China (15). To date, there have been 2,915 deaths in China and 128 deaths outside of China. As of 03 March 2020, in the United States there are 60 confirmed cases (22 travel-related, 11 person-to-person spread, 27 under investigation) and a total of 6 deaths (16). Individuals with COVID-19 can present with fever, and cough, ground-glass opacity on chest computed tomography (CT), and lymphocytopenia (17). The case- fatality rate (CFR) appears to be highly variable with early reports of 8-15% in older adults infected in Hubei Province (CFR is increased in adults with comorbid conditions who develop respiratory symptoms) but likely no higher than 1-2% outside of Hubei (though data is limited and this will depend on accurate detection of those with mild disease) (18). Several risk factors, including age, history of smoking, maximum body temperature on hospital admission, respiratory failure, albumin, and C-reactive protein, appear associated with increased risk for progression to pneumonia among COVID-19 hospitalized patients (19). Risk factors of disease progression and magnitude of clinical severity are incompletely understood and require further study. SARS-CoV-2 is believed to be transmitted primarily via person-to-person, such as through close contact (within about 6 feet) and exposure to respiratory droplets from an infected individual who coughs or sneezes (20). Evidence exists for transmission of SARS-CoV-2 via the oral-fecal route, as viral nucleotide was detected in rectal swabs and blood later in the course of infection when virus was not detected in oral swabs (21). On the population level, there is a poorly-defined risk of transmission via asymptomatic infection (22). The occurrence of asymptomatic infections has raised concerns about the rapid spread of transmission to vulnerable populations (21).

9.4 Objectives/Specific Aims/Research Questions:

General Goals and Objectives:

1. To describe the natural history of clinical disease
2. To identify risk factors for infection and severe clinical course
3. To characterize the host immune response to infection
4. To evaluate the correlation between symptom severity, virologic/microbiologic characteristics, the host immune response and clinical course and outcome
5. To develop diagnostic and prognostic tools for future clinical study of novel pathogens / emerging infectious diseases
6. To establish a clinical and laboratory database, and related specimen repository, for future studies of novel pathogens / emerging infectious diseases
7. To investigate efficacy of emergency Investigational New Drug (eIND) therapies that may be administered to enrolled study participants during the course of clinical care

For COVID-19 / SARS-CoV-2 infection, clinical and demographic data will be evaluated, along with laboratory findings as appropriate, to assess key endpoints of interest to include, but not limited to, the following:

- Incubation period

- Duration of illness / symptoms
- Duration of functional disability
- Duration of shedding from respiratory and gastrointestinal tract
- Incidence and duration of viremia
- Predictors of severe disease: clinical signs, symptoms, laboratory features; host factors, eg smoking, vaping, alcohol consumption, comorbid illness/conditions, occupational health factors influencing lung disease; host biomarkers; virologic (strain/virulence) attributes
- Supportive management required: O_2, HFNC, NIPPV, ventilation; hemodynamics
- Complications: e.g., bacterial pneumonia; bacteremia; AKI; liver injury; CNS involvement, etc.
- Outcomes: resolution of symptoms; return to pre-illness functional status; requirement for O_2; development / exacerbation of reactive airway disease; pulmonary function; resolution of medical complications; new Rx requirements, e.g. bronchodilators; other sequelae
- Development of immunity (i.e., correlates), kinetics, duration of antibody detection
- Relationship between acute illness (or pre-illness) CoV titer and infection, outcome, virologic parameters
- Costs of care, costs of duty days lost

9.5 Study Design:

A prospective, longitudinal cohort study of novel pathogens / emerging infections with pandemic potential

9.6 Target Population:

Study findings will be generalized and applicable to all populations that travel to or reside in disease endemic areas or live within an area that has been exposed to infectious pathogens with pandemic potential; some of which include military personnel and the general public.

9.7 Benefit to the DoD:

Infectious diseases are a significant source of morbidity among military forces. Infectious disease outbreaks compromise force readiness and interfere with training programs. Strategies to characterize and ultimately to treat and prevent the major infectious disease threats to military personnel are urgently needed.

10.0 Study Procedures and Data management

10.1 Study Procedures:

Recruitment and enrollment strategies will be dynamically responsive to the outbreak as the situation evolves. Study volunteers may be recruited from areas of the hospital where they present for care or in the inpatient and isolation settings. Informed consents and enrollments will be conducted by study staff included on the IRB approved protocol, including staff named on delegation log, and trained in study procedures. Clinical evaluations will be performed by the MTF healthcare team and may also be conducted by qualified study team members.

In addition to MTF enrollment, MHS beneficiaries who have been clinically tested for COVID-19 will be invited to participate in a limited schedule of assessments for EPICC, including completion of a series of online surveys and at-home capillary blood sample collection at three timepoints. See Appendix A for further details.

At the time of a participant's initial consultation at an MTF, study activities will consist of explanation of study goals and purpose, and obtaining informed consent (may include consultation with Legally Authorized Representative). The day of enrollment will be defined as Study Day 0. The team will subsequently conduct serial collection of data (demographics, vital signs, clinical findings, diagnostics and other laboratory results, etc.) through study participant interview and from medical record review from the time of onset of illness throughout study duration. Specific risk factors linked to pathogen exposure (e.g., travel history to disease endemic regions, contact with sick individuals or known reservoirs of disease, etc.) will also be acquired from study volunteers, mainly through interview at Day 0. Medical records collected prior to day of enrollment will also be evaluated to ascertain medical history information pertinent to understanding baseline health and analysis of underlying risk factors associated with disease progression and other outcomes. Study team members will collect for research purposes biological specimens (e.g., nasopharyngeal and oropharyngeal swabs, rectal swabs, and blood) from enrolled participants who are laboratory-confirmed cases and Persons Under Investigation (PUIs) at time of enrollment, during the duration of hospitalization and/or isolation, with follow-up specimen collections up to approximately 6 and 12 months post-enrollment.

When possible, the study team will also obtain residual volumes of specimens collected for clinical care that would otherwise be discarded. Sample types include but are not limited to bronchoalveolar lavage, blood, urine, sputum, saliva, stool, cerebrospinal fluid, semen (adults only), or vomit. As available, these residual samples will be obtained from the time of onset of the disease under study. For active duty servicemembers, the study team will also request a pre-illness (up to 2 years prior to enrollment) serum sample from the DoD Serum Repository (DoDSR)

Some subjects may undergo clinical imaging studies as part of regular patient care or as part of this protocol using clinical imaging devices. For example, point-of-care portable ultrasound may be used to image areas such as the lungs, heart, and lower extremity veins, to explore the prognostic use of ultrasound in early detection of pneumonia and cardiovascular sequelae associated with severe disease. These imaging studies, when available, will be part of the collected subject data.

Study participants recruited and enrolled at MTFs who are ambulatory cases may also be evaluated via serial self-collection of samples such as nasal swabs, oropharyngeal swabs, capillary blood samples, and/or other specimens appropriate for self-collection.

Recruitment of individuals who are asymptomatic at high/some risk of exposure may involve study team members approaching family or friends who escorted the sick individual to the hospital as well as healthcare providers who have potentially been exposed to known or suspected cases. Enrollees who are asymptomatic high/some risk will undergo serial collections of clinical and laboratory data as well as biological specimens (e.g., nasopharyngeal, oropharyngeal, and rectal swabs and blood) at enrollment, with follow-up specimen collections up to approximately 12 months post-enrollment. The study team may consider implementing a plan for asymptomatic study volunteers to conduct self-collection of clinical specimens (eg., nasopharyngeal swabs, oropharyngeal swabs, rectal swabs, capillary blood samples) through the follow-up period. Demographic, clinical, and other data as described above for symptomatic volunteers may also be acquired for asymptomatic high/some risk participants.

The Flu-PRO questionnaire (24-27) may be used to record the patient-reported outcome (PRO) on severity of clinical symptoms and the impact of clinical interventions on infection. A web link to the Flu-PRO questionnaire may be provided to participants to record the severity of ILI symptoms daily for 14 days from either the day of enrollment, or the date of onset of symptoms (for subjects who were asymptomatic at enrollment). Paper based versions of the Flu-PRO questionnaire may be administered as needed.

In addition, an online questionnaire will be administered at enrollment and follow-up timepoints to collect data on medical history, comorbidities, and subject-reported recovery status.

If a participant is dually-enrolled in a treatment protocol and scheduled to receive investigational products under an eIND, an additional sample may be collected in the first 48 hours after test article administration. If eligible, study participants may also be considered for enrollment concurrently in an interventional clinical trial evaluating efficacy of treatment regimens. The priority of collection of clinical specimens will be as follows: 1. specimens collected for clinical care; 2. specimens collected in support of an interventional clinical trial; and 3. specimens collected for observational study (i.e., EPICC protocol).

The study team may consider discontinuation of study participants who have a negative test result for the pathogen of interest (based on current standard of care diagnostics) and/or are diagnosed with another respiratory pathogen other than the one of interest and/or the disease of interest is highly unlikely based on the primary clinical team assessment. This approach may need to be taken to conserve study staff time and resources depending upon local epidemiologic circumstances, e.g. very high incidence and subject accrual.

Results obtained from research analyses will not be entered into the participant's medical record. However, the study team will communicate relevant findings to the participant's primary care provider overseeing care of the indicated infection of study. The study volunteer's primary healthcare provider is likely to be a member of the protocol study team, and as such will be directly aware of study findings. If the primary healthcare provider is not a part of the protocol team, relevant findings from the study will be communicated to the healthcare provider verbally in a private setting to protect patient confidentiality or via a secure encrypted electronic communication.

Pregnant participants will be followed monthly until delivery to collect information on pregnancy outcome and health of the child with regards to the disease under study, to the extent practicable. Cord blood (a clinical residual specimen) may be collected for research purposes from enrolled women following childbirth.

Specimens collected through this observational study will be evaluated in various laboratory assays to characterize the epidemiology, immunology, clinical characteristics, and other important outcomes of interest. Associated clinical and demographic data will also be evaluated, along with laboratory findings as appropriate, to assess key endpoints of interest as described above.

Schedule of Patient Evaluation and Data Collection

The table below shows the schedule of assessments for study participants enrolled in EPICC. Given the possibility of critical illness of the patient and the potential increased risk of exposure to medical personnel, assessments that cannot be completed due to patient care considerations, infection control measures, or other logistical challenges will not constitute protocol deviations. In addition, the frequency of specimen and data collection may be reduced to accommodate logistics of acquiring specimens and data among study volunteers who are outpatients.

Ambulatory subjects who do not have restrictions of movement for infection control purposes (i.e., not advised by their care provider/local health authority to remain home in isolation) will be invited to return to the hospital for follow-up assessment and collection of specimens.

If an asymptomatic and/or COVID-19-negative subject develops symptoms or receives a positive test result for COVID-19, they will be asked to contact study staff to report their symptoms/test result and will restart the schedule of assessments from Day 0.

Inpatient MTF-Enrolled Subjects

Specimen/ Data	Enrollment (Day 0)	Days 3, 7 (± 1 day)	Weekly until discharge (± 2 days)	Day 14 if Discharged (± 3 days)	Day 28 (± 7 days)	6 Months (± 14 days)	12 Months (± 28 days)
Virology Samples – Nasal, Throat, and/or Oral specimen*	√	√	√	√			
Virology Sample - Rectal swab	√	√	√		√		
Blood¶	√	√	√	√¶	√¶	√¶	√¶
Residual clinical samples #	√	√	√	√	√	√	√
Radiologic images **	√	√					
Flu-PRO Symptom Diary ***	√	√	√	√			
Online Questionnaires	√				√	√	√

* To detect and analyze virus in the nose, throat, and/or mouth, the specimen collection method employed may change to follow updated best practices as reported in the scientific literature. Example sample types are swabs (nasal, nasopharyngeal, oropharyngeal) and/or saliva that may be collected by study staff or self-collected by subjects.
¶ Blood collection after discharge may be done using a commercial at-home microsampling blood collection kit.
# Residual samples from clinically-indicated lab tests will be collected for research. Sample types may include sputum, saliva, urine, stool, blood, semen (adults only) or vomit. Residuals may be obtained from samples collected starting at the date of disease onset, which may be prior to Study Day 0.
** Lung, cardiac, and/or venous ultrasound may be conducted on hospitalized subjects on days 0, 1, and 3 as resources and local patient care policy allows. Findings from any additional radiologic images obtained during routine clinical care will be collected for research.
*** Subjects aged 7 and older who are experiencing symptoms will be asked to complete the Flu-PRO diary for 14 days.

Outpatient MTF-Enrolled Subjects

Specimen/ Data	Enrollment (Day 0)	Day 7 (± 1 day)	Day 14 (± 3 days)	Day 28 (± 7 days)	6 Months (± 14 days)	12 Months (± 28 days)
Virology Sample – Nasal, Throat, and/or Oral specimen*	√		√			
Blood¶	√	√	√	√	√	√
Flu-PRO Symptom Diary **	√	√	√			
Online Questionnaires	√			√	√	√
Residual clinical samples #	√	√	√	√	√	√

* To detect and analyze virus in the nose, throat, and/or mouth, the specimen collection method employed may change to follow updated best practices as reported in the scientific literature. Example sample types are swabs (nasal, nasopharyngeal, oropharyngeal) and/or saliva that may be collected by study staff or self-collected by subjects.
¶ Blood collection may be done using a commercial at-home microsampling blood collection kit.
# Residual samples from clinically-indicated lab tests will be collected for research. Sample types may include sputum, saliva, urine, stool, blood, semen (adults only) or vomit. Residuals may be obtained from samples collected starting at the date of disease onset, which may be prior to Study Day 0.
** Subjects aged 7 and older who are experiencing symptoms will be asked to complete the Flu-PRO diary for 14 days.

Online Cohort: See Appendix A

Specimen/ Data	Enrollment (Day 0)	Day 28 (± 7 days)	6 Months (± 14 days)	12 Months (± 28 days)
Online Questionnaires	√	√	√	√
Self-collected blood¶		√	√	√

¶ All blood collection for this cohort will be done using a commercial at-home microsampling blood collection kit.

Considerations for Pediatric Participants

- Flu-PRO symptom diary may be completed by subjects aged **7 and older**.
- Pediatric subjects will not be asked to complete the online questionnaires.
- Self-collection of specimens is an option for subjects aged **8 and older**.
- Blood collection volume and frequency may be reduced to comply with safety limits. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.

Research Laboratory Methods

Clinical specimens collected through this observational study will be evaluated in a variety of laboratory assays developed by laboratory partners. Specific laboratory assays to study the virologic, immunologic, and other relevant aspects of infections are unlikely to be available at the time of specimen collection (i. e., during the outbreak) given the time required to establish assays specific for novel, emerging pathogens. Below is a general description of laboratory assay methods and endpoints that may be used; however, this does not represent an exhaustive list to include new methodologies that are later developed in response to assaying novel pathogens.

To characterize virologic correlates (e.g., viremia) of clinical symptoms and disease severity, serum levels of viral genomes can be assessed by real time-PCR at various time points. Serum concentration of viral antigen can be evaluated by enzyme-linked immunosorbent assay (ELISA). These same assays may be utilized in the longitudinal detection of virus in body fluids (e.g., nasal mucosa, sputum, urine, stool, etc.) throughout the course of evaluation/illness.

In addition, serum may be assayed for concentrations of virus-specific IgM and IgG in the acute and convalescent stages of evaluation/illness. Cytokine profiles can be conducted via multiplex assay to describe the pro- versus anti-inflammatory cytokine responses of study volunteers throughout the course of illness. PBMCs may be collected to investigate cell-mediated immune responses in the acute and convalescent phases of illness.

Novel biomarkers, including transcriptomic and proteomic signatures, are promising for diagnosis and prognosis. Collection of appropriate specimens from individuals with well-characterized illnesses is required to inform assay development. Immune factors have been used as biomarkers for predicting clinical course and outcome, as well as the severity of clinical symptoms. For example, higher levels of cytokines IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNFα were observed in patients with SARS-CoV- 2 infection requiring ICU-level care compared to those receiving non-ICU care (21). Host specific responses may be evaluated using real-time PCR and other molecular methods to identify biomarkers, transcriptomic signatures, or other lab-based endpoints indicative of severity of clinical course of disease progression and other risk factors associated with clinical outcomes, etc. In addition, evaluation of host transcriptomic responses among symptomatic versus asymptomatic, high risk exposure individuals may identify an early warning means for identifying asymptomatic people who eventually develop disease.

RNA sequencing of blood specimens may also be conducted to assess viral transcription signatures as biomarkers for clinical outcomes. Blood specimens may also be used in phosphoproteomic assessments by measuring the proportion of host immune system proteins that are phosphorylated to evaluate novel biomarkers associated with activation of protein signaling pathways in response to infection.

10.2 Data Collection:

Diagnostic Tests and Laboratory Analysis to be Obtained:

As available, laboratory data elements, such as but not limited to hematologic, biochemical, and other clinical parameters; clinical images and associated reports; and clinical diagnosis of the pathogen of interest (SARS-CoV-2) and/or alternate or co-infective pathogens, will be abstracted from the medical record of study volunteers on the day of enrollment (Study Day 0), throughout the period of hospitalization (inpatients) and through duration of follow-up. In addition, laboratory and diagnostic data available in medical records prior to Day 0 will be abstracted for study of baseline health, existing co- morbidities, etc.

Data Elements

Case report forms (CRFs) for this protocol have been adapted from those of the International Severe Acute Respiratory Infection Consortium (ISARIC).

There are five main components of the data collection forms, encompassing study participant information at:

1. The time of enrollment (at time of presentation at MTF, hospital admission, or being placed in isolation),
2. Period prior to Day 0, as appropriate, to assess recent medical history,
3. Medical encounter data (inpatient or outpatient),
4. The time of discharge or death
5. Follow-up period (following hospital discharge or being released from isolation setting, or otherwise as an out-patient)

Major data variable categories to be collected include, but are not limited to the following:

- Day of enrollment
    - Demographics (Residence, Occupation, Military Status)
    - Signs and Symptoms at onset of illness and presentation for care
    - Epidemiological Risk Factors and Exposures
    - Pre-admission Medications
    - Review of recent medical history via medical record review
    - Flu-PRO symptom severity questionnaire
- Hospitalization or Isolation Period
    - Daily Observations (i.e., vital signs) and Treatments (i.e., receipt of IV fluids)
    - Daily Laboratory Results
    - Medications and Blood Products
    - Microbiology Results
    - Critical Care
    - Severe Symptoms and Complications
    - Flu-PRO symptom severity questionnaire
- Outcome
    - Final Diagnosis
    - Patient Status at Outcome
    - Follow-up period

Data Collection

Study data will be copied onto electronic CRFs at the study site. These transcriptions will occur on an ongoing basis during the study in accordance with Good Clinical Practice (GCP) guidelines. Data entered on CRFs, as well as corrections made, shall be performed by authorized individuals. This study will make use of electronic case report forms (eCRFs) using tablets with the Research Electronic Data Capture (REDCap). For cases where a tablet cannot be used because of infection control concerns or logistical issues, authorized personnel will capture CRF information using a paper based version and later electronically record results into REDCap. All data transmitted to and from the application is done using a secure, encrypted transmission (SSL/HTTPS). For increased security, the application additionally verifies the SSL certificate of the REDCap server that it is communicating with in order to validate the server's identity. The REDCap mobile application employs encryption-at-rest on the mobile device's hard drive, so all data and information stored on the device is protected from unauthorized users. REDCap has built-in safeguards such as username and PIN, and remote lockout. When resources are available, data from clinical devices including de-identified ultrasound or radiograph images, continuous vital signs, and/or cardiac telemetry will be locally stored on password-protected devices, and if possible, transmitted wirelessly to a secure Cloud backup. Henry Jackson Foundation (HJF) headquarters IT will host deidentified study data on HJF HQ secured servers. HJF is responsible for the purchase, setup, maintenance, and service of any its servers and technology.

The supervising principal investigator, or designated associate investigator, will be responsible for regular maintenance and review of CRFs for accuracy. Access to study files (e.g., CRFs) is restricted to care providers and research team members given specific tasks to perform. The files are kept in a secured location in each study site. Some participant-completed forms such as surveys may be completed through the web-based program. These will be transmitted directly into the study database. Research labs will transmit their testing data to the DCC using standard procedures for data handling and transmission.

Data Management/Quality Control

During the study, the investigator will review study data from their site, including medical records, records detailing the progress of the study for each volunteer, laboratory reports, CRFs, documentation of informed consent and authorization forms for each study volunteer, correspondence with the IRB, adverse event (AE) reports and information regarding volunteer discontinuation of the study. All required study data will be clearly and accurately recorded on source documents or CRFs by either the participant themselves or authorized study personnel. Only designated study personnel shall record or change data on a CRF. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded on the CRFs. Original observations entered directly onto the CRFs are defined as source data. PHI will be removed from clinical devices, such as those used for radiology, and associated data will be labeled with the study participant's ID. For each pivotal analysis (e.g., for a sub-study, manuscript, or presentation), the 'snapshot' (or 'data freeze') database will be made and archived in the study data repository. After the study analysis ends, the database will be locked.

Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for this Study:

Human Biological Specimens

1. Intended Use of the Samples/Specimens/Data

Specimens will be processed and handled as appropriate following applicable MTF enhanced precaution and Infection Control SOPs. Methods for specimen collection, handling, and processing are described in their respective SOPs. Details of specimen storage, maintenance, and QA/QC are described in their respective SOPs.

Samples and data collected under this protocol will be analyzed to study the pathogenesis of disease and the host immune response to infection. Genetic testing on human specimens will be conducted to evaluate the host (subject's) immune response to SARS-CoV-2 infection and its association with clinical outcomes. Additional genetic testing will examine viral genetic markers and transcription signatures as predictors of infection severity, clinical course, and outcomes. Genetic testing results will not be provided to participants.

This is a purely observational study. Any experimental treatments will be done under other protocols for which separate IRB review and approval will be obtained.

2. How Samples/Specimens/Data Will Be Stored

Access to research samples will be limited using locked facilities and monitored freezers. Samples and data will be stored using codes assigned by the investigators or their designee(s). Data will be kept in password-protected computers. Only investigators or their designee(s) will have access to the samples and data.

3. How Samples/Specimens/Data Will Be Stored & Tracked

Samples from individuals found infected with a novel pathogen / emerging infectious disease of interest will be archived and stored at an appropriate biosafety level facility.

Samples acquired as part of the protocol will be tracked using appropriate software, such as Freezer Works, a sample tracking, biorepository management and freezer inventory program.

4. What Will Happen to the Samples/Specimens/Data at the Completion of the Protocol?

In the future, other investigators may wish to study these samples and/or data. IRB approval will be sought prior to any sharing of samples/data. Any clinical information associated with the sample with or without patient identifiers will be shared after IRB approval has been obtained. Upon study closure, all samples and data will be de-identified in accordance with the DHA-approved Data De-Identification Plan.

5. What Circumstances Would Prompt the PI to Report to the IRB Loss or Destruction of Samples/Specimens/Data

Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that compromises the scientific integrity of the study will be reported to the IRB.

6. Storage and Tracking of Samples/Specimens/Data

Research use of data collected through this protocol will be coded and will not contain identifiers. These data will be coded with access maintained by a custodian not affiliated with the study team per IDCRP policy. Much of the subsequent research using the coded materials collected under the auspices of this study will be accomplishable through approved exempt or non-human use sub-studies. All research using the materials collected under this protocol will be conducted in accordance with an IRB-reviewed protocol. Data and specimens released for research use will be labeled by PIN only; data will be shared without any identifiers or links to individuals. The DCC custodian, the DCC Chief, will not share identifying information with investigators without explicit IRB approval.

A Defense Health Agency (DHA) Data Sharing Agreement will be implemented as needed to access MHS data. Appropriate data sharing agreements and other legal agreements will be implemented prior to sharing of data and specimens with collaborators.

Managing Data (Data Management and/or Sharing Plan) and/or Human Biological Specimens for Future Research:

If the study involves collecting, storing, or banking human specimens, data, or documents (either by the Investigator or through an established repository) for FUTURE research, address. How the specimens/data will be used, where and how data/specimens will be stored (including shipping procedures, storage plan, etc.), whether and how consent will be obtained, procedures that will fulfill subjects' request as stated in the consent, whether subjects may withdraw their data/specimens from storage, whether and how subjects may be re-contacted for future research and given the option to decline, whether there will be genetic testing on the specimens, who will have access to the data/specimens, and the linkage, the length of time that data/specimens will be stored and conditions under which data/specimens will be destroyed

Establishment of a specimen repository for future clinical and laboratory investigation for future emerging infectious disease research:

The study aims to build a repository of biological specimens (e.g. whole blood, serum, and PBMCs, as well as nasopharyngeal fluid, sputum, bronchioalveolar lavage fluid, oropharyngeal fluid, saliva, urine, semen, and rectal swab specimens, etc.) from an observational, longitudinal study of emerging infectious diseases. Specimens may be stored in an appropriate biosafety level facility at a protocol-approved biorepository. These specimen repositories will be linked to an extensive de-identified clinical database.

In the future, this repository might be used in the identification and validation of biomarkers for severe infection, identification and molecular characterization of novel pathogens, development and evaluation of new diagnostic platforms, and/or evaluation of host factors that may be associated with susceptibility and outcome. Coupled with detailed clinical history and risk factor information gleaned through electronic medical record review, this repository will ultimately be an invaluable resource for future investigations of emerging infectious diseases in clinical as well as laboratory settings. Moreover, the infrastructure developed through these activities will serve as a broad-based platform for a diverse portfolio of relevant sub-studies into emerging infectious diseases and strategies for its control and prevention.

Study Time Line

This natural history core protocol will be open indefinitely, although sites may close once an epidemic has passed.

11.0 Statistical/Data Analysis Plan

11.1 Statistical Considerations

This is an enhanced surveillance protocol using clinical observations and laboratory testing, supplemented by additional lab testing for research purposes. The frequency and expansion of such outbreaks is unknown, and hence this protocol will be activated on contingencies in many cases. Data from a given outbreak will be compiled into a dataset for descriptive analyses. Risk factors will be identified retrospectively using odds ratios. Comparison between similar outbreaks may be performed if sufficient data allows. It is recognized that a proportion (i.e., not all) of eligible subjects will consent to participate in this study. As such, the limitations and generalizability of the study findings will be acknowledged in associated reports and manuscripts.

11.2 Sample Size Estimation:

As determined by exigencies of the outbreak, and resource availability. Please see Appendix A for sample size considerations for the online cohort.

11.3 Data Analysis Plan:

Student’s t test (or Wilcoxon rank-sum test if appropriate) on continuous variables and Pearson’s chi-square tests (or Fisher’s exact test if appropriate) for categorical variable to summarize key outcomes of interest. Multivariate regression techniques will be used to assess relationships between key variables adjusted for potential confounders.

A data analysis plan will be created to meet the following reporting requirements:

- During an outbreak, descriptive clinical and laboratory data will be summarized per patient on a regular basis as appropriate for all investigators, involved hospitals, the respective service's public health agencies, AFHSB, and DHA leadership as a part of the epidemiologic reporting requirements. It is recognized this data may not be fully cleaned. Aggregate data report will be prepared monthly.

- At conclusion of an outbreak, a summary report per outbreak will be prepared once all data is cleaned. This will take an estimated 90 days.

With respect to the analysis of the study data, the following objectives will be addressed, and more fully articulated, in a statistical analysis plan:

1) Describe natural history of clinical disease.

Data items related to disease duration include duration of fever and other predominant symptoms, and days from onset of symptoms until hospital discharge/return to normal activity. Each variable will be summarized by mean, standard deviation, median, inter-quartile range, minimum and maximum overall and also for each group within several groupings: age categories, adult vs. minor, gender, different race groups, index cases vs. secondary cases, military rank and/or nature of duties for those with active duty military duty status. We will also use symptom severity data from the Flu-PRO questionnaire to explore the extent to which ARI symptom severity scores differs across the cohort.

Adverse binary outcomes where the time to event may also be of interest: These include requirement for ICU admission, development of any super infection, development of oxygen requirement, mortality, etc. For each of these, we will first give a simple summary of the number and percent experiencing each outcome. We will also summarize by different subgroups as above.

2) Identify risk factors for infection and severe clinical course.

One set of analyses will treat laboratory-confirmed diagnosis as the outcome in logistic regression models, with the following covariates: age, sex, race, military rank, service branch, type of exposure, use of personal protective equipment, etc. Similar logistic regression models will be developed to determine predictors of severe clinical course, such as mortality.

Another set of analyses will treat illness duration as a quantitative endpoint to be used as the outcome variable in multiple linear regressions. The key endpoint will be the total symptom score considered at three different time points: at symptom onset, at 48 hours post symptom onset, and at its maximum value whenever that occurs. Other potential confounders to be included as covariates are: age, sex, race, and number of days since presumed exposure.

Complementary methods of subject recruitment and data collection within the study will address the following specific aims:

1. Among COVID-19 confirmed cases, compare risk factors (comorbidities, lifestyle factors, medications, exposure opportunities, general symptoms) for:

    a. Those who get hospitalized vs. those who do not get hospitalized
    b. Those who get hospitalized with a ventilator vs. those who do not get hospitalized and those hospitalized but not placed on a ventilator
    c. Those who get hospitalized in the ICU vs. those who do not get hospitalized and those hospitalized but not in the ICU
    d. Those who go on to die vs. those who do not die
2. Compare <u>risk factors</u> (comorbidities, lifestyle factors, medications, exposure opportunities, general symptoms) for infection among the total COVID-19 tested study population of COVID-19 confirmed cases (test positive) vs. confirmed non-cases (test negative).
3. Compare <u>symptoms</u> between various groups:
    a. Among the total COVID-19 tested study population, COVID-19 confirmed cases vs. confirmed non-cases
    b. Among COVID-19 confirmed cases for those:
        i. who get hospitalized vs. those who do not get hospitalized
        ii. who get hospitalized in the ICU vs. those who are hospitalized, but not in the ICU
        iii. who go on to die vs. those who do not die
4. Among the COVID-19-confirmed cases and an appropriate and hypothesis-specific randomly selected comparison group, using medical record data, identify comorbidities and medications that are associated with reduced occurrence of COVID-19 symptoms as well as with becoming infected with COVID-19.

The proposed analytic strategy will be to report crude (non-adjusted), age and sex-adjusted, and multivariable adjusted probability of hospitalization in those with and without the exposures of interest.

1) Calculate crude (non-adjusted) likelihood of hospitalization in demographic sub-populations of interest (e.g. by age strata, sex, and race/ethnicity)
2) Determine the age-specific prevalence of the major comorbidities (obesity, hypertension, asthma) separately by sex and race/ethnicity
3) The primary model will be logistic regression, calculating adjusted predicted prevalence of hospitalization for those with each comorbidity alone and comorbidity clusters (since comorbidities are correlated)
    a. A priori, evaluate whether risk associated with each comorbidity is similar for men and women and for African Americans vs others (interaction by sex; interaction by race).

Conduct separate models for the active duty population and the beneficiary populations

3) Characterize host immune response.

We will examine the relationship between factors, such as the immune response (e.g., serum antibody concentration) and pathogen infection kinetics, and disease progression, symptom severity, and clinical outcomes of interest. We will look at both cross-sectional relationships (i.e., among people) and within person relationships. For cross-sectional relationships, we will start by plotting the total symptom severity score against the immune response, separately for each of the four blood samples. We anticipate a monotonic relationship, but it is possible for a U-shaped relationship in some cases, i.e., illness severity is greater if there is a too small immune response or a too large one. We will develop regression models with the outcome of the symptom severity or clinical course on the chosen function of

immune response to see if there is a statistically significant relationship. We will add covariates to control for possible confounders, such as age, race, sex, military status, etc. and others mentioned above.

4) Evaluate correlations between symptom severity, virologic/microbiologic characteristics, host immune response and clinical course/outcome.

We will consider two groups: subjects with versus without a severe clinical course, such as mortality. We will compare the proportions with a significant serum antibody ‘response’ between the two groups by contingency table chi-square tests. We anticipate that the categorization of immune response will be binary (i.e., proportion of individuals exceeding a given concentration of serum antibody), in which case we will be comparing a binomial probability between the two groups. These analyses will form the backbone for more elaborate multivariate analyses.

5) Develop diagnostic and prognostic tools.

Data and specimens from this protocol may be used in the development of diagnostic and prognostic assays for novel pathogens. For example, the collection of blood and other body fluids from individuals with laboratory-confirmed infection may aid in the development of novel assays with higher sensitivity and specificity. Additionally, the collection of longitudinal clinical characteristics in addition to clinical laboratory data may aid in the development of prediction models to determine risk factors for severe clinical course, such as mortality.

12.0 Participant Information

Subject Population:

As this is an observational study, subjects of all ages will be enrolled. Military Treatment Facilities see a diverse population including all genders and races, and a wide range of ages. This study will examine the epidemiology of novel pathogens / emerging infectious diseases, seek to identify risk factors for severe disease and elucidate mechanisms of host immunity to these pathogens.

Given the possibility of MTFs being used as surge capacity to support the care of individuals affected by an infectious disease outbreak, this study also allows for enrollment of anyone meeting the inclusion criteria and obtaining care at a participating MTF. Males and females of all ages who are symptomatic or asymptomatic individuals (at high/some risk of exposure) meeting inclusion criteria are eligible for participation.

Participation in the online survey-based cohort is limited to adults only.

12.5 Inclusion Criteria:

Inclusion Criteria for **MTF Enrollment:**

Individuals of any age who are located at a participating MTF and meet at least one of the following criteria:

1. **Laboratory-confirmed presence of the pathogen of interest** (e.g., SARS-CoV-2, influenza A /H7N9, etc.)
   OR
2. **Are a person under investigation (PUI) for the pathogen of interest, as identified per current CDC PUI case definition**

(refer to "Criteria to Guide Evaluation of PUI for COVID-19" at https://www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html
OR

3. **Are asymptomatic and/or have tested negative for the pathogen of interest, but are considered per current CDC guidance to have had recent high/some risk for exposure to pathogen of interest**
   For assessing asymptomatic individuals, refer to guidance provided by the CDC for COVID-19 at: https://www.cdc.gov/coronavirus/2019-ncov/php/public-health-recommendations.html

Inclusion Criteria for **Online Enrollment:**

1. An MHS Beneficiary who has been clinically tested for the pathogen of interest
2. Able to receive email communications and respond to web-based surveys
3. ≥18 years of age

12.6 Exclusion Criteria:

1. Individuals who decline participation in the study
2. Individuals who the study investigators believe are unable to comply with the requirements of the study

13.0 Recruitment and Consent

13.1 Identification and Selection of Subjects:

Given the dynamic nature of novel pathogens/emerging infectious diseases, there is no pre-defined target enrollment for the protocol. The main objective of this protocol is to recruit and enroll a representative proportion of cases of emerging infectious diseases to characterize the epidemiology and clinical characteristics of disease. Site study team members will engage with relevant clinical staff to identify eligible patients in inpatient wards, intensive care units, acute care clinics, and emergency departments. Participants will be identified through screening of medical records to identify individuals with the recent exposures and clinical manifestations of interest, and will be approached following the initial medical evaluation. The study will be described to patients, and their eligibility and willingness to participate will be assessed. For those with confirmed infection or PUI, room access may be limited and consent will be performed by study staff members with patient access.

Individuals presenting for care at MTFs, such as at clinics or in the inpatient and isolation setting, will be recruited and enrolled and will participate in serial collection of biological specimens over a 12-month period, with more intensive (i.e., daily, weekly) data collection occurring during the inpatient or isolation period. Information on current and prior medical conditions and hospitalizations will be collected via medical chart review and/or MDR data extractions. Information on specific epidemiologic risk factors during or related to deployment (e.g., travel history, zoonotic exposures, etc.) will be ascertained through participant interview or review of available medical records (e.g., AHLTA-T, ALTHA, CHCS, Essentris, MHS Genesis, JLV (accessed via AHLTA or MHS Genesis), MDR, DMSS, and/or DRSi). Study participants evaluated at MTFs and who are either 1. ambulatory cases advised to return home and remain home in isolation or 2. classified as asymptomatic individuals with high/some risk for exposure may also be evaluated for follow up through a study design including serial self-collection of nasopharyngeal swabs, oropharyngeal swabs, rectal swabs, or other specimens appropriate for self-collection. A similar schedule of data collection and methods used for enrolled inpatients will be applied for ambulatory cases (non-hospitalized) and asymptomatic individuals as feasible.

Details on the recruitment, consent, and enrollment of subjects participating through the MHS-wide online process are included in Appendix A.

13.2 Recruitment Process:

Recruitment of study volunteers at the MTFs will rely heavily on the direct interface of healthcare providers with potential eligible participants. The study team will develop an MTF communications plan, outlined in the protocol's Manual of Procedures, that will be implemented by local site teams to facilitate identification of potential eligible participants. Study volunteers may be recruited from clinics and inpatient or isolation settings (to include PUI). In addition, recruitment of individuals who are asymptomatic at high/some risk of exposure may involve study team members approaching family or friends who escorted the sick individual to the hospital. Another group of individuals who may be approached for recruitment as asymptomatic high/some risk participants, are healthcare providers who are in close contact with and potentially exposed to known cases. Adults and children are eligible for participation.

The study team will use multiple means of communicating study awareness to enhance recruitment efforts, including periodic briefings delivered by the site PI's and their study teams to their critical care and infectious disease staff and inpatient nursing staff. Information notices with points of contacts will be posted in clinical sites such as inpatient units, acute care clinics, and several points in the emergency room. Research team members who are Active Duty military personnel will not wear visible signs of their rank during recruitment. Site PI's or their authorized designees will conduct daily queries of MTF electronic health heath records (including infectious disease reportables list, DRSi, and searching in CHCS for patients with coronavirus lab code) to identify individuals who tested positive for disease of interest and will review daily admissions into the ICU, general pediatric and internal medicine wards with their study personnel to identify new inpatients. Information from infection control, preventive medicine and infectious disease departments may be obtained to find patients who may be managed / followed outside of the MTF.

Depending on age, severity of infection, and MTF requirements, study staff will determine if a potential subject is capable and/or allowed to provide a signed consent, verbal consent, or if an LAR is required according to IRB, HIPAA, and local MTF requirements.

13.3 Compensation for Participation:

In accordance with 24 USC 30, enrolled subjects will be provided compensation in the form of a gift card. Subjects will receive:

- $50 for each outpatient venipuncture
- $10 for each self-collected blood sample and swab
- $10 for completion of online surveys and Flu-PRO

13.4 Eligibility Assessment Process:

As part of medical record review, the team will assess the eligibility of potential subjects. Individuals identified as having known risk factors for an emerging infectious disease (e.g., recent travel to a disease-endemic region, or close contact with a known or suspected infected individual or a returning traveler) who present to a participating MTF for medical care (e.g., febrile illness) or are placed into isolation are eligible for study participation. Additionally, asymptomatic high/some risk for exposure individuals may be enrolled at MTFs. Given the possibility of MTFs being used as surge capacity to support the care of individuals affected by an infectious disease outbreak, this study allows for enrollment of anyone meeting the inclusion criteria and obtaining care at a participating MTF. Eligibility will be assessed and a final determination of subject eligibility will be made during the consent process. Please refer to section 12.5 for the inclusion criteria.

13.5 Consent Process:

ICD and Assent Regulatory Requirements

Are you requesting a waiver or alteration of informed consent?

☑ Yes ☐ No

What type?

☑Waiver of documentation of informed consent

☐ Waiver or alteration of informed consent

Due to the contagious nature of the emergent infectious diseases being studied under this protocol, study staff may not always be allowed work near the potential and/or active study subjects or handle any items that they may have come in contact with per local MTF infection control policies. Therefore, obtaining signed ICDs and HIPAA Authorizations for subjects enrolled in this study may not always be feasible or safe for study staff. The study team is requesting a waiver of documentation of informed consent as well as a full Waiver of HIPAA Authorization to use as appropriate based on the circumstances of the disease being studied and MTF requirements. This study falls under the following criteria noted in 32 CFR 219.117: “That the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context."

Please explain the consent process:

ICD and Assent Regulatory Requirements

The Informed Consent Document (ICD) and Assent Form, including a HIPAA authorization, will be reviewed and approved by the IRB prior to initiation of the study. The ICD will contain a full explanation of the possible advantages and risks of study participation. The ICD will be compliant with applicable DoD regulations, 32 CFR 219, and the Belmont Principles, and the Health Insurance Portability and Accountability Act (HIPAA) Authorization. Informed consent includes the principle that it is critical the participant be informed about the principal potential risks and benefits. This information will allow the participant to make a personal risk versus benefit decision and understand the following general principles:

- Participation is entirely voluntary,
- Participants may withdraw from participation at any time,
- Refusal to participate involves no penalty; and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

An ICD or Assent Form (with parental permission) will be signed by the participant before any study-related procedures are initiated for each participant. The investigators or their designees will present the protocol in lay terms to the participants. If access is limited, an authorized member of the study team accompanied by a witness may conduct the informed consent process. Questions on the nature of the protocol, the means by which it is to be accomplished, and the risks to the participants will then be solicited. Any question that cannot be answered will be referred to the principal investigator (PI). All participants will be given the opportunity to ask questions. No participant should grant consent until questions have been answered to his/her satisfaction. Documented parental permission will be received prior to obtaining assent from a minor. If rules allow, a copy should be provided to the volunteer or their guardian.

Minors:
An assent form (with parental permission) will be used for children aged 7-17. The ICD and Assent Form (with parental permission) indicate that by signature, the participant permits access to relevant medical records by the study investigators and regulatory authorities. Documented parental permission is required prior to assent by a minor using the ICD. A Waiver of Documentation of Assent will be used for children 6 and under, documenting that the study was explained to the child in age-appropriate terms and that the child has either expressed verbal assent or does not have the capacity to do so.

For adults who are incapacitated by the illness:
Consent may be obtained by a Legally Authorized Representative (LAR). The patients should be consented themselves at such time that they are able to provide consent.

For Hospital Staff who are Active Duty Military:
Hospital staff who are active duty military, and therefore eligible for care at a military treatment facility, may be approached for consenting to the study due to their potential exposure to coronavirus. If hospital staff who are active duty military are approached for participation in the study, supervisors will not be present at any human subject recruitment sessions or during the consent process in which hospital staff who are active duty military under their supervision are afforded the opportunity to participate as human subjects. Supervisors (e.g., military and civilian supervisors or anyone in the supervisory structure) will not influence the decisions of their subordinates regarding their participation as subjects in this study.

Verbal Informed Consent:
Due to the contagious nature of the emergent infectious diseases being studied under this protocol, study staff may not always be allowed work near the potential and/or active study subjects or handle any items that they may have come in contact with per local MTF infection control policies. Therefore, obtaining signed ICDs and HIPAA Authorizations for subjects enrolled in this study may not always be feasible or safe for study staff.

The study team is requesting a waiver of documentation of informed consent as well as a full Waiver of HIPAA Authorization to use as appropriate based on the circumstances of the disease being studied and MTF requirements. When possible, the subject will provide signed ICD and HIPAA authorization. If the subject is unable to provide signed authorizations, the full Waiver of HIPAA Authorization will be utilized. If study personnel are unable to work near the potential and/or active subject for safety reasons, the study personnel will contact the patient according to the requirements of the MTF. Options may include but are not limited to speaking with the patient from a safe distance in person, contacting the patient by phone in person with a barrier in between the potential and/or active subject and the study personnel, contacting the patient by phone from a separate location, or having a study physician trained in infectious disease safety procedures meet with the potential and/or active subject in close proximity (if allowed by the MTF).

Regardless of the method of delivery, the potential and/or active subject would be thoroughly briefed on the study's Informed Consent and HIPAA information. They would also be provided with an opportunity to ask any questions and an unsigned copy of the informed consent if permissible by the MTF. In turn, study staff will thoroughly document the informed consent process and the subject's verbal approval of the ICD in the study chart. The study personnel will sign and file the ICD. The study personnel will also include a note in the study chart summarizing the informed consent process.

13.7 Withdrawal from Study Participation:

A subject may voluntarily end participation in the study at any time. If a subject withdraws, the investigator will make a reasonable effort to determine the reason for the subject's withdrawal from the study. Telephone calls, registered letters, and/or e-mail correspondence will be considered reasonable effort. Data and specimens collected up to the time of withdrawal will be retained and de-identified in accordance with IRB, HIPAA, and Safe Harbor requirements.

Subjects may be discontinued by the study team if there is a determination by the on-site physician that the volunteer may be unable to comply with the requirements of the study, or in the interest of the military mission.

14.0 Risks and Benefits

Risks of Harm:

Risks associated with obtaining specimens:

- Obtaining specimens could be associated with discomfort from a needle stick, swab, or other procedure, and occasionally associated with bleeding, bruising, local or systemic infection.

- Genetic testing may reveal sensitive information that subjects may not want disclosed, and may cause some emotional distress for the participant.

Risks associated with privacy:

- Despite meticulous precautions, breaches of confidential information (including genetic information) do occasionally occur, although the chances of such a breach are unlikely.

Expected Adverse Events from Research Risks and Reporting

- Rare (Event Rate < 1%)
  - Secondary infection and syncope from vasovagal reactions occur from blood collection in rare cases. Risks of nasopharyngeal swab, rectal swab, cheek swab could include bleeding or mucous membrane irritation.
- Less Likely (1% ≤ Event Rate < 5%)
  - No known risks
- Likely (5% ≤ Event Rate < 10%)
  - Bruising and bleeding may occur as a result of blood collection procedures.
- More likely (Event Rate ≥10%)
  - No known risks

Measures to Minimize Risks of Harm (Precautions, safeguards):

Participation in this protocol will impose minimal risks to the participant. As feasible, the study team will coordinate the collection of research purpose blood draws during timing of blood collection for clinical care to minimize discomfort to the patient. Blood volume collection per visit will be approximately 25 mL total. Total blood volumes collected over the 6 month duration of study will not exceed limitations for adults and children outlined in the CFR §46.110: Expedited review procedures for certain kinds of research involving no more than minimal risk, and for minor changes in approved research. Blood draw volumes will be reduced for pediatric subjects, small adults, and pregnant women in accordance with minimal risk and safety guidelines, and as needed per guidance from the primary care provider overseeing care of enrolled patients.

Staff safety in sample collection and handling is a concern that will be closely monitored by the Infection Control staff at the facility. Risk to staff is mitigated by minimizing an additional phlebotomy beyond that being done for clinical care. Specimen collection should be harmonized with the clinician’s requirements, and missed samples will not be re-drawn.

Unanticipated problems involving risk to subjects or others (UPIRTSO) may also occur that are not related to a described harm (adverse event) to study subject, rather a potential harm (such as loss of accountability of PII/PHI) or risks to others including medical staff. Specifically in this study is a concern that collection of infection fluids may cause harm to study personnel. Collection of samples for this study must only be conducted by individuals trained and authorized to collect and handle serious biohazardous specimens wearing proper personal protective equipment (PPE). PPE training will be conducted and documented for all personnel who may interact directly with patients and/or samples in order to mitigate any UPIRTSO related to accidental exposure of study staff and other individuals in the facility.

Confidentiality Protections (for research records, data and/or specimens):

A certificate of confidentiality will not be used in this protocol.

The only electronic links established between the participant name, SSN, and the Study ID number will be in the data management system managed by DCC and on a tracking spreadsheet used for scheduling purposes, which will both require a password to access and will only be accessed by authorized study personnel. During the study, source documents will be held in a secured cabinet with restricted access. Team members with access to the documents are counseled on the importance of confidentiality of participant medical records. All staff involved in this study have completed the required human subjects protection and ethics training. In all electronic records for data analysis, the participant is not referenced by name, but only Study ID number. This method is designed to protect the privacy of study participant medical information. All CRFs will be held at the individual study sites in the research spaces in secured cabinets with restricted access.

The only hard copy link established between the participant’s name, SSN, and the study code number will be held in a secured cabinet with access restricted to research team members with a need to use the Registry Form or Contact Information Form. Team members with access to CRFs are counseled on the

importance of confidentiality of participant medical records. In all electronic records for data analysis, the participant is not referenced by name, but only study code number.

The only electronic link will be stored on a secure password protected server. The file is only accessible directly to the DCC data custodian. The designated CRC at each site and two backups at DCC will also have access via DCC’s data management system.

The study team may share coded data with collaborators at non-DoD facilities, and may work with participating MTFs to assist with appropriate public health data reporting requirements.

Potential Benefits:

If the individuals in the research are considered experimental subjects (per 10 USC 980), and they cannot provide their own consent, the protocol must describe the intent to directly benefit all subjects

This is an observational study that does not test interventions that would offer direct benefit to study volunteers.

Study volunteers may indirectly benefit from enhanced clinical testing, physical examination, radiographic studies, and longitudinal follow up, or improved access to related trials of therapeutics.

Privacy for Subjects:

When possible, all means necessary will be taken to ensure participant privacy. This may entail conducting study procedures in a private area and keeping study documents coded. Non-coded study documents will be kept in a locked drawer and/or locked file in a locked room. Subjects will disclose information at their consent, if subjects choose not to disclose information, the study team will respect subject privacy and not inquire further.

Incidental or Unexpected Findings:

The study team will share relevant findings from research labs/imaging with the patient’s healthcare provider(s).

Aspects of this study may include epidemiologic reporting to AFHSB and public health authorities at the respective services, state and local health departments, and CDC. That data transfer is not subject to Data Use Agreement or HIPAA requirements.

15.0 Study Monitoring

Data Monitoring Plan:

The supervising principal investigator, or designated associate investigator, will be responsible for regular maintenance and review of CRFs for accuracy. Access to study files (e.g., CRFs) is restricted to care providers and research team members given specific tasks to perform. The files are kept in a secured location in each study site. Some participant-completed forms such as surveys may be completed through the web-based program. These will be transmitted directly into the study database. Research labs will transmit their testing data to the DCC using standard procedures for data handling and transmission.

During the study, the investigator will review study data from their site, including medical records, records detailing the progress of the study for each volunteer, laboratory reports, CRFs, signed ICD and authorization forms for each study volunteer, correspondence with the IRB, adverse event (AE) reports and information regarding volunteer discontinuation of the study. All required study data will be clearly and accurately recorded on source documents or CRFs by either the participant themselves (questionnaires) or authorized study personnel. Only designated study personnel shall record or change data on a CRF. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded on the CRFs. Original observations entered directly onto the CRFs are defined as source data. A Quality Management Plan (QMP) will be used for this study. The QMP will include a QC subject data review checklist, a QC Subject data review checklist, as well as automatic queries from the electronic database based on expected parameters for data entry, noting possible inconsistencies or omissions. The QC subject data review checklist will be completed at each site on 100% of subject’s study files. This will be completed by another member of the study team who did not complete the original source document or CRF. A QA subject data review checklist will also be used on a small subset of study records at each site to ensure that quality management processes are being conducted appropriately for the study. QA review will be conducted by an individual on the study who is not involved in the day-to-day activities of the study.

Responsibilities of the Data Coordination Center (DCC)

1. Provides review of protocol, CRFs, data security plan and data management SOPs
2. Complies with protocol, SOPs, and all federal and local regulations and policies
3. Develops clinical database
4. Entry and quality control of CRF based study data
5. Receipt and management of specimen tracking and laboratory data
6. Provides study database to IDCRP Program Coordination Center (PCC)
7. Locks final study database

Safety Monitoring Plan:

Participation in this protocol will impose minimal risks to the participant. Staff safety in sample collection and handling is a concern that will be closely monitored by the Infection Control staff at the facility. Risk to staff is mitigated by minimizing an additional phlebotomy beyond that being done for clinical care.

Specimen collection must be harmonized with the clinician’s requirements, and missed samples will not be re-drawn. Proper safety training, following hospital Infection Control requirements, of study team members who come in contact with study participants will be implemented to mitigate disease transmission risk.

Termination of study participation will take place through 1) an expressed desire by the volunteer to terminate participation, 2) a determination by the on-site physician that the volunteer risks his/her health by further participation in the study, or 3) in the interest of the military mission.

16.0 Reportable Events

Reportable Events:

Reporting Protocol Deviations

The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or study-specific procedure. Investigators will report protocol deviations to the USU IRB within 2 business days that:

- relate to participant safety
- relate to the informed consent process
- are any other protocol deviations which, in the opinion of the PI, should be promptly reported to the IRB.

A log of all deviations will be reported annually in the continuing review report to the IRB and in the Final Study Report.

Reportable events will also be submitted to reporting site's regulatory authorities as required.

Reporting Adverse Events and Serious Adverse Events

An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research. For this minimal risk observational study, AEs that meet all of the following criteria will be recorded by the study team and reported to the IRB at the annual Continuing Review:

- At least possibly related to study procedures
- Unexpected (i.e. not included in the list of known risks in section 14)
- Moderate or greater severity

Additional AEs may be reported if determined by the study site investigator to be of sufficient significance to notify the IRB.

Prompt reporting is required for any related serious adverse events, and any unanticipated problem involving risk to subject or others (UPIRTSO).

AEs are recorded and inspected by the site PI or designee to determine severity and relatedness to study participation (definite, probable, possible, or unlikely). The physician investigator is responsible for directly managing or coordinating management of adverse events.

Because this is proposed to be a minimal risk study, the reporting of adverse events is limited to an analysis at the time of annual reporting. Prompt reporting is required for unless it is a related serious adverse event or unanticipated problem involving risk to subject or others (UPIRTSO).

Reporting unanticipated problems involving risk to subjects or others

Unanticipated problems involving risk to subjects or others (UPIRTSO) may also occur that are not related to a described harm (adverse event) to study subject, rather a potential harm (such as loss of accountability of PII/PHI) or risks to others including medical staff. Specifically in this study is a concern that collection of infection fluids may cause harm to study personnel. Collection of samples for this study must only be conducted by individuals trained and authorized to collect and handle serious biohazardous specimens wearing proper personal protective equipment. Any sample collection for research purposes (as compared to clinical care) that results in an exposure to clinical staff is considered a UPIRTSO.

All UPIRTSOs will be collected and reported to the IRB within 24 hours of the PI recognition of the problem.

20.0 References and Glossary

References:

1) Van Kerkhove MD, Mumford E, Mounts AW, Bresee J, Ly S, Bridges CB, et al. Highly pathogenic avian influenza (H5N1): pathways of exposure at the animal-human interface, a systematic review. PloS one. 2011;6 (1):e14582. Epub 2011/02/02. doi: 10.1371/journal.pone.0014582. PubMed PMID: 21283678; PubMed Central PMCID: PMC3025925.
2) WHO. Weekly Epidemiological Record: Update on human cases of highly pathogenic avian influenza A (H5N1) virus infection, 2011. Geneva, Switzerland: World Health Organization, 2012 30 Mar. Report No.
3) Cowling BJ, Jin L, Lau EH, Liao Q, Wu P, Jiang H, et al. Comparative epidemiology of human infections with avian influenza A H7N9 and H5N1 viruses in China: a population-based study of laboratory- confirmed cases. Lancet. 2013;382 (9887):129-37. Epub 2013/06/28. doi: 10.1016/s0140-6736 (13) 61171-x. PubMed PMID: 23803488; PubMed Central PMCID: PMC3777567.

4) WHO. WHO Risk Assessment: Human infections with avian influenza A (H7N9) virus. Geneva, Switzerland: World Health Organization, 2014 Oct 2. Report No.
5) Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. The New England journal of medicine. 2012;367 (19):1814-20. Epub 2012/10/19. doi: 10.1056/NEJMoa1211721. PubMed PMID: 23075143.
6) Assiri A, McGeer A, Perl TM, Price CS, Al Rabeeah AA, Cummings DA, et al. Hospital outbreak of Middle East respiratory syndrome coronavirus. The New England journal of medicine. 2013;369(5):407-16. Epub 2013/06/21. doi: 10.1056/NEJMoa1306742. PubMed PMID: 23782161; PubMed Central PMCID: PMC4029105.
7) Memish ZA, Zumla AI, Al-Hakeem RF, Al-Rabeeah AA, Stephens GM. Family cluster of Middle East respiratory syndrome coronavirus infections. The New England journal of medicine. 2013;368 (26):2487- 94. Epub 2013/05/31. doi: 10.1056/NEJMoa1303729. PubMed PMID: 23718156.
8) Breban R, Riou J, Fontanet A. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. Lancet. 2013;382 (9893):694-9. Epub 2013/07/09. doi: 10.1016/s0140-6736 (13)61492-0. PubMed PMID: 23831141.
9) Cauchemez S, Fraser C, Van Kerkhove MD, Donnelly CA, Riley S, Rambaut A, et al. Middle East respiratory syndrome coronavirus: quantification of the extent of the epidemic, surveillance biases, and transmissibility. The Lancet Infectious diseases. 2014;14 (1):50-6. Epub 2013/11/19. doi: 10.1016/s1473-3099 (13)70304-9. PubMed PMID: 24239323; PubMed Central PMCID: PMC3895322.
10) Hemida MG, Chu DK, Poon LL, Perera RA, Alhammadi MA, Ng HY, et al. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis. 2014;20 (7):1231-4. Epub 2014/06/26. doi: 10.3201/eid2007.140571. PubMed PMID: 24964193; PubMed Central PMCID: PMC4073860.
11) WHO. Summary of probably SARS cases with onset of illness from 1 November 2002 to 31 July 2003 Switzerland, Geneva: World Health Organization; 2003 [cited 2014 Oct 31]. Available from: http://www.who.int/csr/sars/country/table2004_04_21/en/.
12) Ge X-Y, Li J-L, Yang X-L, Chmura AA, Zhu G, Epstein JH, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013;503 (7477):535-8. doi: 10.1038/nature12711.
13) WHO. Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). Geneva, Switzerland: World Health Organization, Response DoCDSa; 2003.
14) Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. The New England journal of medicine. 2003;348 (20):1967-76. Epub 2003/04/12. doi: 10.1056/NEJMoa030747. PubMed PMID: 12690091.
15) WHO. Coronavirus Disease Situation Report, 2020. Geneva, Switzerland: World Health Organization, 2020 Mar 2. Report No 42. Available from: https://www.who.int/docs/default-source/coronaviruse/20200302-sitrep-42-covid-19.pdf?sfvrsn=d863e045_2
16) CDC. Coronavirus Disease 2019 (COVID-19) in the U.S. https://www.cdc.gov/coronavirus/2019- ncov/cases-in-us.html. Accessed 03 March 2020
17) Wei-jie G., Zheng-yi N., Yu H., Wen-hua L., Chun-quan O., Jian-xing H., et al. Clinical Characteristics of Coronavirus Disease 2019 in China. 2020. Epub 2020/02/28. The New England Journal of Medicine. doi: 10.1056/NEJMoa2002032
18) del Rio C, Malani PN. COVID-19—New Insights on a Rapidly Changing Epidemic. JAMA. Published online February 28, 2020. doi:10.1001/jama.2020.3072
19) Liu W, Tao ZW, Lei W, Ming-Li Y, Kui L, Ling Z, Shuang W, Yan D, Jing L, Liu HG, Ming Y, Yi H. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chin Med J (Engl). 2020 Feb 28. doi: 10.1097/CM9.0000000000000775.
20) CDC. How COVID-19 Spreads. 2020. https://www.cdc.gov/coronavirus/2019-ncov/about/transmission.html
21) Wei Zhang, Rong-Hui Du, Bei Li, Xiao-Shuang Zheng, Xing-Lou Yang, Ben Hu, Yan-Yi Wang, Geng- Fu Xiao, Bing Yan, Zheng-Li Shi & Peng Zhou (2020) Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes, Emerging Microbes & Infections, 9:1, 386-389, DOI: 10.1080/22221751.2020.1729071
22) Rothe C, Schunk M, Sothmann P, Bretzel G, Froeschl G, Wallrauch C, Zimmer T, Thiel V, Janke C, Guggemos W, Seilmaier M, Drosten C, Vollmar P, Zwirglmaier K, Zange S, Wolfel R, Hoelscher M. Transmission of 2019-nCoV Infection from an Asymptomatic Contact in Germany. N Engl J Med. 2020. Epub 2020/02/01. doi: 10.1056/NEJMc2001468.

23) Chan JF Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet (London, England). 2020. Epub 2020/01/28. doi: 10.1016/s0140-6736 (20) 30154- 9
24) Powers JH, Bacci ED, Leidy NK, Poon JL, Stringer S, Memoli MJ, Han A, Fairchok MP, Coles C, Owens J, Chen WJ, Arnold JC, Danaher PJ, Lalani T, Burgess TH, Millar EV, Ridore M, Hernández A, Rodríguez-Zulueta P, Ortega-Gallegos H, Galindo-Fraga A, Ruiz-Palacios GM, Pett S, Fischer W, Gillor D, Moreno Macias L, DuVal A, Rothman R, Dugas A, Guerrero M. Performance of the inFLUenza Patient-Reported Outcome (FLU-PRO) diary in patients with influenza-like illness (ILI). PLoS One. 2018 Mar 22; 13(3):e0194180.
25) Coles C, Millar EV, Burgess T, Ottolini MG.The Acute Respiratory Infection Consortium: A Multi- Site, Multi-Disciplinary Clinical Research Network in the Department of Defense. Mil Med. 2019 Nov 1;184 (Supplement 2):44-50. doi: 10.1093/milmed/usz174.
26) Han A, Poon JL, Powers JH, Leidy NK, Yu R, Memoli MJ. Using the Influenza Patient-reported Outcome (FLU-PRO) diary to evaluate symptoms of influenza viral infection in a healthy human challenge model. BMC Infect Dis. 2018 Jul 28;18(1):353. doi: 10.1186/s12879-018-3220-8.
27) Yu J, Powers JH, Vallo D, Falloon J. Evaluation of Efficacy Endpoints for a Phase IIb Study of a Respiratory Syncytial Virus Vaccine in Older Adults Using Patient-Reported Outcomes With Laboratory Confirmation. Value Health. 2020 Feb;23(2):227-235. doi: 10.1016/j.jval. 2019.09.2747.

Abbreviations and Acronyms:

AE - Adverse Event
AFHSB - Armed Forces Health Surveillance Branch
AHLTA - Armed Forces Health Longitudinal Technology Application
AIDS/HIV - Acquire Immunodeficiency Syndrome/Human Immunodeficiency Virus
APTT - Activated Partial Thromboplastin Time
ARI - Acute Respiratory Infection
ALT/SGPT - Alanine Aminotransferase/Serum Glutamic Pyruvic Transaminase
AST/SGOT - Aspartate Aminotransferase/Serum Glutamic Oxaloacetic Transaminase
BSL - Biosafety Level
CDC - Centers for Disease Control and Prevention
CoV - Coronavirus
COVID-19 - Coronavirus Disease 2019
CRC - Clinical Research Coordinator
CRF - Case Report Form
DARPA - Defense Advanced Research Projects Agency
DCC - Data Coordination Center
DEERS - Defense Enrollment Eligibility Reporting System
DHA - Defense Health Agency
DoD - Department of Defense
DoDSR - DoD Serum Repository
eIND - Emergency Investigational New Drug
EVD - Ebola Virus Disease
FDA - Food and Drug Administration
GCP - Good Clinical Practice
GEIS - Global Emerging Infections Surveillance and Response System
HIPAA - Health Insurance Portability and Accountability Act
HRPP - Human Research Protection Program
ICD - Informed Consent Document
ICU - Intensive Care Unit
ICH - International Conference on Harmonization
IDCRP - Infectious Disease Clinical Research Program
IFN - Interferon

Ig - Immunoglobulin
IL - Interleukin
IND - Investigational New Drug
IRB - Institutional Review Board
ISARIC - International Severe Acute Respiratory Infection Consortium
MCP - Macrophage Chemo-attractant Protein
MDR - Military Health System Data Repository
MERS-CoV - Middle East Respiratory Syndrome-Coronavirus
MHRP - Military HIV Research Program
MIP - Macrophage Inflammatory Protein
MTF - Military Treatment Facility
NIAID - National Institute of Allergy and Infectious Diseases
NIH - National Institutes of Health
NMCP - Naval Medical Center Portsmouth
NMCSD - Naval Medical Center San Diego
OCRPRO - Office of Clinical Research Policy and Regulatory Operations
OHRP - Office for Human Research Protections
OUA - Operation United Assistance
PBMC - Peripheral Blood Mononuclear Cell
PCC - Program Coordination Center
PCR - Polymerase Chain Reaction
PHD - Predicting Health and Disease
PI - Principal Investigator
PHI - Protected Health Information
PII - Personally Identifiable Information
PPE - Personal Protective Equipment
PTT - Partial Thromboplastin Time
PUI - Person under Investigation
RCT - Randomized Controlled Trial
RNA - Ribonucleic Acid
SAE - Serious Adverse Event
SARI - Secure Acute Respiratory Infection
SARS - Severe Acute Respiratory Syndrome
SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2
SOP - Standard Operating Procedure
SPL - Specimen Processing Laboratory
SSN - Social Security Number
Study ID - Study Identification Number
TNF - Tumor Necrosis Factor
UPIRTSO - Unanticipated Problems Involving Risk to Subjects or Others
US - United States
USU - Uniformed Services University of the Health Sciences
WHO - World Health Organization

IDCRP-085 Appendix A – MHS-Wide Online Recruitment

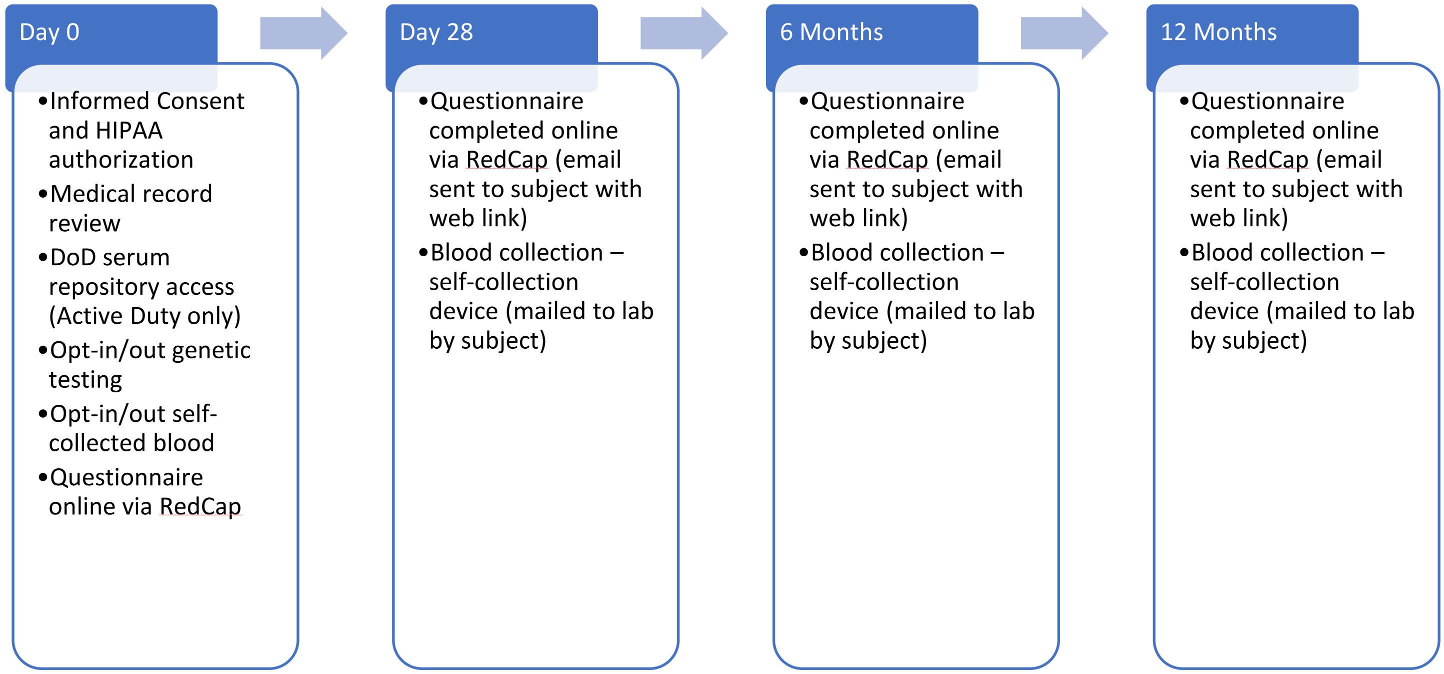
Target population: COVID-19-tested individuals who have not had contact with IDCRP study staff including at non-EPICC sites or do not wish to participate in MTF-based enrollment

Benefit: Increased enrollment and participation in limited survey; improved targeting of risk factors for infection

Procedures:

1) Consent completed electronically
    a) Medical record access
    b) DoD serum repository access, if active duty
    c) Opt-in/out self-collected blood
    d) Opt-in/out genetic testing
2) Day 0, Day 28, 6 months, 12 months – survey via REDCap
3) Day 28, 6 months, 12 months – self-collected blood

Figure 1, study schema:



Day 0
- Informed Consent and HIPAA authorization
- Medical record review
- DoD serum repository access (Active Duty only)
- Opt-in/out genetic testing
- Opt-in/out self-collected blood
- Questionnaire online via RedCap

Day 28
- Questionnaire completed online via RedCap (email sent to subject with web link)
- Blood collection – self-collection device (mailed to lab by subject)

6 Months
- Questionnaire completed online via RedCap (email sent to subject with web link)
- Blood collection – self-collection device (mailed to lab by subject)

12 Months
- Questionnaire completed online via RedCap (email sent to subject with web link)
- Blood collection – self-collection device (mailed to lab by subject)

MHS-wide Online Recruitment:

Study advertising via MHS CarePoint Information Portal messaging and MTF based flyers targeting individuals tested for either COVID19 PCR-based and/or serology tests

Recruitment approaches:

1. All adults within the MHS who have been tested for COVID-19 (irrespective of result) are identified within the Defense Health Agency (DHA) CarePoint Information Portal. In accordance with an approved DHA

DSA a weekly list of adult MHS beneficiaries who have been clinically tested for COVID-19 will be provided through secure file transfer to IDCRP Data Custodian. The list will contain the following information: patient name, DOB, DoD Number, COVID-19 test collection date, and email address.

2. An IRB-approved email message will be sent to each eligible DoD beneficiary containing a web link to consent and questionnaire. If there is no response after 1 week a second email invitation will be sent. The message to all eligible participants would inform them of eligibility in the study and direct them to contact the study website for further information and enrollment. The website will offer a description of the study objectives and procedures, as well as a consent form.
3. Upon local approval, IRB-approved recruitment flyers, with the link to the EPICC enrollment website, will also be handed out at MTFs COVID-19 evaluation/testing sites participating in the EPICC study.

Informed Consent

1. Patients will be given an opportunity to provide informed consent to enroll in the EPICC study. The prospective subject will be instructed through information on the website. There will be an opportunity to request a call from study personnel to answer questions; however, this is not required for enrollment.
2. The subject will be asked to provide their name, date of birth, last 4 of SSN, and contact information (email and phone number) in order to permit future contact as well as review and abstraction from the MHS electronic medical records related to management and outcomes of COVID-19 illness.
3. For active duty servicemembers, consent will be requested to obtain a pre-illness (up to 2 years prior) serum sample from the DoD Serum Repository (DoDSR) as well as a post-illness sample (2 months out to 1 year) in order to assess seroconversion
4. Subjects will be asked to provide an opt in to evaluate genetic susceptibility to COVID-19 (similar to current approved EPICC consent).

Study Procedures

1. Subjects will be directed on the website to completion of a questionnaire which will collect information on demographics, the illness or exposure that prompted COVID-19 testing, comorbidities, medications, and other risk factors. The subject will be contacted via email at 1, 6, and 12 months from enrollment with a link to questionnaires regarding resolution and outcomes related to the COVID-19 illness. Questionnaires will be administered at enrollment, Day 28 (± 1 week), 6 Months (± 2 weeks), and 12 Months (± 4 weeks)
2. Self-collected blood collection at home will be done using the Neoteryx Mitra® Collection Kits or similar. A subject who opts into this procedure will be mailed the collection kit closer to time of collection. The subject will collect 40 µL via finger prick using the all-in-one provided device (or a comparable microsampling kit). The Neoteryx sample dries on the collection tip. The subject will be asked to send their sample in a pre-paid mailer to the study laboratory. Information regarding the Neoteryx collection kits is available at URL: https://www.neoteryx.com/home-blood-blood-collection-kits-dried-capillary-blood (last accessed: 20 April 2020). Subjects will be provided the blood collection kits along with pre-addressed mailers for follow-up timepoints.

Sample Size Considerations and Analytic Strategy

Part I: Source Study Population

The study population will consist of MHS beneficiaries who have received SARS-CoV-2 testing. The following figures on TRICARE beneficiaries indicate the potential distribution of this population

Figure 2:

Population	Count	%
Active Duty Service Members	1.38 Million	15%
Active Duty Family Members	1.68 Million	18%
National Guard and Reserve	190,000	2%
Family Members National Guard and Reserve	780,000	8%
Retirees and Family Members < 65	3.2 Million	34%
Retirees and Family Members >= 65	2.29 Million	24%
Total	**9.5 Million**	**100%**

Uncertainties: Are all beneficiaries identifiable via DHA search or are some (e.g. retirees, dependents) who go outside of MHS for testing be captured? Uncertainty about how many minors are included in above. Source: https://www.health.mil/I-Am-A/Media/Media-Center/Patient-Population-Statistics/Patients-by-Beneficiary-Category

Part II: Approximate Demographics (Age and Sex) of Source Study Population

Tables 2a, 2b provide further details about the demographics of the TRICARE beneficiary population (active duty and other beneficiaries). Table 2a is the entire beneficiary population and Table 2b is the Active Component Population. Note from Table 2b that only about 6% of the AD population is older than 45 while 50-55% of the total TRICARE population is older than 45 (Table 2a).

Table 2a: Demographics of TRICARE Beneficiary / Adult Beneficiary Population

Age	Male	Female	Male*	Female*
<=4	6%	6%		
5-14	11%	12%		
15-17	4%	4%		
18-24	16%	11%	19%	13%
25-34	12%	11%	15%	13%
35-44	9%	10%	11%	12%
45-64	23%	24%	28%	29%
65+	19%	22%	23%	27%

*percentage of adults >= 18; Source: https://www.ncbi.nlm.nih.gov/books/NBK259169/; Estimated from Figure 2

Table 2b: Demographics of Active Component Population

Age	Male	Female	Total
15-19	5%	6%	5%
20-24	32%	34%	32%
25-29	26%	25%	26%
30-34	12%	13%	12%
35-39	11%	10%	11%
40-44	8%	8%	8%
45-49	3%	2%	3%
50-54	2%	1%	2%
55-59	1%	1%	1%

Note: 84% male; Source: Estimated from MSMR Figure 1; November 2013 Vol 20 Number 11

Table 2c: Age Distribution of Active Duty and Dependent / Retiree Population

Age	Active Duty
<=25	45%
26-30	21%

31-35	15%
36-40	10%
41+	8%

Source: file:///C:/Users/Admin/Desktop/EPICC/Stats/2017-demographics-report.pdf

Part III: Count of SARS-CoV-2 Test Positives on April 3 and April 8 2020

Limited surveillance data have been provided thus far about test results. The two tables below indicate an approximate doubling between April 3 and April 8 for military screen-positive patients. For the purpose of the sample size calculations, assume that approximately 2500 individuals (2000 active component) have been tested as of April 8 with hospitalization reported in approximately 5% thus far.

DoD COVID-19 Update April 3 2020

	Current Cases	**Hospitalized**	**Recovered**	**Deaths**
Military	978	34 (3.5%)	73	1
Dependent*	256	16 (6.3%)	12	1

DoD COVID-19 Update April 8 2020

	Current Cases	**Hospitalized**	**Recovered**	**Deaths**
Military	1975	61 (3.1%)	144	1
Dependent*	347	15 (4.3%)	35	1?

*does not include retirees as per Dr. Shauna Stahlman. Approximately the same number of retirees as dependents have been tested thus far.

To get updates: osd.pa.dutyofficer@mail.mil

https://www.militarytimes.com/news/your-military/2020/04/08/pentagon-reports-nearly-1000-new-troops-with-coronavirus-in-last-five-days/

Part IV: Estimated Prevalence of Selected Comorbid Conditions in the Active Duty and Beneficiary Population

Table 1 shows the prevalence of common comorbidities based on reported prevalence in the general population and in the active duty population.

Table 1: Estimated Prevalence of Selected Comorbid Conditions in the Active Duty (AD) and Beneficiary Population

Condition	**AD**	**AD Incidence***	**Dependent (<65)**	**Dependent (>=65)**
Hypertension	4%	15.3	20%	60%
Diabetes	1.2%	0.8	10%	27%
Asthma	2.5%	5.3	8%	8%
BMI>=30	17%	19.1	35%	35%

*per 1000 person-years; Sources: Obesity, OSA from 2018 HEALTH OF THE DOD FORCE

Asthma, diabetes, hypertension DMED Population-level prevalence estimates listed below

Additional background information on comorbidities

Prevalence of Diabetes [CDC National Diabetes Statistics Report, 2020]

Age	Prevalence (%)
18-44	4%
45-64	18%
65+	27%

Prevalence of Selected Respiratory Diseases, National Health Interview Survey, 2018

https://ftp.cdc.gov/pub/Health_Statistics/NCHS/NHIS/SHS/2018_SHS_Table_A-2.pdf

https://www.cdc.gov/nchs/fastats/asthma.htm

Percent of adults aged 18 and over who currently have asthma: 7.7%

Age	Emphysema	Hx of Asthma	Current Asthma	Hay Fever	Sinusitis	Chronic Bronchitis
18-44	0.2%	14.7%	7.2%	5%	8.5%	2.2%
45-64	1.6%	12.6%	8.3%	10.8%	14.5%	4.5%
65-74	4.1%	12.5%	8.6%	9.8%	14.8%	5.1%
75+	4.5%	10.6%	6.7%	6.8%	12.5%	5.6%

Hypertension Prevalence, NCHS Data Brief No 289 October 2017

https://www.cdc.gov/nchs/data/databriefs/db289.pdf

Age	Men	Women	Total
18-39	9.2%	5.6%	7.5%
40-59	37.2%	29.4%	33.2%
60+	58.5%	66.8%	63.1%
Total	**30.2%**	**27.7%**	**29.0%**

Among those with hypertension, percentage controlled

Age	Men	Women	Total
18-39	15.5%	62.6%	32.5%
40-59	48.1%	54.2%	50.8%
60+	49.7%	49.2%	49.4%
Total	**45.7%**	**52.5%**	**48.3%**

Prevalence of obesity

https://www.cdc.gov/nchs/products/databriefs/db131.htm

Age	Men	Women	Total
30-39	29%	32%	30%
40-59	39%	40%	39%
60+	32%	38%	35%
Total	**34%**	**36%**	**35%**

Part V: Sample Size Calculation

The primary outcome of interest is hospitalization. We will explore whether hospitalization is associated with demographic factors (age, sex, race). We will determine whether risk is related to the presence and severity of underlying comorbidities and whether this risk is modified by the degree of controls, and medications taken, for comorbidities.

Assumptions:

- Approximately equal numbers of retirees as family member beneficiaries will access testing
- Prevalence of comorbidities in non-active duty beneficiaries will be approximately equal to the general population
- Likelihood of hospitalization is approximately 5% (although note that there are no data yet about the likelihood of hospitalization in the retiree population)
- Sample size calculations assume a study population of 5,000
- Sample size calculations based on 80% power with given study population to detect a change in prevalence of hospitalization from assumed prevalence of 5%

Prevalence of Risk Factor*	Examples	Hospitalization in Non-Exposed	Hospitalization in Exposed
20%	Hypertension, Obesity	5%	7.5%
15%	African American / All Others	5%	9%
5%	Asthma	5%	10%
2%	Diabetes	5%	15%

*Estimated weighted prevalence for tested population, assuming over-representation of AD vs beneficiaries / retirees

Interpretation:

Common risk factor (prevalence 20%): With a study population of 5000, there is 80% power to detect an approximate 1.5X risk of hospitalization vs baseline risk (e.g. 7.5% risk in those with hypertension vs 5% risk in others).

Rare risk factor (prevalence 2%): With a study population of 5000, there is 80% power to detect an approximate 3X risk of hospitalization vs baseline risk (e.g. 15% risk in those with diabetes vs 5% risk in others).

From: Simon Pollett on behalf of Simon Pollett <spollett@idcrp.org>
To: Munster, Vincent (NIH/NIAID) [E]; Eric Laing; Brian Agan; Caroline English
Cc: Broder, Chris (USU-DoD); De wit, Emmie (NIH/NIAID) [E]; Cammarata, Stephanie M CTR USN NAVMEDCEN SAN CA (USA)
Subject: RE: nhps and CoV-2 question
Date: Wednesday, October 21, 2020 11:53:54 AM
Attachments: IDCRP-085_CORE_AM14_Approval_20201016.pdf
IDCRP-085_CORE_Protocol_v11_EIRB v1.15_20200914.pdf

Hi all

Following up on the below:

1/ See current protocol and IRB approval to inform NIAID NHSR determination

2/ CCing in Stephanie Cammarata for agreements

3/ Eric and Chris: to confirm, is the proposed project synopsis essentially wt VNT of n = ?25 SARS-CoV-2 positive sera with and without cross binding to MERS and SARS-1 with an aim to examine how cross-binding correlates with wildtype cross-neutralization?

Thanks

Simon

From: Simon Pollett
Sent: Monday, October 5, 2020 7:59 AM
To: 'Munster, Vincent (NIH/NIAID) [E]' <vincent.munster@nih.gov>; Eric Laing <eric.laing@usuhs.edu>; Brian Agan <BAgan@idcrp.org>; Caroline English <CEnglish@idcrp.org>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
Subject: RE: nhps and CoV-2 question

Dr Munster,

I am one of the AIs on the EPICC study and the director of the COVID19 research area at IDCRP. Pleased to meet you and looking forward to this EPICC subproject. Dr Brian Again is the EPICC PI and deputy science director of IDCRP and Caroline English the CRM of EPICC. Stephanie Cammarata is our IDCRP Agreements Officer (among other roles). Below are the relatively simple next logistic steps required to execute this really interesting serology subproject.

1. Ethics review (NIAID NHSR determination) - Attached is the EPICC protocol v 9 for your preliminary review. I will send ICF and protocol for version 10. Caroline are you able to send the relevant DoD determination?

2. I discussed with Stephanie Cammarata and this should just require a SLA between USU and NIAID. We can initiate this on our side.

3. Study synopsis – for the purposes of #2, we'd need a brief synopsis. Eric and Chris, this sounds like wt VNT of n = ?25 SARS-CoV-2 positive sera with and without cross binding to MERS and SARS-1 with an aim to examine how cross-binding correlates with wildtype cross-neutralization?

Happy to assist on all the above as needed

Simon

Dr. Simon Pollett, MBBS
Associate Scientific Director, HJF
Infectious Disease Clinical Research Program
Department of Preventive Medicine and Biostatistics
Uniformed Services University of the Health Sciences
Phone: 310-895-8048
Email: spollett@idcrp.org

From: Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>
Sent: Tuesday, September 29, 2020 3:09 PM
To: Eric Laing <eric.laing@usuhs.edu>; Simon Pollett <spollett@idcrp.org>; Brian Agan <bagan@idcrp.org>; Caroline English <CEnglish@idcrp.org>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
Subject: [EXTERNAL] RE: nhps and CoV-2 question

Thanks Eric,

Sounds good, would be easy to determine any neutralizing cross-reactivity with MERS, SARS-2/2

Cheers,

Vincent Munster, PhD
Chief Virus Ecology Section
Rocky Mountain Laboratories
NIAID/NIH

From: Laing, Eric <eric.laing@usuhs.edu>
Sent: Tuesday, September 29, 2020 12:42 PM

To: Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>; Simon Pollett <spollett@idcrp.org>; Agan, Brian (IDCRP) <bagan@idcrp.org>; Caroline English <CEnglish@idcrp.org>
Cc: Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
Subject: Re: nhps and CoV-2 question

Hi Vincent,

Looping back to this topic regarding MERS/SARS-CoV VNs/PRNTs and bringing in the IDCRP leadership that can begin an approval process for non-human research determination.

We have a handful of SARS-CoV-2 IgG positive serum samples (~15 - 20) with fairly high cross-reactivity to SARS-CoV-1 and MERS-CoV. Some of these serum samples are longitudinal collections from the same subject; over time, the cross-reactivity decreases as the IgG response matures and becomes monotypic for SARS-CoV-2. It would be interesting to write a short letter about cross-reactivity and cross-neutralization between SARS-CoV-2 IgG antibodies and high priority zoonotic bCoVs.

- Eric

Eric D. Laing, Ph.D.
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eric.laing@usuhs.edu

On Thu, Sep 10, 2020 at 9:47 AM Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov> wrote:

> Hi Chris,
>
> Would be easy to do some MERS and SARS-CoV-2 VNs or prnts.
>
> Cheers,
>
> Vincent Munster, PhD
> Chief Virus Ecology Section

> Rocky Mountain Laboratories
> NIAID/NIH
>
> **From:** Laing, Eric <eric.laing@usuhs.edu>
> **Sent:** Wednesday, September 9, 2020 8:34 PM
> **To:** Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov>
> **Cc:** Broder, Chris (USU-DoD) <christopher.broder@usuhs.edu>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>
> **Subject:** Re: nhps and CoV-2 question
>
> Hi Vincent and Emmie,
>
> Any interest or bandwidth for testing SARS-CoV-2 patient serum samples that are highly cross-reactive with MERS-CoV and SARS-CoV-1 spike proteins to assess cross-neutralization potential? We have a handful of SARS-CoV-2 seroconverts that have a strong polyclonal response to SARS-CoV-1 and MERS-CoV, could be an interesting short translational letter if the antisera retains neutralizing antibodies.
>
> - Eric
>
> Eric D. Laing, Ph.D.
> Research Assistant Professor
> Department of Microbiology and Immunology
> Uniformed Services University
> 4301 Jones Bridge Road
> Bethesda, MD 20814
> cell: (301) 980-8192
> office: (301) 295-9884
> lab: (301) 295-9618
>
> eric.laing@usuhs.edu
>
> On Fri, Sep 4, 2020 at 9:42 AM Munster, Vincent (NIH/NIAID) [E] <vincent.munster@nih.gov> wrote:
>
>> Hi Chris no plans for that,
>>
>> Housing the animals for 6 months after challenge into high containment would make it unfeasible. There are reports of back challenge after a month or so, but I think we should start getting data from humans soon,
>>
>> Cheers,
>>
>> Vincent Munster, PhD

> Chief Virus Ecology Section
> Rocky Mountain Laboratories
> NIAID/NIH
>
> ---
>
> **From:** "Broder, Christopher" <christopher.broder@usuhs.edu>
> **Date:** Thursday, September 3, 2020 at 10:01 AM
> **To:** "vincent.munster@nih.gov" <vincent.munster@nih.gov>, Emmie De wit <emmie.dewit@nih.gov>
> **Cc:** Eric Laing <eric.laing@usuhs.edu>
> **Subject:** nhps and CoV-2 question
>
> hi Vincent / Emmie.
>
> hope all is continuing to go so great.
>
> are you all going to try an NHP SARS-2 back challenge exp after
> waiting 6 or more months after primary infection and recovery?   so expensive i know,
> might provide very informative data on the ab response / longevity / anamnestic response ect..
>
> chris

MEETING HIGHLIGHTS
Therapeutics and Prevention

Nipah@20: Lessons Learned from Another Virus with Pandemic Potential

AQ: au **Raúl Gómez Román,**[a] **Lin-Fa Wang,**[b] **Benhur Lee,**[c] **Kim Halpin,**[d] **Emmie de Wit,**[e] **Christopher C. Broder,**[f] **Mahmudur Rahman,**[g] **Paul Kristiansen,**[a] **Melanie Saville**[a]

[a]Coalition for Epidemic Preparedness and Innovations (CEPI), Oslo, Norway
[b]Duke-NUS Medical School, Singapore, Singapore
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[e]Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, Hamilton, Montana, USA
[f]Department of Microbiology and Immunology, Uniformed Services University, Bethesda, Maryland, USA
[g]Independent Consultant, Dhaka, Bangladesh

ABSTRACT Nipah disease is listed as one of the WHO priority diseases that pose the greatest public health risk due to their epidemic potential. More than 200 experts from around the world convened in Singapore last year to mark the 20th anniversary of the first Nipah virus outbreaks in Malaysia and Singapore. Most of these AQ: A experts are now involved in responding to the coronavirus disease 2019 (COVID-19) pandemic. Here, members of the Organizing Committee of the 2019 Nipah Virus International Conference review highlights from the Nipah@20 Conference and reflect on key lessons learned from Nipah that could be applied to the understanding of the COVID-19 pandemic and to preparedness against future emerging infectious diseases (EIDs) of pandemic potential.

KEYWORDS CEPI, COVID-19, Hendra virus, Nipah virus, epidemic, henipavirus, pandemic, paramyxovirus, public health, surveillance studies, vaccines, zoonotic infections

In December 2019, the Nipah Virus International Conference (Nipah@20) was cohosted by the Coalition for Epidemic Preparedness and Innovations (CEPI), World Health Organization (WHO), U.S. National Institute of Allergy and Infectious Disease (NIAID) of the National Institutes of Health (NIH), and Duke-NUS Medical School AQ: B (Duke-NUS). The conference marked the 20th anniversary of the first outbreak of Nipah virus infection, an emerging infectious disease (EID) that, together with coronavirus disease 2019 (COVID-19), is currently listed as one of the WHO priority diseases (1) that pose the greatest public health risk due to their epidemic potential and the insufficient countermeasures to mitigate them.

Since its first identification in Malaysia (1998) and Singapore (1999), Nipah virus infection has caused multiple outbreaks that have thus far been limited to the Asian continent. Nipah@20 provided a forum to review the history and key scientific findings over the last 20 years and to understand the current challenges in developing Nipah diagnostics, therapeutics, and vaccines. To foster international collaboration in the context of epidemic preparedness, the conference brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines, and Singapore) were represented at the conference, with their delegations accounting for 46% of all attendees.

Citation Román RG, Wang L-F, Lee B, Halpin K, de Wit E, Broder CC, Rahman M, Kristiansen P, Saville M. 2020. Nipah@20: lessons learned from another virus with pandemic potential. mSphere 5:e00602-20. https://doi.org/10.1128/mSphere.00602-20.

Editor Michael J. Imperiale, University of Michigan—Ann Arbor

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For an editorial on this article, see [zrel;;5; e00633-20;10.1128/mSphere.00633-20]https://doi.org/10.1128/mSphere.00633-20[zrelx].

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sph-sph/sph00420/sph3327d20z | xppws | S=5 | 6/25/20 | 16:41 | ArtID: | DOI:10.1128/mSphere.00602-20 | CE: CB

In terms of outcomes, the 2-day conference created a scientific evidence-based framework (i) to inform discussions between global health stakeholders participating in CEPI's Joint Coordination Group (JCG) on 11 December 2019 (2), (ii) to discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (iii) to identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

The proceedings from the conference are now publicly available and may be consulted using the link in reference 3. Highlights and commentaries from members of the International Organising Committee follow, with an emphasis on lessons learned from Nipah that could be applied to our understanding of the current COVID-19 pandemic and to preparedness against future emerging infectious diseases (EIDs) of pandemic potential.

Lesson 1. Make a sound investment case for preparedness. Nipah@20 brought together a community of scientists who, over the past 20 years, have passionately studied the epidemiology, virology, pathogenesis, and therapeutics of viruses that have pandemic potential. Many of the invited speakers were involved in understanding and responding to the 2002–2003 severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) outbreak. It is no surprise that most of these scientists are currently involved in responding to the COVID-19 pandemic. For the past 15 years, this scientific community has been heavily involved in warning governments about the possible emergence of pathogen X and about the need to invest in preparedness against such a pathogen.

In terms of policy and implementation, however, prior to the emergence of SARS-CoV-2, this scientific community had struggled to convince policy makers about the benefits to invest in research, development, and vaccine manufacturing preparedness against pandemics. As an example, even though Nipah disease has been known for more than 20 years, investment on Nipah vaccine development has been limited. The development of a sound business/investment case remains a priority to conduct advocacy and to move forward the development of effective Nipah countermeasures. CEPI has estimated that, to bring four Nipah vaccine candidates through successful phase 2a clinical trials, an investment of up to 200 million U.S. dollars (USD) would be required, assuming no risk of failure (4). This figure may seem high, but it pales in comparison to the economic and nonfinancial impact that a pandemic can create, as currently being demonstrated by COVID-19. Studies estimating the economic impact of past and present henipavirus outbreaks could provide valuable data to model a sound investment case for medical countermeasures against Nipah virus, Hendra virus, and other henipaviruses with pandemic potential. To maximize investments, the possibility of a pan-henipavirus vaccine should also be considered, similar to programs considering the development of pan-sarbecovirus vaccines (5).

Lesson 2. Articulate how to transform surveillance in animals into impact for humans. Regarding progress and challenges in surveillance, scientists from several countries presented their research on Nipah and Henipavirus-like sequences found in bat samples throughout Asia and Africa. In addition, the Indian Council for Medical Research (ICMR) has found several new viruses among bats and is exploring their EID potential. Opinions may vary on how to translate bat surveillance into policy and prevention of disease. However, it is clear that international collaboration and further investment on surveillance research are needed to propose policy and to prepare against potential new pathogens that will continue to emerge from bats and other animals in areas with high biodiversity (6).

Lesson 3. Articulate diagnostics use cases and target product profiles. In terms of Nipah diagnostics, the need for international reference standards was highlighted. Organizing and coordinating the collection of sera and virus isolates in the midst of an epidemic are of great importance for the timely establishment of standard material. International standards are needed for assay validation, calibration, comparison, and quality controls, but cost-effective diagnostic kits and devices are also needed. For example, a mobile, nucleic acid-based Nipah diagnostic system is being used and validated in India and Bangladesh, with potential applications in clinical trials and outbreak response against other EIDs. These and other methods for Nipah diagnostics were presented at the conference, together with a draft Nipah diagnostics target product profile (TPP) developed by the WHO R&D Blueprint team (7).

In the absence of an effective, Nipah-specific treatment, it could be argued that there is no clinical justification or direct clinical benefit to an individual patient when testing. However, what may not benefit the individual patient will undoubtedly benefit public health at large, and thus, save lives. Two use cases for Nipah diagnostics include rapid detection of Nipah virus infections at a peripheral health center or hospital and confirmation of active Nipah virus infection at a centralized laboratory. For the former, a TPP would envision a near-patient/point-of-care (NPT/POC) test for rapid screening; for the latter, the TPP would envision a NPT/POC for rapid confirmation in settings with higher infrastructures (8).

NPT/POC testing, contact tracing, and proactive quarantine of suspected cases are indicated at the onset of an outbreak and can quickly halt further spread, as evidenced by the Nipah outbreaks that were successfully contained by Indian authorities in Kerala in 2018 and 2019 (9). In an outbreak situation involving a novel pathogen of pandemic potential, referring to previously developed diagnostics use cases and TPPs can help to quickly articulate diagnostics strategies. In the early stages of a pandemic, widespread NPT/POC diagnostics may need to prioritize sensitivity at the expense of specificity, if needed. Widespread sensitive diagnostic testing combined with appropriate prevention and control measures has so far been the fastest way to bring the COVID-19 reproduction number (R_0) to less than 1 in some countries.

Lesson 4. Diversify the preclinical testing portfolio and create direct dialogue between lab scientists, pathologists, and health workers in the field. Two conference sessions were dedicated to Nipah pathogenesis in animal models and to transmission and case management in humans. Data were presented on aerosol and intranasal challenges in African green monkeys (AGM), intratracheal challenge in cynomolgus macaques, and intranasal challenge in ferrets. Although models vary in recapitulating specific aspects of Nipah disease observed in humans, there was no specific mention of myocarditis observed in heart tissue. Myocarditis has been a consistent observation among Nipah virus-infected patients in India and Bangladesh, but this has not yet been reported in animal models. It is possible that this difference may be due to early humane endpoints not allowing the heart tissue from infected animals to reach the level of myocarditis deterioration observed in some Nipah virus-infected humans. There was also mention of relapse and late onset of infection observed in Malaysian patients several years after the acute infection. There is no evidence of renewed viremia during relapsed encephalitis in humans, and there is currently no animal model for relapsing Nipah disease, although many investigators have proposed such research and its importance.

For Nipah and henipaviral diseases, it is conceivable that an animal rule or similar regulatory pathway may be necessary to achieve licensure of vaccines and other countermeasures. Direct dialogue between preclinical scientists, pathologists, and health workers can better inform the design of animal models suitable for EID countermeasure development. Importantly, henipavirus research has benefited from having a diverse preclinical testing portfolio, as many different animal species are susceptible AQ: C to disease, which means that preclinical studies do not have to be limited to the use of nonhuman primates (NHPs). This is also proving important for SARS-CoV-2, as research with NHPs, hamsters, mice, and ferrets ensures that COVID-19 vaccines can be tested simultaneously, according to each model, in a wider range of biosafety level 3 (BSL3) laboratory facilities for large and small animals across the world (10). It is conceivable that there will be different animal models for different endpoints such as infection, disease, and transmission. In addition, the use of human organoids is also being explored as a model to better understand SARS-CoV-2 biology in human tissue (11).

Lesson 5. Plan for clinical trials by bringing together local and regional regulators. Three sessions were dedicated to epidemiological needs for clinical trials and to

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review progress in the development of Nipah medical countermeasures. There are several therapeutics in development, including the following: lipid-anchored peptides to prevent viral fusion, low-cost compounds such as heparin, and a nasal spray with compounds for potential prophylactic use. There are also two monoclonal antibodies that can neutralize the virus and protect against Henipavirus infection and disease *in vivo*. One monoclonal antibody (m102.4) has completed a phase I trial in Australia and has been approved for compassionate use in humans. Since antibodies do not cross the blood-brain barrier, for monoclonal antibody-based therapeutics to be effective, virus neutralization will likely have to occur in the respiratory tract, blood, and other tissues before the virus disseminates into the brain. However, multiple Nipah challenge models including NHPs indicate that passive immunization with Nipah neutralizing antibodies can also prevent central nervous system (CNS) involvement if administered at the first signs of Nipah disease.

There was discussion on what the optimal Nipah clinical trial design would be in the context of an EID with very low incidence, i.e., in the absence of feasible randomized controlled trials (RCTs), observational studies such as case-control studies could still provide valuable evidence. Relevant clinical trials for Nipah countermeasures will require engagement of local regulatory authorities in Bangladesh (Directorate General of Drug Administration [DGDA]) and India (Drug Controller General of India [DCGI]). Partnerships are being built locally, with a focus on equitable access and low-cost interventions. Recognizing that pathways to Nipah vaccine licensure are likely to vary across countries, early dialogue between regulators from Asia, Europe, and North America was facilitated during the conference, and an international, Nipah-focused regulatory group was created. Similarly, bringing regulators together early during COVID-19 medical countermeasure development could result in the identification of actions that can be harmonized or accelerated to meet common requirements or convergences along the pathway to licensure across multiple national regulatory agencies.

Lesson 6. Invest in diversified platform technologies that can be harnessed for speed, scale, and access during a pandemic. One Nipah@20 session was devoted to the Nipah vaccine pipeline. At least 13 vaccine candidates have been confirmed to be under development in preclinical stages (4). Five of these active vaccine developers presented their latest preclinical data, with two speakers presenting on platform technologies that are being used for the development of COVID-19 vaccines.

AQ: D Keith Chappell and Paul Young from the University of Queensland, in Brisbane, Australia, presented their molecular clamp approach to produce a highly stable prefusion form of the Nipah virus F glycoprotein, which is responsible for membrane fusion, and could be an additional target for neutralizing antibodies. The molecular clamp is a novel way of locking synthetic versions of viral surface proteins into the same shape that appears on the virus surface. This technology has been designed as a platform approach to generate vaccines against a range of human and animal viruses and has shown promising results in the laboratory targeting viruses such as influenza virus, Ebola virus, Nipah virus, and Middle East respiratory syndrome (MERS) coronavirus. This same principle is also being supported by CEPI to develop a recombinant, spike protein-based COVID-19 vaccine, with phase I trials expected to begin by July 2020.

Sarah Gilbert from the Jenner Institute, at Oxford University, United Kingdom, presented the ChAdOx1 Nipah vaccine, a simian adenovirus-based vaccine encoding Nipah virus (NiV) glycoprotein (G) Bangladesh. ChAdOx1 is a replication-deficient simian adenoviral vector which has been used for the development of vaccines against many pathogens in preclinical and clinical studies. It is safe for use in all ages and in patients who are immunocompromised, inducing strong and well-maintained humoral and T cell responses against the encoded antigen without the requirement for an adjuvant. A single dose of a ChAdOx1-vectored Nipah vaccine protects against homologous (Bangladesh strain) and heterologous (Malaysia strain) change in the intraperitoneal Syrian golden hamster challenge model (12). Sarah Gilbert's team is now using the same chimpanzee adenoviral platform technology to develop a COVID-19 vaccine candidate, supported in part by CEPI. A single dose of the vaccine, which encodes the spike protein of SARS-CoV-2, may confer protection against disease in a rhesus macaque pneumonia model (13). This COVID-19 vaccine candidate has now entered phase I/II trials in the United Kingdom (EU Clinical Trials Register [https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-001072-15/GB]), thus becoming the first CEPI-supported vaccine technology platform to move from pathogen sequence availability to phase I trials in less than 18 weeks.

AQ: E

Investment in many other vaccine platform technologies has resulted in an unprecedented speed, moving quickly from pathogen sequence availability in early 2020 to a diverse portfolio of more than 70 vaccine candidates in less than 4 months (14). Although regulatory agencies are adapting to keep up with such a rapidly evolving vaccine pipeline, preparedness for future pandemics may necessitate regulatory frameworks to preapprove specific platforms with consistent safety data in order to save precious time when plugging in a novel pathogen sequence into a vaccine platform technology. For many COVID-19 platform technologies, dialogue is ongoing to articulate strategies for large-scale manufacturing (15) and access (16).

Lesson 7. Engage regionally and locally, not just globally. The final conference session was dedicated to collaboration and synergy. Zhengli Shi, from the Wuhan Institute of Virology/Chinese Academy of Sciences (CAS), described how a Sino-French agreement to collaborate on EIDs was established in 2004, following the SARS-CoV outbreak. BSL4 labs were established in the Wuhan Institute of Virology as a result of the SARS-CoV outbreak. Prior to the COVID-19 pandemic, the institute had an annual call launched around April each year for external organizations wanting access to the facility to test their own samples or to have access to samples from the in-house repositories. The many years of research experience and international collaborations built by the CAS since 2004 have provided a preparedness framework through which Chinese scientists have responded and continue to respond to the SARS-CoV-2 epidemic 16 years later.

Nivedita Gupta, from the Indian Council of Medical Research (ICMR), described the Regional Enabler for the Southeast Asia Research Collaboration for Health (RESEARCH) platform. The RESEARCH platform was established on 28 August 2019, with ICMR as the secretariat. The goal of the platform is to facilitate clinical research across the region, leveraging the unique capabilities of each country and sharing expertise. Of the 11 member states in the WHO Southeast Asia Region, 10 participated in the second RESEARCH platform meeting. With the platform in place, it will be easier to mount rapid responses to new outbreaks and to conduct clinical trials—not only on Nipah virus, but also other EIDs.

Epilogue. Unbeknownst to the scientists attending the Nipah@20 conference in Singapore in December 2019, the COVID-19 outbreak had already begun. We started the year 2020 confronted with a new EID that required rapid mobilization for vaccine development. Nipah@20 was a scientific drill, a timely dialogue about the need to invest in the development of vaccines and medical countermeasures against EIDs with pandemic potential. CEPI has since mobilized early investment for several COVID-19 vaccine candidates. As stated earlier, for two awardees, this builds on work already undertaken with Nipah virus vaccine projects which were showcased at the Nipah@20 conference.

Transparency and a collaborative spirit underpinned the Nipah@20 conference. Although Nipah virus may not seem like a priority in the current COVID-19 context, we hope that the scientific momentum will continue, with high-value EID-related research being undertaken and published. When will Henipavirus experts next meet? An unofficial suggestion has been to commemorate the 30th anniversary of the discovery of Hendra virus, which was the founding member of the Henipavirus genus, sometime in 2024.

ACKNOWLEDGMENTS

We thank Tung Thanh Le for his comments on the preparation of this summary.

Emmie de Wit is supported by the Intramural Research Program of NIAID, NIH.

REFERENCES

1. World Health Organization. 2020. WHO Priority Diseases. World Health Organization, Geneva, Switzerland. https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts. AQ: F
2. Coalition for Epidemic Preparedness and Innovations. 2019. CEPI Joint Coordination Group Nipah Meeting Summary, Singapore, December 11, 2019. Coalition for Epidemic Preparedness and Innovations, Oslo, Norway. https://cepi.net/wp-content/uploads/2020/01/JCG-Nipah-Meeting-Summary.pdf.
3. Coalition for Epidemic Preparedness and Innovations. 2019. Nipah@20. Proceedings from Nipah Virus International Conference, Singapore, 9 to 11 December 2019. https://cepi.net/wp-content/uploads/2020/06/2019-CEPI-Duke-WHO-NIAID-Nipah-Conference_FINAL.pdf.
4. Gouglas D, Le TT, Henderson K, Kaloudis A, Danielsen T, Hammersland NC, Robinson JM, Heaton PM, Røttingen J-A. 2018. Estimating the cost of vaccine development against epidemic infectious diseases: a cost minimisation study. Lancet Glob Health 6:e1386–e1396. https://doi.org/10.1016/S2214-109X(18)30346-2.
5. Burton DR, Walker LM. 2020. Rational vaccine design in the time of COVID-19. Cell Host Microbe 27:695–698. https://doi.org/10.1016/j.chom.2020.04.022.
6. Daszak P, Olival KJ, Li H. 2020. A strategy to prevent future epidemics similar to the 2019-nCoV outbreak. Biosaf Health 2:6–8. https://doi.org/10.1016/j.bsheal.2020.01.003.
7. World Health Organization. 2019. Nipah diagnostics target product profile. World Health Organization, Geneva, Switzerland. https://www.who.int/news-room/articles-detail/nipah-diagnostics-target-product-profile.
8. World Health Organization. 2019. WHO R&D Blueprint: Priority Diagnostics for Nipah Use Cases and Target Product Profiles. World Health Organization, Geneva, Switzerland. https://www.who.int/docs/default-source/blue-print/call-for-comments/who-nipah-dx-tpps-d.pdf?sfvrsn=8a856311_4.
9. Arunkumar G, Chandni R, Mourya DT, Singh SK, Sadanandan R, Sudan P, Bhargava B, Nipah Investigators People and Health Study Group. 2019. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 219:1867–1878. https://doi.org/10.1093/infdis/jiy612.
10. Cohen J. 13 April 2020. Mice, hamsters, ferrets, monkeys. Which lab animals can help defeat the new coronavirus? Science https://www.sciencemag.org/news/2020/04/mice-hamsters-ferrets-monkeys-which-lab-animals-can-help-defeat-new-coronavirus.
11. Lamers MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, van Schayck JP, Mykytyn AZ, Duimel HQ, van Donselaar E, Riesebosch S, Kuijpers HJH, Schippers D, van de Wetering WJ, de Graaf M, Koopmans M, Cuppen E, Peters PJ, Haagmans BL, Clevers H. 2020. SARS-CoV-2 productively infects human gut enterocytes. Science 2020: eabc1669. https://doi.org/10.1126/science.abc1669.
12. van Doremalen N, Lambe T, Sebastian S, Bushmaker T, Fischer R, Feldmann F, Haddock E, Letko M, Avanzato VA, Rissanen I, LaCasse R, Scott D, Bowden TA, Gilbert S, Munster V. 2019. A single-dose ChAdOx1-vectored vaccine provides complete protection against Nipah Bangladesh and Malaysia in Syrian golden hamsters. PLoS Negl Trop Dis 13:e0007462. https://doi.org/10.1371/journal.pntd.0007462.
13. van Doremalen N, Lambe T, Sebastian S, Bushmaker T, Fischer R, Feldmann F, Haddock E, Letko M, Avanzato VA, Rissanen I, LaCasse R, Scott D, Bowden TA, Gilbert S, Munster V. 2020. ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2 pneumonia in rhesus macaques. bioRxiv https://doi.org/10.1101/2020.05.13.093195.
14. Le TT, Andreadakis Z, Kumar A, Román RG, Tollefsen S, Saville M, Mayhew S. 2020. The COVID-19 vaccine development landscape. Nat Rev Drug Discov 19:305–306. https://doi.org/10.1038/d41573-020-00073-5.
15. World Health Organization. 2020. WHO South-East Asia Region meeting of vaccine manufacturers and regulators. World Health Organization, Geneva, Switzerland. https://www.who.int/southeastasia/news/detail/29-04-2020-who-convenes-manufacturers-regulatory-authorities-meet-on-covid-19-vaccines.
16. Yamey G, Schäferhoff M, Hatchett R, Pate M, Zhao F, Kennedy McDade K. 2020. Ensuring global access to COVID-19 vaccines. Lancet 395:1405–1406. https://doi.org/10.1016/S0140-6736(20)30763-7.

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AQA—Proofread the entire article carefully to ensure that sense was not inadvertently changed incorrectly during processing of electronic file and editing. Check that abbreviations have been introduced or spelled out correctly throughout. Check symbols (Greek symbols, diacritical marks, etc.) throughout. Correct city locations added for Duke-NUS Medical School and Icahn School of Medicine at Mount Sinai? City location must be given per ASM style.

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HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID)	AI123449, AI125536, AI071002	Benhur Lee	https://doi.org/10.13039/100000060
HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID)	Intramural Research Program	Emmie de Wit	https://doi.org/10.13039/100000060

AUTHOR QUERIES

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From: Raul Gomez Roman on behalf of Raul Gomez Roman <raul.gomezroman@cepi.net>
To: Wang Linfa; Lee, Benhur; Halpin, Kim (AAHL, Geelong AAHL); De wit, Emmie (NIH/NIAID) [E]; Broder, Christopher; Mahmudur Rahman; Mahmudur Rahman; Paul Kristiansen; Melanie Saville; Jodie Rogers
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Dear All,

Please find the proof for our mSphere paper, which will be published in the July/August issue of the journal. Kindly have a quick look for any last minute *editing* changes. The Editor is asking for a 48 hour turnaround; so I will send your compiled changes (if any) on Saturday 5pm, CET(Oslo). In addition:

1. @De wit, Emmie (NIH/NIAID) [E] & @Lee, Benhur, please see the question on funding sources for cross-referencing and kindly advise.
2. @Jodie Rogers, please advise if anything else should be included in the Acknowledgements section.

Also, please note that the Editor will include an accompanying editorial piece. He is currently writing this.

Many thanks to all for the final push in this.

Best wishes,

Raúl

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Target audience: scientific (mSphere)

Nipah@20: Lessons learned from another virus with pandemic potential

In December 2019, the Nipah Virus International Conference (Nipah@20) was co-hosted by the Coalition for Epidemic Preparedness Innovations (CEPI), the World Health Organization (WHO), the U.S. National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Duke-NUS Medical School (Duke-NUS). The Conference marked the 20th anniversary of the first outbreak of Nipah, an emerging infectious disease (EID) that, together with COVID-19, is currently listed as one of the WHO priority diseases that pose the greatest public health risk due to their epidemic potential and the insufficient countermeasures to mitigate them.

Since its first identification in Malaysia (1998) and Singapore (1999), Nipah virus infection has caused multiple outbreaks that have thus far been limited to the Asian continent. Nipah@20 provided a forum to review the history and key scientific findings over the last 20 years, and to understand the current challenges in developing Nipah diagnostics, therapeutics and vaccines. To foster international collaboration in the context of epidemic preparedness, the conference brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all Henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines and Singapore) were represented in the Conference, with their delegations accounting for 46% of all attendees.

In terms of outcomes, the two-day conference created a scientific evidence-based framework to: (a) inform discussions between global health stakeholders participating in *CEPI's Joint Coordination Group (JCG) on December 11, 2019*, (b) discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (c) identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

The Proceedings from the Conference are now publicly available and may be consulted using this link. Highlights and commentaries from members of the International Organising Committee follow, with an emphasis on lessons learned from Nipah that could be applied to our understanding of the current COVID-19 pandemic and to preparedness against future emerging infectious diseases (EIDs) of pandemic potential.

Lesson 1: Make a sound investment case for preparedness.

Nipah@20 brought together a community of scientists who, over the past 20 years, have passionately studied the epidemiology, virology, pathogenesis and therapeutics of viruses that have pandemic potential. Many of the invited speakers were involved in understanding and responding to the 2002-2003 SARS-CoV-1 outbreak. And it is no surprise that most of them are currently involved in responding to the COVID-19 pandemic. For the past 15 years, this scientific community has been heavily involved in warning governments about the possible emergence of Pathogen X, and about the need to invest in preparedness against such pathogen.

In terms of policy and implementation, however, prior to the emergence of SARS-CoV-2, this scientific community had struggled to convince policy makers about the benefits to invest in research, development and vaccine manufacturing preparedness against pandemics. As an example, even though Nipah has existed for over 20 years, investment on Nipah vaccine development has been limited. The development of a sound business/investment case remains a priority to conduct advocacy and to move forward the development of effective Nipah countermeasures. CEPI has estimated that, to bring four Nipah vaccine candidates through successful Phase 2a clinical trials, an investment of up to 200 million USD would be required, assuming no risk of failure (*Gouglas et al. 2018*). This figure may seem high, but it pales in comparison to the economic and non-financial impact that a pandemic can create, as currently being demonstrated by COVID-19. Studies estimating the economic impact of past and present henipavirus outbreaks could provide valuable data to model a sound investment case for medical countermeasures against Nipah, Hendra, and other henipaviruses with pandemic potential. To maximise investments, the possibility of a pan-henipavirus vaccine should also be considered, similar to programs considering the development of pan-sarbecovirus vaccines (*Burton & Walker, 2020*).

Lesson 2: Articulate how to transform surveillance in animals into impact for humans.

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References:

1. WHO priority diseases
2. *CEPI's Joint Coordination Group (JCG) on December 11, 2019*
3. *2019 Nipah Virus International Conference – Conference Proceedings*
4. *Gouglas et al. 2018*
5. *Burton & Walker, 2020*
6. *Daszak et al. 2020*
7. *WHO Nipah Diagnostics webpage*
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Nipah@20: Lessons learned from another virus with pandemic potential

In December 2019, the Nipah Virus International Conference (Nipah@20) was co-hosted by the Coalition for Epidemic Preparedness Innovations (CEPI), the World Health Organization (WHO), the U.S. National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Duke-NUS Medical School (Duke-NUS). The Conference marked the 20th anniversary of the first outbreak of Nipah, an emerging infectious disease (EID) that, together with COVID-19, is currently listed as one of the WHO priority diseases that pose the greatest public health risk due to their epidemic potential and the insufficient countermeasures to mitigate them.

Since its first identification in Malaysia and Singapore in 1999, Nipah virus infection has caused multiple outbreaks that have thus far been limited to the Asian continent. Nipah@20 provided a forum to review the history and key scientific findings over the last 20 years, and to understand the current challenges in developing Nipah diagnostics, therapeutics and vaccines. To foster international collaboration in the context of epidemic preparedness, the conference brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all Henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines and Singapore) were represented in the Conference, with their delegations accounting for 46% of all attendees.

In terms of outcomes, the two-day conference created a scientific evidence-based framework to: (a) inform discussions between global health stakeholders participating in *CEPI's Joint Coordination Group (JCG) on December 11, 2019*, (b) discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (c) identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

The Proceedings from the Conference are now publicly available and may be consulted in the following link: *(in progress, under construction by CEPI Communications)*. Highlights and commentaries from members of the International Organising Committee follow, with an emphasis on lessons learned from Nipah that could be applied to our understanding of the current COVID-19 pandemic and to preparedness against future emerging infectious diseases (EIDs) of pandemic potential.

Lesson 1: Make a sound investment case for preparedness.

Nipah@20 brought together a community of scientists who, over the past 20 years, have passionately studied the epidemiology, virology, pathogenesis and therapeutics of viruses that have pandemic potential. Many of the invited speakers were involved in understanding and responding to the 2002-2003 SARS-CoV-1 outbreak. And it is no surprise that most of them are currently involved in responding to the COVID-19 pandemic. For the past 15 years, this scientific community has been heavily involved in warning governments about the possible emergence of Pathogen X, and about the need to invest in preparedness against such pathogen.

In terms of policy and implementation, however, prior to the emergence of SARS-CoV-2, this scientific community had struggled to convince policy makers about the benefits to invest in research, development and vaccine manufacturing preparedness against pandemics. As an example, even though Nipah has existed for over 20 years, investment on Nipah vaccine development has been limited. The development of a sound business/investment case remains a priority to conduct advocacy and to move forward the development of effective Nipah countermeasures. CEPI has

estimated that, to bring four Nipah vaccine candidates through successful Phase 2a clinical trials, an investment of up to 200 million USD would be required, assuming no risk of failure (*Gouglas et al. 2018*). This figure may seem high, but it pales in comparison to the economic and non-financial impact that a pandemic can create, as currently being demonstrated by COVID-19. Studies estimating the economic impact of past and present henipavirus outbreaks could provide valuable data to model a sound investment case for medical countermeasures against Nipah, Hendra, and other henipaviruses with pandemic potential. To maximise investments, the possibility of a pan-henipavirus vaccine should also be considered, similar to programs considering the development of pan-sarbecovirus vaccines (*Burton & Walker, 2020*).

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In terms of Nipah diagnostics, the need for international reference standards was highlighted. Organizing and coordinating the collection of sera and virus isolates in the mist of an epidemic is of great importance for the timely establishment of standard material. International standards are needed for assay validation, calibration, comparison and quality controls; but cost-effective diagnostic kits and devices are also needed. For example, a mobile, nucleic acid based Nipah diagnostic system is being used and validated in India & Bangladesh, with potential applications in clinical trials and outbreak response against other EIDs. These and other methods for Nipah diagnostics were presented in the Conference, together with a draft Nipah Diagnostics Target Product Profile (TPP) developed by the WHO R&D Blueprint team (*see WHO Nipah Diagnostics webpage*).

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References:

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Subject: final version for mSphere
Date: Wednesday, June 10, 2020 9:00:10 AM
Attachments: Summary for mSphere_v9_FINAL_withproceedingslink.docx
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Dear Benhur, Dear All,
Thank you all for your final comments.
All co-authors have now agreed to submit the attached version to *mSphere*. Benhur has kindly agreed to submit this and will let us know if anything else is needed.
Thanks again to all for your continued patience and contributions in this effort.
Best wishes,
Raúl

cc Jodie – CEPI Coms
cc Ana Paul – CEPI Publications Manager

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From: Lee, Benhur <benhur.lee@mssm.edu>

Sent: onsdag 3. juni 2020 08:28
To: Raul Gomez Roman <raul.gomezroman@cepi.net>
Cc: LinFa Wang Linfa <linfa.wang@duke-nus.edu.sg>; Halpin, Kim (AAHL, Geelong AAHL) <Kim.Halpin@csiro.au>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>; Broder, Christopher <christopher.broder@usuhs.edu>; rahman.mahmudur@icddrb.org; Mahmudur Rahman <mahmudur57@gmail.com>; Lai Yih Shin <yihshin.lai@duke-nus.edu.sg>
Subject: Re: final version for mSphere
Importance: High

Thanks, Raul. I'm acknowledging receipt and will standby.

benhur

#BeSafe #FlattenTheCurve #SocialDistancingWorks

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> On Jun 2, 2020, at 9:00 AM, Raul Gomez Roman <raul.gomezroman@cepi.net> wrote:
>
> USE CAUTION: External Message.
>
> Dear All,
> I hope this email finds you well. Here is the latest version of our summary to *mSphere*. It has now incorporated ***all*** your comments. It has also undergone significant internal review at CEPI and should be considered the near final version. Could you please:
>
> 1. Review and indicate any ***major*** changes that absolutely need to be made prior to submission
> 2. Approve the authorship order and this near final version for submission
>
> Benhur and I will follow up with *mSphere*. Apologies this has taken so long (nearly 6 months after the Nipah Conference!), but we have all been swamped with COVID-19 activities – and so have been most Nipah researchers, I understand…Kindly send your reply ***by this Friday, June 5.***
> Best wishes,
> Raúl
>
> **RAUL GOMEZ ROMAN**
> Preclinical Vaccine Scientist
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> <image002.png>

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<image004.png> <image006.png>

<Summary for mSphere_v8.docx>

Confidential ***Draft June 02.2020***

Authors: Raúl Gómez Román, Linfa Wang, Benhur Lee*, Kim Halpin, Emmie de Wit, Christopher C. Broder, Mahmudur Rahman, Paul Kristiansen and Melanie Saville.*
**corresponding authors*
Target audience: scientific (mSphere)
Expected length: No more than 5 pages/2800 words (currently 2800 words)

Nipah@20: Lessons learned from another virus with pandemic potential

In December 2019, the Nipah Virus International Conference (Nipah@20) was co-hosted by the Coalition for Epidemic Preparedness Innovations (CEPI), the World Health Organization (WHO), the U.S. National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Duke-NUS Medical School (Duke-NUS). The Conference marked the 20th anniversary of the first outbreak of Nipah, an emerging infectious disease (EID) that, together with COVID-19, is currently listed as one of the WHO priority diseases that pose the greatest public health risk due to their epidemic potential and the insufficient countermeasures to mitigate them.

Since its first identification in Malaysia and Singapore in 1999, Nipah virus infection has caused multiple outbreaks that have thus far been limited to the Asian continent. Nipah@20 provided a forum to review the history and key scientific findings over the last 20 years, and to understand the current challenges in developing Nipah diagnostics, therapeutics and vaccines. To foster international collaboration in the context of epidemic preparedness, the conference brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all Henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines and Singapore) were represented in the Conference, with their delegations accounting for 46% of all attendees.

Commented [MR1]: Please check, Malaysia outbreak was in 1998

In terms of outcomes, the two-day conference created a scientific evidence-based framework to: (a) inform discussions between global health stakeholders participating in *CEPI's Joint Coordination Group (JCG) on December 11, 2019*, (b) discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (c) identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

The Proceedings from the Conference are now publicly available and may be consulted in the following link: *(in progress, under construction by CEPI Communications)*. Highlights and commentaries from members of the International Organising Committee follow, with an emphasis on lessons learned from Nipah that could be applied to our understanding of the current COVID-19 pandemic and to preparedness against future emerging infectious diseases (EIDs) of pandemic potential.

Lesson 1: Make a sound investment case for preparedness.

Nipah@20 brought together a community of scientists who, over the past 20 years, have passionately studied the epidemiology, virology, pathogenesis and therapeutics of viruses that have pandemic potential. Many of the invited speakers were involved in understanding and responding to the 2002-2003 SARS-CoV-1 outbreak. And it is no surprise that most of them are currently involved in responding to the COVID-19 pandemic. For the past 15 years, this scientific community has been heavily involved in warning governments about the possible emergence of Pathogen X, and about the need to invest in preparedness against such pathogen.

In terms of policy and implementation, however, prior to the emergence of SARS-CoV-2, this scientific community had struggled to convince policy makers about the benefits to invest in research, development and vaccine manufacturing preparedness against pandemics. As an example, even though Nipah has existed for over 20 years, investment on Nipah vaccine development has been limited. The development of a sound business/investment case remains a priority to conduct advocacy and to move forward the development of effective Nipah countermeasures. CEPI has

estimated that, to bring four Nipah vaccine candidates through successful Phase 2a clinical trials, an investment of up to 200 million USD would be required, assuming no risk of failure (*Gouglas et al. 2018*). This figure may seem high, but it pales in comparison to the economic and non-financial impact that a pandemic can create, as currently being demonstrated by COVID-19. Studies estimating the economic impact of past and present henipavirus outbreaks could provide valuable data to model a sound investment case for medical countermeasures against Nipah, Hendra, and other henipaviruses with pandemic potential. To maximise investments, the possibility of a pan-henipavirus vaccine should also be considered, similar to programs considering the development of pan-sarbecovirus vaccines (*Burton & Walker, 2020*).

Lesson 2: Articulate how to transform surveillance in animals into impact for humans.

Regarding progress and challenges in surveillance, scientists from several countries presented their research on Nipah and Henipavirus-like sequences found in bat samples throughout Asia and Africa. In addition, the Indian Council for Medical Research (ICMR) has found several new viruses among bats and is exploring their EID potential. Opinions may vary on how to translate bat surveillance into policy and prevention of disease. However, it is clear that international collaboration and further investment on surveillance research is needed to propose policy and to prepare against potential new pathogens that will continue to emerge from bats and other animals in areas with high biodiversity (*Daszak et al. 2020*).

Lesson 3: Articulate Diagnostics Use Cases and Target Product Profiles.

In terms of Nipah diagnostics, the need for international reference standards was highlighted. Organizing and coordinating the collection of sera and virus isolates in the mist of an epidemic is of great importance for the timely establishment of standard material. International standards are needed for assay validation, calibration, comparison and quality controls; but cost-effective diagnostic kits and devices are also needed. For example, a mobile, nucleic acid based Nipah diagnostic system is being used and validated in India & Bangladesh, with potential applications in clinical trials and outbreak response against other EIDs. These and other methods for Nipah diagnostics were presented in the Conference, together with a draft Nipah Diagnostics Target Product Profile (TPP) developed by the WHO R&D Blueprint team (*see WHO Nipah Diagnostics webpage*).

In the absence of an effective, Nipah-specific treatment, it could be argued that there is no clinical justification or direct clinical benefit to an individual patient when testing. However, what may not benefit the individual patient will undoubtedly benefit public health at large, and thus, save lives. Two Use Cases for Nipah diagnostics include: rapid detection of Nipah virus infections at a peripheral health center or hospital, and confirmation of active Nipah virus infection at a centralized laboratory. For the former, a TPP would envision a near-patient/point-of-care (NPT/POC) test for rapid screening; for the latter, the TPP would envision a NPT/POC for rapid confirmation in settings with higher infrastructures (*see WHO draft Nipah Diagnostics TPPs*).

NPT/POC testing, contact-tracing and proactive quarantine of suspected cases is indicated at the onset of an outbreak and can quickly halt further spread, as evidenced by the Nipah outbreaks that

were successfully contained by Indian authoritities in Kerala in 2018 and 2019 (*Arunkumar et al. 2019*). In an oubreak situation involving a novel pathogen of pandemic potential, referring to previously developed Diagnostics Use Cases and TPPs can help to quickly articulate diagnostics strategies. In the early stages of a pandemic, wide-spread NPT/POC diagnostics may need to prioritise sensitivity at the expense of specificity, if needed. Wide-spread sensitive diagnostic testing combined with appropriate prevention and control measures has so far been the fastest way to bring the COVID-19 reproduction number (R_o) to less than 1 in some countries.

Lesson 4: Diversify the pre-clinical testing portfolio and create direct dialogue between lab scientists, pathologists and health workers in the field.

Two Conference sessions were dedicated to Nipah pathogenesis in animal models and to Transmission & Case-management in humans. Data were presented on aerosol and intranasal challenges in African green monkeys (AGM), intratracheal challenge in cynomolgus macaques, and intranasal challenge in ferrets. Although models vary in recapitulating specific aspects of Nipah disease observed in humans, there was no specific mention of myocarditis observed in heart tissue. Myocarditis has been a consistent observation among Nipah-infected patients in India and Bangladesh, but this has not yet been reported in animal models. It is possible that this difference may be due to early humane endpoints not allowing the heart tissue from infected-animals to reach the level of myocarditis deterioration observed in some Nipah-infected humans. There was also mention of relapse and late-onset of infection observed in Malaysian patients several years after the acute infection. There is no evidence of renewed viremia during relapsed encephalitis in humans, and there is currently no animal model for relapsing Nipah disease although many investigators have proposed such research and its importance.

For Nipah and henipaviral diseases, it is conceivable that an Animal Rule or similar regulatory pathway may be necessary to achieve licensure of vaccines and other countermeasures. Direct dialogue between preclinical scientists, pathologists and health workers can better inform the design of animal models suitable for EID countermeasure development. Importantly, Henipavirus research has benefited from having a diverse pre-clinical testing portfolio, as many different animal species are susceptible to disease, which means that pre-clinical studies do *not* have to be limited to the use of non-human primates (NHPs). This is also proving important for SARS-CoV-2, as research in NHPs, hamsters, mice and ferrets ensures that COVID-19 vaccines can be tested simultaneously, according to each model, in a wider range of BSL3 laboratory facilities for large and small animals across the world (*Cohen, J. 2020*). It is conceivable that there will be different animal models for different endpoints such as infection, disease, and transmission. In addition, the use of human organoids is also being explored as a model to better understand SARS-CoV-2 biology in human tissue (*Lamers et al., 2020*).

Lesson 5: Plan for clinical trials by bringing together local and regional regulators.

Three sessions were dedicated to epidemiological needs for clinical trials and to review progress in the development of Nipah medical countermeasures. There are several therapeutics in development, including: lipid-anchored peptides to prevent viral fusion, low-cost compounds such as heparin, and a nasal spray with compounds for potential prophylactic use. There are also two

monoclonal antibodies that can neutralize the virus and protect against Henipavirus infection and disease *in vivo*. One (m102.4) has completed a Phase I trial in Australia and has been approved for compassionate use in humans. Since antibodies do not cross the blood-brain barrier, for monoclonal antibody-based therapeutics to be effective, virus neutralization will likely have to occur in the respiratory tract, blood, and other tissues before the virus disseminates into the brain. However, multiple Nipah challenge models including NHPs indicate that passive immunization with Nipah neutralizing antibodies can also prevent CNS involvement if administered at the first signs of Nipah disease.

There was discussion on what the optimal Nipah clinical trial design would be in the context of an EID with very low incidence, i.e. in the absence of feasible randomised controlled trials (RCTs), observational studies such as case-control studies could still provide valuable evidence. Relevant clinical trials for Nipah countermeasures will require engagement of local regulatory authorities in Bangladesh (Directorate General of Drug Administration, DGDA) and India (Drug Controller General of India, DCGI). Partnerships are being built locally, with a focus on equitable access and low-cost interventions. Recognising that pathways to Nipah vaccine licensure are likely to vary across countries, early dialogue between regulators from Asia, Europe and North America was facilitated during the Conference, and an international, Nipah-focused regulatory group was created. Similarly, bringing regulators together early during COVID-19 medical countermeasure development could result in the identification of actions that can be harmonised or accelerated to meet common requirements or convergences along the pathway to licensure across multiple national regulatory agencies.

Lesson 6: Invest in diversified platform technologies that can be harnessed for speed, scale and access during a pandemic.

One Nipah@20 session was devoted to the Nipah vaccine pipeline. At least 13 vaccine candidates have been confirmed to be under development in pre-clinical stages (*Gouglas et al. 2018*). Five of these active vaccine developers presented their latest pre-clinical data, with two speakers presenting on platform technologies that are being used for the development of COVID-19 vaccines.

Dr. Keith Chappell and Professor Paul Young from the University of Queensland, in Brisbane, Australia presented their molecular clamp approach to produce a highly stable pre-fusion form of the Nipah virus F glycoprotein, which is responsible for membrane fusion, and could be an additional target for neutralising antibodies. The molecular clamp is a novel way of locking synthetic versions of viral surface proteins into the same shape that appears on the virus surface. This technology has been designed as a platform approach to generate vaccines against a range of human and animal viruses and has shown promising results in the laboratory targeting viruses such as influenza, Ebola, Nipah and MERS coronavirus. This same principle is also being supported by CEPI to develop a recombinant, spike protein-based COVID-19 vaccine, with Phase I trials expected to begin by July 2020.

Professor Sarah Gilbert from the Jenner Institute, at Oxford University, United Kingdom, presented the ChAdOx1 Nipah vaccine, a simian adenovirus-based vaccine encoding NiV glycoprotein (G) Bangladesh. ChAdOx1 is a replication-deficient simian adenoviral vector which has been used for the development of vaccines against many pathogens in preclinical and clinical studies. It is safe for use in all ages and in patients who are immunocompromised, inducing strong and well maintained

humoral and T cell responses against the encoded antigen without the requirement for an adjuvant. A single dose of a ChAdOx1-vectored Nipah vaccine protects against homologous (Bangladesh strain) and heterologous (Malaysia strain) change in the intraperitoneal Syrian Golden Hamster challenge model (*van Doremalen et al. 2019*). Professor Gilbert's team is now using the same chimpanzee adenoviral platoform technology to develop a COVID-19 vaccine candidate, supported in part by CEPI. A single dose of the vaccine, which encodes the Spike protein of SARS-CoV-2, may confer protection against disease in a rhesus macaque pneumonia model (*van Doremalen et al. 2020*). This COVID-19 vaccine candidate has now entered Phase I/II trials in the UK (*EU Clinical Trials Register*), thus becoming the first CEPI-supported vaccine technology platform to move from pathogen sequence availability to Phase I trials in less than 18 weeks.

Investment in many other vaccine platform technologies has resulted in an unprecedented speed, moving quickly from pathogen sequence availability in early 2020 to a diverse portfolio of more than 70 vaccine candidates in less than 4 months (*Tung Thanh Le et al. 2020*). Although regulatory agencies are adapting to keep up with such a rapidly evolving vaccine pipeline, preparedness for future pandemics may necessitate regulatory frameworks to pre-approve specific platforms with consistent safety data in order to save precious time when plugging in a novel pathogen sequence into a vaccine platform technology. For many COVID-19 platform technologies, dialogue is ongoing to articulate strategies for large-scale manufacturing (*see WHO Manufacturers meeting*) and access (*Yamey et al. 2020*).

Lesson 7: Engage regionally and locally, not just globally.

The final Conference session was dedicated to collaboration and synergy. Professor Zhengli Shi, from the Wuhan Institute of Virology/Chinese Academy of Sciences (CAS), described how a Sino-French agreement to collaborate on EIDs was established in 2004, following the SARS-CoV outbreak. BSL4 labs were established in the Wuhan Institute of Virology as a result of the SARS-CoV outbreak. Prior to the COVID-19 pandemic, the Institute had an annual call launched around April each year for external organisations wanting access to the facility to test their own samples or to have access to samples from the in-house repositories. The many years of research experience and international collaborations built by the CAS since 2004 have provided a preparedness framework through which Chinese scientists have responded and continue to respond to the SARS-CoV-2 epidemic sixteen years later.

Dr. Nivedita Gupta, from the Indian Council of Medical Research (ICMR), described the Regional Enabler for the Southeast Asia Research Collaboration for Health (RESEARCH) Platform. The RESEARCH platform was established on August 28, 2019, with ICMR as secretariat. The goal of the platform is to facilitate clinical research across the region, leveraging the unique capabilities of each country and sharing expertise. Of the 11 member states in the WHO Southeast Asia Region, 10 participated in the second RESEARCH platform meeting. With the platform in place, it will be easier to mount rapid responses to new outbreaks, and to conduct clinical trials – not only on Nipah virus, but also other EIDs.

Epilogue

Authors: Raúl Gómez Román, Linfa Wang, Benhur Lee*, Kim Halpin, Emmie de Wit, Christopher C. Broder, Mahmudur Rahman, Paul Kristiansen and Melanie Saville.*
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Target audience: scientific (mSphere)
Expected length: No more than 5 pages/2800 words (currently 2800 words)

Unbeknownst to the scientists attending the Nipah@20 conference in Singapore in December 2019, the COVID-19 outbreak had already begun. We started the year 2020 confronted with a new EID that required rapid mobilisation for vaccine development. Nipah@20 was a scientific drill, a timely dialogue about the need to invest in the development of vaccines and medical countermeasures against EIDs with pandemic potential. CEPI has since mobilised early investment for several COVID-19 vaccine candidates. As stated earlier, for two awardees, this builds on work already undertaken with Nipah virus vaccine projects which were showcased at the Nipah@20 conference.

Transparency and a collaborative spirit underpinned the Nipah@20 conference. Although Nipah virus may not seem like a priority in the current COVID-19 context, we hope that the scientific momentum will continue, with high value EID-related research being undertaken and published. When will Henipavirus experts next meet? An unofficial suggestion has been to commemorate the 30th anniversary of the discovery of Hendra virus, which was the founding member of the Henipavirus genus, sometime in 2024.

***Acknowledgments**: The authors wish to thank Tung Thanh Le for his comments in the preparation of this summary. Emmie de Wit is supported by the Intramural Research Program of NIAID, NIH.*

References:

1. WHO priority diseases
2. *CEPI's Joint Coordination Group (JCG) on December 11, 2019*
3. *in progress, under construction by CEPI Communications*
4. *Gouglas et al. 2018*
5. *Burton & Walker, 2020*
6. *Daszak et al. 2020*
7. *WHO Nipah Diagnostics webpage*
8. *see WHO draft Nipah Diagnostics TPPs*
9. *Arunkumar et al. 2019*
10. *Cohen, J. 2020*
11. *Lamers et al., 2020*
12. *van Doremalen et al. 2019*
13. *van Doremalen et al. 2020 (pre-print pending peer review)*
14. *EU Clinical Trials Register*
15. *Tung Thahn Le et al. 2020*
16. *WHO Manufacturers meeting*
17. *Yamey et al. 2020*

From: Mahmudur Rahman on behalf of Mahmudur Rahman <mahmudur57@gmail.com>
To: Raul Gomez Roman
Cc: LinFa Wang Linfa; Lee, Benhur; Halpin, Kim (AAHL, Geelong AAHL); De wit, Emmie (NIH/NIAID) [E]; Broder, Christopher; rahman.mahmudur@icddrb.org; Lai Yih Shin
Subject: Re: final version for mSphere
Date: Wednesday, June 3, 2020 7:11:12 AM
Attachments: Summary for mSphere_v8 comments Mahmud.docx
image002.png
image004.png
image006.png

Dear Raul,

Greetings from Bangladesh !!!

Thanks for sharing the final version. I have one issue to raise, please check the year of Nipah outbreak in Malaysia. To my knowledge is was in 1998.

I concur with the content and agree to be a co-author and is also in agreement with the authorship order.

Best regards,

Mahmud

Professor Dr. Mahmudur Rahman
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In terms of policy and implementation, however, prior to the emergence of SARS-CoV-2, this scientific community had struggled to convince policy makers about the benefits to invest in research, development and vaccine manufacturing preparedness against pandemics. As an example, even though Nipah has existed for over 20 years, investment on Nipah vaccine development has been limited. The development of a sound business/investment case remains a priority to conduct advocacy and to move forward the development of effective Nipah countermeasures. CEPI has

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For Nipah and henipaviral diseases, it is conceivable that an Animal Rule or similar regulatory pathway may be necessary to achieve licensure of vaccines and other countermeasures. Direct dialogue between preclinical scientists, pathologists and health workers can better inform the design of animal models suitable for EID countermeasure development. Importantly, Henipavirus research has benefited from having a diverse pre-clinical testing portfolio, as many different animal species are susceptible to disease, which means that pre-clinical studies do *not* have to be limited to the use of non-human primates (NHPs). This is also proving important for SARS-CoV-2, as research in NHPs, hamsters, mice and ferrets ensures that COVID-19 vaccines can be tested simultaneously, according to each model, in a wider range of BSL3 laboratory facilities for large and small animals across the world (*Cohen, J. 2020*). It is conceivable that there will be different animal models for different endpoints such as infection, disease, and transmission. In addition, the use of human organoids is also being explored as a model to better understand SARS-CoV-2 biology in human tissue (*Lamers et al., 2020*).

Lesson 5: Plan for clinical trials by bringing together local and regional regulators.

Three sessions were dedicated to epidemiological needs for clinical trials and to review progress in the development of Nipah medical countermeasures. There are several therapeutics in development, including: lipid-anchored peptides to prevent viral fusion, low-cost compounds such as heparin, and a nasal spray with compounds for potential prophylactic use. There are also two

monoclonal antibodies that can neutralize the virus and protect against Henipavirus infection and disease *in vivo*. One (m102.4) has completed a Phase I trial in Australia and has been approved for compassionate use in humans. Since antibodies do not cross the blood-brain barrier, for monoclonal antibody-based therapeutics to be effective, virus neutralization will likely have to occur in the respiratory tract, blood, and other tissues before the virus disseminates into the brain. However, multiple Nipah challenge models including NHPs indicate that passive immunization with Nipah neutralizing antibodies can also prevent CNS involvement if administered at the first signs of Nipah disease.

There was discussion on what the optimal Nipah clinical trial design would be in the context of an EID with very low incidence, i.e. in the absence of feasible randomised controlled trials (RCTs), observational studies such as case-control studies could still provide valuable evidence. Relevant clinical trials for Nipah countermeasures will require engagement of local regulatory authorities in Bangladesh (Directorate General of Drug Administration, DGDA) and India (Drug Controller General of India, DCGI). Partnerships are being built locally, with a focus on equitable access and low-cost interventions. Recognising that pathways to Nipah vaccine licensure are likely to vary across countries, early dialogue between regulators from Asia, Europe and North America was facilitated during the Conference, and an international, Nipah-focused regulatory group was created. Similarly, bringing regulators together early during COVID-19 medical countermeasure development could result in the identification of actions that can be harmonised or accelerated to meet common requirements or convergences along the pathway to licensure across multiple national regulatory agencies.

Lesson 6: Invest in diversified platform technologies that can be harnessed for speed, scale and access during a pandemic.

One Nipah@20 session was devoted to the Nipah vaccine pipeline. At least 13 vaccine candidates have been confirmed to be under development in pre-clinical stages (*Gouglas et al. 2018*). Five of these active vaccine developers presented their latest pre-clinical data, with two speakers presenting on platform technologies that are being used for the development of COVID-19 vaccines.

Dr. Keith Chappell and Professor Paul Young from the University of Queensland, in Brisbane, Australia presented their molecular clamp approach to produce a highly stable pre-fusion form of the Nipah virus F glycoprotein, which is responsible for membrane fusion, and could be an additional target for neutralising antibodies. The molecular clamp is a novel way of locking synthetic versions of viral surface proteins into the same shape that appears on the virus surface. This technology has been designed as a platform approach to generate vaccines against a range of human and animal viruses and has shown promising results in the laboratory targeting viruses such as influenza, Ebola, Nipah and MERS coronavirus. This same principle is also being supported by CEPI to develop a recombinant, spike protein-based COVID-19 vaccine, with Phase I trials expected to begin by July 2020.

Professor Sarah Gilbert from the Jenner Institute, at Oxford University, United Kingdom, presented the ChAdOx1 Nipah vaccine, a simian adenovirus-based vaccine encoding NiV glycoprotein (G) Bangladesh. ChAdOx1 is a replication-deficient simian adenoviral vector which has been used for the development of vaccines against many pathogens in preclinical and clinical studies. It is safe for use in all ages and in patients who are immunocompromised, inducing strong and well maintained

humoral and T cell responses against the encoded antigen without the requirement for an adjuvant. A single dose of a ChAdOx1-vectored Nipah vaccine protects against homologous (Bangladesh strain) and heterologous (Malaysia strain) change in the intraperitoneal Syrian Golden Hamster challenge model (*van Doremalen et al. 2019*). Professor Gilbert's team is now using the same chimpanzee adenoviral platoform technology to develop a COVID-19 vaccine candidate, supported in part by CEPI. A single dose of the vaccine, which encodes the Spike protein of SARS-CoV-2, may confer protection against disease in a rhesus macaque pneumonia model (*van Doremalen et al. 2020*). This COVID-19 vaccine candidate has now entered Phase I/II trials in the UK (*EU Clinical Trials Register*), thus becoming the first CEPI-supported vaccine technology platform to move from pathogen sequence availability to Phase I trials in less than 18 weeks.

Investment in many other vaccine platform technologies has resulted in an unprecedented speed, moving quickly from pathogen sequence availability in early 2020 to a diverse portfolio of more than 70 vaccine candidates in less than 4 months (*Tung Thanh Le et al. 2020*). Although regulatory agencies are adapting to keep up with such a rapidly evolving vaccine pipeline, preparedness for future pandemics may necessitate regulatory frameworks to pre-approve specific platforms with consistent safety data in order to save precious time when plugging in a novel pathogen sequence into a vaccine platform technology. For many COVID-19 platform technologies, dialogue is ongoing to articulate strategies for large-scale manufacturing (*see WHO Manufacturers meeting*) and access (*Yamey et al. 2020*).

Lesson 7: Engage regionally and locally, not just globally.

The final Conference session was dedicated to collaboration and synergy. Professor Zhengli Shi, from the Wuhan Institute of Virology/Chinese Academy of Sciences (CAS), described how a Sino-French agreement to collaborate on EIDs was established in 2004, following the SARS-CoV outbreak. BSL4 labs were established in the Wuhan Institute of Virology as a result of the SARS-CoV outbreak. Prior to the COVID-19 pandemic, the Institute had an annual call launched around April each year for external organisations wanting access to the facility to test their own samples or to have access to samples from the in-house repositories. The many years of research experience and international collaborations built by the CAS since 2004 have provided a preparedness framework through which Chinese scientists have responded and continue to respond to the SARS-CoV-2 epidemic sixteen years later.

Dr. Nivedita Gupta, from the Indian Council of Medical Research (ICMR), described the Regional Enabler for the Southeast Asia Research Collaboration for Health (RESEARCH) Platform. The RESEARCH platform was established on August 28, 2019, with ICMR as secretariat. The goal of the platform is to facilitate clinical research across the region, leveraging the unique capabilities of each country and sharing expertise. Of the 11 member states in the WHO Southeast Asia Region, 10 participated in the second RESEARCH platform meeting. With the platform in place, it will be easier to mount rapid responses to new outbreaks, and to conduct clinical trials – not only on Nipah virus, but also other EIDs.

Epilogue

Authors: Raúl Gómez Román, Linfa Wang, Benhur Lee*, Kim Halpin, Emmie de Wit, Christopher C. Broder, Mahmudur Rahman, Paul Kristiansen and Melanie Saville.*
**corresponding authors*
Target audience: scientific (mSphere)
Expected length: No more than 5 pages/2800 words (currently 2800 words)

Unbeknownst to the scientists attending the Nipah@20 conference in Singapore in December 2019, the COVID-19 outbreak had already begun. We started the year 2020 confronted with a new EID that required rapid mobilisation for vaccine development. Nipah@20 was a scientific drill, a timely dialogue about the need to invest in the development of vaccines and medical countermeasures against EIDs with pandemic potential. CEPI has since mobilised early investment for several COVID-19 vaccine candidates. As stated earlier, for two awardees, this builds on work already undertaken with Nipah virus vaccine projects which were showcased at the Nipah@20 conference.

Transparency and a collaborative spirit underpinned the Nipah@20 conference. Although Nipah virus may not seem like a priority in the current COVID-19 context, we hope that the scientific momentum will continue, with high value EID-related research being undertaken and published. When will Henipavirus experts next meet? An unofficial suggestion has been to commemorate the 30th anniversary of the discovery of Hendra virus, which was the founding member of the Henipavirus genus, sometime in 2024.

Acknowledgments: *The authors wish to thank Tung Thanh Le for his comments in the preparation of this summary. Emmie de Wit is supported by the Intramural Research Program of NIAID, NIH.*

References:

1. WHO priority diseases
2. *CEPI's Joint Coordination Group (JCG) on December 11, 2019*
3. *in progress, under construction by CEPI Communications*
4. *Gouglas et al. 2018*
5. *Burton & Walker, 2020*
6. *Daszak et al. 2020*
7. *WHO Nipah Diagnostics webpage*
8. *see WHO draft Nipah Diagnostics TPPs*
9. *Arunkumar et al. 2019*
10. *Cohen, J. 2020*
11. *Lamers et al., 2020*
12. *van Doremalen et al. 2019*
13. *van Doremalen et al. 2020 (pre-print pending peer review)*
14. *EU Clinical Trials Register*
15. *Tung Thahn Le et al. 2020*
16. *WHO Manufacturers meeting*
17. *Yamey et al. 2020*

From: Halpin, Kim (AAHL, Geelong ACDP) on behalf of Halpin, Kim (AAHL, Geelong ACDP) <Kim.Halpin@csiro.au>
To: Raul Gomez Roman; LinFa Wang Linfa; Lee, Benhur; De wit, Emmie (NIH/NIAID) [E]; Broder, Christopher; rahman.mahmudur@icddrb.org; Mahmudur Rahman
Cc: Lai Yih Shin
Subject: RE: final version for mSphere
Date: Tuesday, June 2, 2020 7:50:21 PM
Attachments: Summary for mSphere_v8_KH.docx
image001.png
image002.png
image003.png

Hi Raul

Thanks for sending through the final version. This article is looking great. I just found one grammatical error:
Page 2 – Lesson 3: it should be the "midst" of an epidemic (not mist). I have also captured this in the attached document for tracking changes.

I am happy with the authorship order.

Kind regards
Kim

From: Raul Gomez Roman <raul.gomezroman@cepi.net>
Sent: Tuesday, 2 June 2020 11:00 PM
To: LinFa Wang Linfa <linfa.wang@duke-nus.edu.sg>; Lee, Benhur <benhur.lee@mssm.edu>; Halpin, Kim (AAHL, Geelong ACDP) <Kim.Halpin@csiro.au>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>; Broder, Christopher <christopher.broder@usuhs.edu>; rahman.mahmudur@icddrb.org; Mahmudur Rahman <mahmudur57@gmail.com>
Cc: Lai Yih Shin <yihshin.lai@duke-nus.edu.sg>
Subject: final version for mSphere
Importance: High

Dear All,
I hope this email finds you well. Here is the latest version of our summary to *mSphere*. It has now incorporated ***all*** your comments. It has also undergone significant internal review at CEPI and should be considered the near final version. Could you please:

1. Review and indicate any ***major*** changes that absolutely need to be made prior to submission
2. Approve the authorship order and this near final version for submission

Benhur and I will follow up with *mSphere*. Apologies this has taken so long (nearly 6 months after the Nipah Conference!), but we have all been swamped with COVID-19 activities – and so have been most Nipah researchers, I understand…Kindly send your reply ***by this Friday, June 5.***
Best wishes,
Raúl

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Authors: Raúl Gómez Román, Linfa Wang, Kim Halpin, Emmie de Wit, Christopher C. Broder, Benhur Lee and --.

Target audience: scientific (mSphere)
Expected length: No more than 5 pages/2400 words (currently 2152 words)

Nipah@20: Lessons learned from another virus with pandemic potential

In December 2019, a Nipah Virus International Conference (Nipah@20) was co-hosted by the Coalition for Epidemic Preparedness Innovations (CEPI), the World Health Organization (WHO), the U.S. National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Duke-NUS Medical School (Duke-NUS).

The Conference marked the 20th anniversary of the discovery of Nipah virus. Since its first identification in Malaysia and Singapore in 1999, the understanding of Nipah virus infection and its pandemic potential has advanced substantially. The Conference provided a forum to review the history and key scientific findings over the last 20 years, and to understand the current challenges in developing Nipah diagnostics, therapeutics and vaccines.

To foster international collaboration in the context of epidemic preparedness, Nipah@20 brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all Henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines and Singapore) were represented in the Conference, with their delegations accounting for 46% of all attendees.

In terms of outcomes, the two-day Conference created a scientific evidence-based framework to: (a) inform discussions between global health stakeholders participating in CEPI's Joint Coordination Group (JCG) on December 11, 2019, (b) discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (c) identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

The Proceedings from the Conference are now publicly available and may consulted in the following link: *(in progress, under construction by CEPI Communications)*. Highlights and commentaries from members of the International Organising Committee follow, with an emphasis on lessons learned from Nipah that could be applied to our understanding of the current COVID-19 pandemic and to preparedness against future emerging infectious diseases (EIDs) of pandemic potential.

Lesson 1: Make a sound investment case for preparedness.

Nipah@20 brought together a community of scientists who, over the past 20 years, have passionately studied the epidemiology, virology, pathogenesis and therapeutics of viruses that have pandemic potential. Many of the invited speakers were involved in understanding and responding to the 2003 SARS-CoV-1 outbreak. And it is no surprise that most of them are currently involved in responding to the COVID-19 pandemic. For the past 15 years, this scientific community has been heavily involved in warning governments about the possible emergence of Pathogen X, and about the need to invest in preparedness against such pathogen.

In terms of policy and implementation, however, prior to the emergence of SARS-CoV-2, this scientific community had struggled to convince policy makers about the benefits to invest in research, development and vaccine manufacturing preparedness against pandemics. As an example, even though Nipah has existed for over 20 years, investment on Nipah vaccine development has been limited. The development of a sound business/investment case remains a priority to conduct advocacy and to move forward the development of effective Nipah countermeasures. CEPI has

estimated that, to bring four Nipah vaccine candidates through successful Phase 2a clinical trials, an investment of up to 1300 million USD would be required (*Gouglas et al. 2018*). This figure may seem high, but it pales in comparison to the economic impact that a pandemic can create. Studies estimating the economic impact of past and present Nipah outbreaks could provide valuable data to model a sound investment case for medical countermeasures against Nipah and other henipaviruses with pandemic potential.

Lesson 2: Articulate how to transform surveillance in animals into impact for humans.

With regard to progress and challenges in surveillance, scientists from several countries presented their research on Nipah and Henipavirus-like sequences found in bat samples throughout Asia and Africa. In addition, the Indian Council for Medical Research (ICMR) has found several new viruses among bats and is exploring their EID potential. Opinions may vary on how to translate bat surveillance into policy and prevention of disease. However, it is clear that international collaboration and further investment on surveillance research is needed to prepare against potential new pathogens that will continue to emerge from bats and other animals in areas with high biodiversity.

Lesson 3: Articulate a strategy for a potential diagnostics conundrum.

In terms of diagnostics, the need for international reference standards was highlighted. International standards are needed for assay validation, calibration, comparison and quality controls; but cost-effective diagnostic kits and devices are also needed. For example, a mobile, nucleic acid based Nipah diagnostic system is being used and validated in India & Bangladesh, with potential applications in clinical trials and outbreak response against other EIDs. In the absence of an effective, Nipah-specific treatment, however, it could be argued that there is no clinical justification or clinical incentive for testing. This is a dilemma that may also apply to other EIDs of pandemic potential: testing and surveillance are beneficial for containment, contact-tracing and rapid response; but in the absence of a specific treatment, what are the benefits to the patient, and how should health providers prioritise diagnostic allocation in resource-constrained settings? Though all countries *should*, not all countries *can* provide diagnostics to test suspect cases of Nipah or other EIDs of pandemic potential. Clear guidance must be developed on how to prioritise diagnostic resources when resources are limited in a pandemic situation in which resources are likely to be limited.

Lesson 4: Diversify the pre-clinical testing portfolio and create direct dialogue between lab scientists, pathologists and health workers in the field.

Two Conference sessions were dedicated to Nipah pathogenesis in animal models and to Transmission & Case-management in humans. Data were presented on aerosol and intranasal challenges in African green monkeys (AGM), intratracheal challenge in cynomolgus macaques, and intranasal challenge in ferrets. Although models vary in recapitulating specific aspects of Nipah disease observed in humans, there was no specific mention of myocarditis observed in heart tissue. Myocarditis has been a consistent observation among Nipah-infected patients in India and Bangladesh, but this has not yet been reported in animal models. It is possible that this difference may be due to early humane endpoints not allowing the heart tissue from infected-animals to reach the level of myocarditis deterioration observed in some Nipah-infected humans. There was also mention of relapse and late-onset of infection observed in Malaysian patients several years after the acute infection. There is no evidence of renewed viremia during relapsed encephalitis in humans, and there is currently no animal model for relapsing Nipah disease although many investigators have proposed such research and its importance. Direct dialogue between preclinical scientists, pathologists and health workers can better inform the design of animal models suitable for EID countermeasure development. Importantly, Henipavirus research has benefited from having a diverse pre-clinical testing portfolio, as many different animal species are susceptible to disease, which means that pre-clinical studies do not have to be limited to the use of non-human primates (NHPs). This is also proving important for SARS-CoV-2, as research in NHPs, hamsters, mice and ferrets ensures that COVID-19 vaccines can be tested simultaneously in a wider range of BSL3 laboratory facilities for large and small animals across the world.

Lesson 5: Plan for therapeutics and vaccine clinical trials by engaging locally.

Three sessions were dedicated to epidemiological needs for clinical trials and to review progress in the development of Nipah vaccines and therapeutics. There are several therapeutics in development, including: lipid-anchored peptides to prevent viral fusion, low-cost compounds such as heparin, and a nasal spray with compounds for potential prophylactic use. There are also two monoclonal antibodies that can neutralize the virus and protect against Henipavirus infection and disease *in vivo*, and one (m102.4) has completed a Phase I trial in Australia and approved for compassionate use in humans. Preclinical data from five Nipah vaccine candidates were also presented, along with one Nipah vaccine candidate, a recombinant subunit G glycoprotein, has already entered Phase I clinical trial in United States. Since antibodies do not cross the blood-brain barrier, for monoclonal antibody-based therapeutics to be effective, virus neutralization will likely have to occur in the respiratory tract, blood, and other tissues before the virus disseminates into the brain. However, multiple Nipah challenge models including NHPs indicate that vaccine-induced antibodies prevent CNS invasion, and passive immunization with Nipah neutralizing antibodies can also prevent CNS involvement if administered at least at the first signs of Nipah disease.

There was discussion on what the optimal Nipah clinical trial design would be in the context of an EID with very low incidence, i.e. in the absence of feasible randomised controlled trials (RCTs), observational studies such as case-control studies could still provide valuable evidence. Relevant clinical trials for Nipah countermeasures will require engagement of local regulatory authorities in Bangladesh (Directorate General of Drug Administration, DGDA) and India (Drug Controller General of India, DCGI). Partnerships are being built locally, with a focus on equitable access and low-cost interventions.

Lesson 6: Invest in platform technologies that can be harnessed during a pandemic.

Two Nipah@20 Conference speakers presented on Nipah vaccines using technology that has recently been used for the development of COVID-19 vaccines.

Professor Sarah Gilbert from the Jenner Institute, at Oxford University, UK presented the ChAdOx1 Nipah vaccine, a simian adenovirus-based vaccine encoding NiV glycoprotein (G) Bangladesh. The ChAdOx1 is a replication deficient simian adenoviral vector which has been used for the development of vaccines against many pathogens, in preclinical and clinical studies. It is safe for use in all ages and in patients who are immunocompromised, including strong and well maintained humoral and T cell responses against the encoded antigen without the requirement for an adjuvant. Professor Gilbert's team is now using the same approach to develop a COVID-19 vaccine candidate, supported by CEPI.

Dr. Keith Chappell and Professor Paul Young from the University of Queensland, in Brisbane, Australia presented their molecular clamp approach to produce a highly stable pre-fusion form of the F glycoprotein which is responsible for membrane fusion, and could be an additional target for neutralising antibodies. The molecular clamp is a novel way of locking synthetic versions of viral surface proteins into the same shape that appears on the virus surface. This technology has been designed as a platform approach to generate vaccines against a range of human and animal viruses and has shown promising results in the laboratory targeting viruses such as influenza, Ebola, Nipah and MERS coronavirus. This same principle is also being supported by CEPI to develop a recombinant spike protein which will be used as a COVID-19 vaccine candidate.

Lesson 7: Engage regionally and locally, not just globally.

The final Conference session was dedicated to collaboration and synergy. Professor Zhengli Shi, from the Wuhan Institute of Virology/Chinese Academy of Sciences, described how a Sino-French agreement to collaborate on EIDs was established in 2004, following the SARS-CoV outbreak. BSL4 labs were established in the Wuhan Institute of Virology as a result of the SARS-CoV outbreak. Prior to the COVID-19 pandemic, the Institute had an annual call launched around April each year for external organisations wanting access to the facility to test their own samples or to have access to samples from the in-house repositories. The many years of research experience and international collaborations built by the CAS since 2004 have provided a preparedness framework through which Chinese scientists have responded and continue to respond to the SARS-CoV-2 epidemic sixteen years later.

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Epilogue

Unbeknownst to the scientists attending the Nipah@20 conference in Singapore in December 2019, the COVID-19 outbreak had already begun in China. We started the year 2020 confronted with a new EID that required rapid mobilisation for vaccine development. Nipah@20 was a scientific drill, a timely dialogue about the need to invest in the development of vaccines and medical countermeasures against EIDs with pandemic potential. CEPI has since mobilised early investment for several COVID-19 vaccine candidates. As stated earlier, for two awardees, this builds on work already undertaken with Nipah virus vaccine projects which were showcased at the Nipah@20 conference.

Transparency and a collaborative spirit underpinned the Nipah@20 conference. Although Nipah virus may not seem like a priority in the current COVID-19 context, we hope that the scientific momentum will continue, with high value EID-related research being undertaken and published. When will Henipavirus experts next meet? An unofficial suggestion has been to commemorate the 30th anniversary of the discovery of Hendra virus, which was the founding member of the Henipavirus genus, sometime in 2024.

***Acknowledgments**: The authors wish to thank --- for their comments in preparation of this summary.*

From:	Raul Gomez Roman on behalf of Raul Gomez Roman <raul.gomezroman@cepi.net>
To:	LinFa Wang Linfa; Halpin, Kim (AAHL, Geelong AAHL); Lee, Benhur; Broder, Christopher; Cristina Cassetti; De wit, Emmie (NIH/NIAID) [E]; rgangakhedkar.hq@icmr.gov.in; Dr. Nivedita Gupta; Emily Gurley; Higgs, Elizabeth (NIH/NIAID) [E]; PREZIOSI, Marie-pierre; Mahmudur Rahman; Ravindran Thayan; mahmudur57@gmail.com
Cc:	Jodie Rogers; Ana Paula De Almeida Aranha
Subject:	Nipah Conference Summary - v3
Date:	Tuesday, April 14, 2020 4:50:19 PM
Attachments:	Summary for mSphere_v3.docx image004.png image006.png image008.png image010.png image012.png image016.png

Dear All,

Thanks again to those of you who have sent contributions to earlier versions on the summary from the Nipah Conference.

Please see the third version of the document here. Co-authors are those who have provided specific feedback, comments and content.

This version will now go for internal review at CEPI prior to submission to mSphere.

I wish to acknowledge the remaining IOC members who are not able to contribute to authorship this time. I understand all of us are swamped with the current pandemic situation, and not all of you will be available to comment or contribute this time.

Best wishes,

Raúl

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From: Raul Gomez Roman
Sent: torsdag 2. april 2020 23:44
To: 'Halpin, Kim (AAHL, Geelong AAHL)' <Kim.Halpin@csiro.au>; Lee, Benhur <benhur.lee@mssm.edu>; benassiv <benassiv@who.int>; Broder, Christopher <christopher.broder@usuhs.edu>; Cristina Cassetti <CCassetti@niaid.nih.gov>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>; rgangakhedkar.hq@icmr.gov.in; Dr. Nivedita Gupta <guptanivedita.hq@icmr.gov.in>; Emily Gurley <egurley1@jhu.edu>; Higgs, Elizabeth (NIH/NIAID) [E] <ehiggs@niaid.nih.gov>; PREZIOSI, Marie-pierre <preziosim@who.int>; Mahmudur Rahman <rahman.mahmudur@icddrb.org>; Ravindran Thayan <rthayan@gmail.com>; mahmudur57@gmail.com
Cc: LinFa Wang Linfa <linfa.wang@duke-nus.edu.sg>; Jodie Rogers <Jodie.rogers@cepi.net>; Ana Paula De Almeida Aranha <AP.AlmeidaAranha@cepi.net>
Subject: RE: Proceedings from Nipah Virus International Conference

Dear All,

We would like to thank those of you who have sent your comments and suggestions. Please find the second draft of the Nipah@20 summary paper for *mSphere*. This incorporates comments received thus far into a more refined version in which the table has been removed.

Kindly send any comments/edits back to us **before April 10th**. We will only include as authors those of you who have expressed a wish to co-write this summary. We will include the remaining IOC members in the acknowledgements section.

Dear Benhur and All – please hang in there and receive our solidarity in these difficult times.

Best wishes,
Raúl

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From: Halpin, Kim (AAHL, Geelong AAHL) <Kim.Halpin@csiro.au>
Sent: tirsdag 31. mars 2020 09:43
To: Lee, Benhur <benhur.lee@mssm.edu>
Cc: Raul Gomez Roman <raul.gomezroman@cepi.net>; benassiv <benassiv@who.int>; Broder, Christopher <christopher.broder@usuhs.edu>; Cristina Cassetti <CCassetti@niaid.nih.gov>; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>; rgangakhedkar.hq@icmr.gov.in; Dr. Nivedita Gupta <guptanivedita.hq@icmr.gov.in>; Emily Gurley <egurley1@jhu.edu>; Higgs, Elizabeth (NIH/NIAID) [E] <ehiggs@niaid.nih.gov>; PREZIOSI, Marie-pierre <preziosim@who.int>; Mahmudur Rahman <rahman.mahmudur@icddrb.org>; Ravindran Thayan <rthayan@gmail.com>; LinFa Wang Linfa <linfa.wang@duke-nus.edu.sg>; Jodie Rogers <Jodie.rogers@cepi.net>; Ana Paula De Almeida Aranha <AP.AlmeidaAranha@cepi.net>
Subject: RE: Proceedings from Nipah Virus International Conference

Dear Benhur

Wow! How confronting. It is one thing to see a clip on the news, but another to hear it from someone in the hot zone. I am so sorry that you, and the rest of your colleagues, are experiencing this. Modern medicine wasn't supposed to be like this. This really is a tsunami.

Please don't hesitate to reach out if I can help you in anyway.

Thinking of you,
Kindest regards
Kim

Sent from Mail for Windows 10

From: Lee, Benhur
Sent: Tuesday, 31 March 2020 4:43 PM
To: Halpin, Kim (AAHL, Geelong AAHL)
Cc: Raul Gomez Roman; benassiv; Broder, Christopher; Cristina Cassetti; De wit, Emmie (NIH/NIAID) [E]; rgangakhedkar.hq@icmr.gov.in; Dr. Nivedita Gupta; Emily Gurley; Higgs, Elizabeth (NIH/NIAID)

[E]; PREZIOSI, Marie-pierre; Mahmudur Rahman; Ravindran Thayan; LinFa Wang Linfa; Jodie Rogers; Ana Paula De Almeida Aranha
Subject: Re: Proceedings from Nipah Virus International Conference

Benhur no longer remembers what day it is anymore. Raul, you'll have to remind me.

As of yesterday we have 1,249 COVID-19 positive patients in our hospitals. That includes 233 patients in our ICUs. We only had ~130 ICU beds . . now we walk by this in our lobby (everything is public so no confidentiality breached here) as every square inch gets gradually taken up by temp beds for COVID-19 patients. If New York was a country, we would be No. 5 in the world in no. of COVID-19 cases. By the time I wake up tomorrow, NY would have surpass the maximal number of cases in Hubei province.

We are trying innovative ways of re-using N95s and/or impregnating them with virucides. Innovative ways of trying to consent patients for longitudinal studies, developing serology testing to identify sero-converters for both human plasma therapy and de-risk front-line responders . . .our dept works as one now. On staggered shifts as we all work only on COVID-19 related research while still maintaining social distancing

I know this is not related to our Nipah conference but thought you all might like to know what it is being in the middle of the world's hottest zone. Being spat on by strangers while taking the subway to work does not make it easier. I no longer take public transport.

#BeSafe #FlattenTheCurve #SocialDistancingWorks

Benhur

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> On Mar 30, 2020, at 12:28 AM, Halpin, Kim (AAHL, Geelong AAHL) <Kim.Halpin@csiro.au> wrote:
>
> USE CAUTION: External Message.
>
> Hi there
>
> I'm not sure if others have contributed to the *mSphere* article as requested by Raul (email below), but please find my contribution.
> Others are welcome to build on this, if they have time. It would be great to see a few

more comments to round out the summary of this amazing (and somewhat prophetic!) conference.

I think Benhur wanted this by 30th March – so I'm cutting this a bit fine – sorry.

Thanks everyone
Kind regards
Kim

From: Raul Gomez Roman <raul.gomezroman@cepi.net>
Sent: Thursday, 19 March 2020 9:57 PM
To: benassiv <benassiv@who.int>; Broder, Christopher <christopher.broder@usuhs.edu>; CCassetti@niaid.nih.gov; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>; rgangakhedkar.hq@icmr.gov.in; Dr. Nivedita Gupta <guptanivedita.hq@icmr.gov.in>; Emily Gurley <egurley1@jhu.edu>; Halpin, Kim (AAHL, Geelong AAHL) <Kim.Halpin@csiro.au>; Higgs, Elizabeth (NIH/NIAID) [E] <ehiggs@niaid.nih.gov>; Lee, Benhur <benhur.lee@mssm.edu>; PREZIOSI, Marie-pierre <preziosim@who.int>; Mahmudur Rahman <rahman.mahmudur@icddrb.org>; Ravindran Thayan <rthayan@gmail.com>
Cc: LinFa Wang Linfa <linfa.wang@duke-nus.edu.sg>; Jodie Rogers <Jodie.rogers@cepi.net>; Ana Paula De Almeida Aranha <AP.AlmeidaAranha@cepi.net>
Subject: [WARNING : MESSAGE ENCRYPTED]Proceedings from Nipah Virus International Conference

Dear International Organizing Committee (IOC) members for the Nipah Virus International Conference,

Please find a ***draft*** (pdf) with the Proceedings from the Nipah Virus International Conference, which took place in Singapore last December. The password for this document is [redacted]. Please consider this document under 'embargo' until cleared by the the respective Communications departments from CEPI and its Conference organizing partners (WHO, NIAID, Duke-NUS).

We hope this email finds you well. We apologise for not sharing the Proceedings with you sooner, but we hope you will understand all of us have been swamped with the present COVID-19 situation. Although we do appreciate the Nipah Conference may be perceived as less of a global priority now comparted to COVID-19, we believe the Proceedings contain relevant insights that should be shared with the global community in a pandemic situation.

Our plan is to upload a refined and cleared version of the Proceedings into a suitable online platform for distribution with our partners, ideally within 4 months of the Conference having taken place (i.e. before April 11th).

In addition, and thanks to Benhur Lee, the Editor-in-Chief for *mSphere* has also agreed to publish a *summary*version of this and we have started working on that. Please also find the *word* document with a *draft* summary of Conference. We would like to invite you to co-author this summary and edit/add comments to this **by Monday, March 30**. If you do not wish to co-author the summary, please let us know.

Again, we do recognise that this may be perceived as less of a priority now, but we hope the insights from the Conference can shed light in some of the scientific issues being discussed now. We would like to thank you again for your valuable contributions to this Conference, and we look forward to your participation in this process.

Best wishes,
Raúl

RAUL GOMEZ ROMAN
Preclinical Vaccine Scientist

<image001.png>

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<image002.png> <image003.png>

<Summary and Outline for mSphere_v1_KH.docx>

Confidential *Draft 19.03.2020*
Authors: Members of the International Organising Committee for Nipah@20
Target audience: scientific (mSphere)
Expected length: ideally no more than 5 pages

Draft summary/outline for mSphere

The Nipah Virus International Conference of 2019 (Nipah@20) was co-hosted by the Coalition for Epidemic Preparedness Innovations (CEPI), the World Health Organization (WHO), the U.S. National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Duke-NUS Medical School (Duke-NUS).

The Conference marked the 20th anniversary of the discovery of Nipah virus. Since its first identification in Malaysia and Singapore in 1999, the understanding of Nipah ~~disease~~ virus infection and its pandemic potential has advanced substantially. The Conference provided a forum to review the history and key scientific findings over the last 20 years, and to understand the current challenges in developing Nipah diagnostics, therapeutics and vaccines.

To foster international collaboration in the context of epidemic preparedness, Nipah@20 brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines and Singapore) were represented in the Conference, with their delegations accounting for 46% of all attendees.

In terms of outcomes, the two-day Conference created a scientific evidence-based framework to: (a) inform discussions between global health stakeholders participating in CEPI's Joint Coordination Group (JCG) on December 11, 2019, (b) discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (c) identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

Highlights from the Conference are summarized in **Table 1.** The page numbers refer to the Proceedings from the Conference, which are publicly available at (**CEPI Communications** working on the branded document and website for citation).

Table 1. Highlights from Nipah Virus International Conference

	Session	Highlighted Topics in Proceedings	Page number in Proceedings
1	Historic Review of Nipah Outbreaks		
2	WHO Nipah R&D Roadmap	Policy and implementation issues: 1) Without effective therapy available at a medical facility, there is no clinical justification or ~~/~~incentive for testing and~~/~~surveillance. 2) Development of a sound business case for product development is needed to move medical countermeasures forward.	21-22
3	Progress and challenges in surveillance of Nipah virus	1) ICMR has found several new viruses among bats and is exploring EID potential. Pathogen X implications and possible candidates for test runs of rapid response.	27, 29

	Session	Highlighted Topics in Proceedings	Page number in Proceedings
		2) A Ghanian henipavirus sequence has been isolated in S. Africa. 3) A lot of work on bat surveillance, but unclear how to translate into impact.	
4	Progress and challenges in diagnostics	1) International reference standards needed for validation, calibration, comparison, and quality controls. 2) A mobile diagnostic system (MolBio) is being used in India & Bangladesh and could have applications in epi, clinical trials, and outbreak response. ~11USD per test. 3) FLI ELISAs use henipavirus sG proteins expressed in *Leishmania tarentolae.* Could be alternative for sourcing protein for assays.	34, 35, 38
5	Pathogenesis and animal models	1) NIH working on **aerosol** challenge model in AGMs. Importantly, no apparent role for antibodies in protection against challenge! 500 pfu used. 2) UTMB using an ***intranasal*** atomization device. 2,000 – 20,000 pfu uniformly lethal in AGMs. 500,000 ***intratracheal*** in cynos, not uniformly lethal. 3) CSIRO working on ***intranasal*** challenge in ferrets. Shedding not sufficient to cause transmission, arguing against aerosol route. No animal model is yet exploring myocarditis – a key clinical attribute of the disease observed in India & Bangladesh.	41-47
6	Transmission/Case management	1) Several key clinical observations that are currently not being contemplated in animal model development, for example, myocarditis and hypoxia in ABG analysis. Would be easy to implement in animal models. 2) Relapse and late-onset henipavirus infections observed in Malaysian patients several years after the acute infection – unclear if there is viremia during relapse. No animal model for relapsing disease.	48-49, 51
7	Epidemiological needs for clinical trials	1) In the absence of randomised controlled trials (RCTs), observational studies such as case-control studies can provide valuable evidence, according to WHO.	54-55, 56, 57

	Session	Highlighted Topics in Proceedings	Page number in Proceedings
		2) A case-control study with vaccination in Faridpur and Rajbari (Bangladesh) and recruitment of 10 matched controls whenever a case is identified would require only 7 years to be completed (provided 70 % vaccine coverage), according to S. Luby. 3) DGDA, the regulatory authority in Bangladesh, has several requirements outlined on top of pg. 56. 4) 3 of 279 close contacts from the 2018 Kerala outbreak had subclinical infection. Similarly, 8 % of Nipah-infected Malaysian patients had subclinical infection with positive serology. Could be important to analyse immune responses in subclinical infections, to try to emulate this type of immunity with a vaccine.	
8	Progress and challenges in vaccine development	Someone asked if the PHV/Crozet VSV-based vaccine caused neurovirulence. G. Hepner replied neurovirulence studies are planned and will be discussed with authorities.	67-68
9	Progress and challenges in therapeutics development	1) Monoclonal antibody shipped from Australia to be tested therapeutically in the outbreak was left unused. 2) To facilitate access of therapeutics to future outbreaks, ICMR has taken the lead in ensuring free production and supply, as well as putting in place shipping agreements with manufacturers. Interesting equitable access example. 3) Other CMCs: antibodies to sG and F, though abs don't cross the brain-blood barrier; heparin as low-cost method; lipid-anchored peptides to prevent viral fusion. Working on a nasal spray with these compounds to be used prophylactically vs NiV. Keep an eye on non-vaccine MCM portfolio.	69, 73
10	Collaboration and Synergy	1) A Sino-French agreement to collaborate on emerging infectious diseases was established in 2004, following the SARS outbreak. *BSL4 labs were established in Wuhan as a result of SARS.*	75-76

	Session	Highlighted Topics in Proceedings	Page number in Proceedings
		2) (Before the COVID-19 days), the Wuhan BSL-4 facility has an annual call around April for external organisations wanting access to the facility to test their own samples or use samples from the in-house repositories. 3) The Regional Enabler for the Southeast Asia Research Collaboration for Health (RESEARCH) Platform was established on August 28, 2019, with ICMR as secretariat. The goal of the platform is to facilitate clinical research across the region, leveraging the unique capabilities of each country and sharing expertise. With the platform in place, it will be easier to mount rapid responses to new outbreaks, and to conduct clinical trials – not only on NiV, but also other EIDs.	

Opinion/Commentary – (open for comments from IOC)

The Nipah@20 conference brought together a community of scientists passionate about infectious diseases – focusing on the epidemiology, virology, pathogenesis and therapeutics of a virus that has pandemic potential. The WHO R&D Nipah Blueprint and Roadmap provides a solid framework from which to organise, collaborate and prioritise the different research areas. The global approach to developing a vaccine for Nipah has involved a multi-pronged approach for vaccine candidates, many of which were presented at the Nipah@20 conference.

The Nipah@20 conference facilitated early mobilisation for vaccine development with COVID-19, with CEPI leading the way, investing in seven potential vaccine candidates. For two groups, this builds on work already undertaken with Nipah virus vaccine projects which were showcased at the Nipah@20 conference.

Professor Sarah Gilbert from the Jenner Institute, at Oxford University, UK presented their ChAdOx1 Nipah vaccine, a simian adenovirus-based vaccine encoding NiV glycoprotein (G) Bangladesh. The ChAdOx1 is a replication deficient simian adenoviral vector which has been used for the development of vaccines against many pathogens, in preclinical and clinical studies. It is safe for use in all ages and in patients who are immunocompromised, including strong and well maintained humoral and T cell responses against the encoded antigen without the requirement for an adjuvant. Professor Gilbert's team are using the same approach with their COVID-19 vaccine candidate.

Dr Keith Chappell and Professor Paul Young from the University of Queensland, in Brisbane, Australia presented their approach which uses a molecular clamp to produce a highly stable pre-fusion form of the F protein which is responsible for membrane fusion, and is one of the main targets for neutralising antibodies (and therefore a major focus of vaccine research). The molecular clamp is a novel way of locking synthetic versions of viral surface proteins into the same shape that appears on the virus surface. The technology has been designed as a platform approach to generate vaccines against a range of human and animal viruses and has shown promising results in the laboratory targeting viruses such as influenza, Ebola, Nipah and MERS coronavirus. This same principle is being used to develop a recombinant spike protein which will be used in their COVID-19 vaccine candidate.

Transparency, and a collaborative spirit underpinned the Nipah@20 conference, and it is hoped that the momentum will continue, and we will see high value Henipavirus research being undertaken and published. When will we next meet? An unofficial suggestion has been to commemorate the 30th anniversary of the discovery of Hendra virus which was the founding member of the Henipavirus genus, sometime in 2024.

Formatted: Superscript

CEPI

Nipah@20

Nipah Virus International Conference

9th and 10th December 2019

Singapore

Meeting report prepared by

Christine Berge
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Day 1

Day 2

Acronyms and Abbreviations

A – answer
AAHL – Australian Animal Health Laboratory
Ad – adenovirus
AEFI – adverse events following immunisation
AES – acute encephalitis syndrome
Ag – antigen
AGM – African green monkey
AMES – Acute Meningo Encephalitis Surveillance system
APVMA – Australian Pesticides and Veterinary Medicines Authority
ARDS – Acute Respiratory Distress Syndrome
BBB – blood-brain barrier
BSA – baseline situation analysis
BSL – biosafety level
C – comment
CD – cluster of differentiation
CDC – Centers for Disease Control and Prevention
CDSCO – Central Drugs Standard Control Organization
CDV – canine distemper virus
CFIA – Canadian Food Inspection Agency
CFR – case fatality rate
CHO – chinese hamster ovary
CM – cynomolgus monkeys
CNS – central nervous system
CoA – certificates of analysis
CRO – clinical research organisation
CSF – cerebrospinal fluid
CT – computed tomography
CTA – clinical trial agreement
CTM – clinical trial material
DC – dendritic cell
DGDA – Directorate General of Drug Administration (Bangladesh)
DHR – Department of Health Research (India)
DIC – disseminated intravascular coagulation
DPI – days post infection
DRC – Democratic Republic of Congo
DSMB – Data and Safety Monitoring Board
Dx – diagnostics
EBOV – Ebola virus
EGFP – enhanced green fluorescent protein
EID – emerging infectious disease
ELISA – enzyme-linked immunosorbent assay
EMA – European Medicines Agency
ERC – ethical review committee
EUA – emergency use authorisation (FDA)
EUL – emergency use listing (WHO)
FDA – Food and Drug Administration
FIND – Foundation for Innovative New Diagnostics
G – NiV glycoprotein
GAG – glycosaminoglycans
GCM – global coordination mechanism
GLP – good laboratory practice
GHSA – Global Health Security Agenda
GMO – genetically modified organisms
GMP – good manufacturing practice
gp – glycoprotein
F – NiV fusion protein
HCW – health care workers
HeV – Hendra virus
HRC – C-terminal Heptad repeat
HRN – N-terminal Heptad repeat
HRP – horseradish peroxidase
HS – heparan sulphate
HV – herpes virus
icddr,b – International Centre for Diarrhoeal Disease Research, Bangladesh
ICF – informed consent form
ICMR – Indian Council of Medical Research
ICP – infection control practice
ICTV – International Committee on the Taxonomy of Viruses
ICU – intensive care unit
IDRI – Infectious Disease Research Institute (U.S.)
IEDCR – Institute of Epidemiology, Disease Control and Research (Bangladesh)
IFAT – indirect fluorescence antibody test
IFN-γ – interferon gamma
ILI – influenza-like illness
IND – investigational new drug
ip – intraperitonea
IRB – institutional review board
JE – Japanese encephalitis
km – kilometres
LASV – Lassa virus
LMICs – low- and middle income countries
mAb – monoclonal antibody
mcsF – molecular clamp-stabilised NiV F
MERS – middle east respiratory syndrome
MERS-CoV – MERS coronavirus
MLV – modified live virus
MoH – Ministry of Health
MRI – magnetic resonance imaging
MTA – material transfer agreement
MV – measles virus
NAT – nucleic acid test

NCFAD – National Centre for Foreign Animal Disease (Canada)
NCID – National Centre for Infectious Diseases (Singapore)
NCPV – National Collection of Pathogenic Viruses
NGS – next-generation sequencing
NHP – non-human primate
NIAID – National Institute of Allergy and Infectious Diseases
NIID – National Institute of Infectious Diseases (Japan)
Nipah@20 – Nipah Virus International Conference
NiV – Nipah virus
NiV-B – Bangladesh isolate of NiV
NiV-M – Malaysian isolate of NiV
NIV – National Institute of Virology (India)
NPT – near-patient
OIE – World Organisation for Animal Health
PEG – polyethyleneglycol
PEP – post-exposure prophylaxis
pfu – plaque-forming units
PHEIC – public health emergencies of international concern
POC – point of care / proof of concept
PPE – personal protective equipment
PRIME – PRIority MEdicines
PRNT – plaque reduction neutralization test
PRRSV – porcine reproductive and respiratory syndrome virus
PTT – partial thromboplastin time
Q – question
Q&A – questions and answers
R 0 – basic reproduction number
R&D – research and development
RCT – randomised controlled trial
RFP – request for proposals
RITM – Research Institute of Tropical Medicine (Philippines)
RNA – ribonucleic acids
RRC – Research Review Committee
RSV – respiratory syncytial virus
RT-PCR – reverse transcriptase polymerase chain reaction
RVF – rift valley fever
rVSV – recombinant VSV
SAG – scientific advisory group
SARS – Severe Acute Respiratory Syndrome
SEAR – WHO South-East Asia Region
SEARO – South-East Asia Regional Office of WHO
sG – soluble G protein
SNT – serum neutralisation test
SOC – standard of care
SOP – standard operating procedure
SPEAC – Safety Platform for Emergency vACcines
SSPE – subacute sclerosing panencephalitis
TNF-α – tumour necrosis factor alpha
TPP – target product profile
Tx – therapeutics
UTMB – University of Texas Medical Branch
VRDL – Virus Research and Diagnostic Laboratory (India)
VSPB – Viral Special Pathogens Branch
VSV – vesicular stomatitis virus
Vx – vaccines
vWF – von Willebrand factor
WHO R&D Blueprint – WHO research and development Blueprint for action to prevent epidemics
ZEBOV – Zaire Ebola virus

Executive Summary

The Nipah Virus International Conference of 2019 (Nipah@20) marked the 20-year anniversary of the first Nipah outbreak in humans, which happened in Malaysia and Singapore in 1998-1999 and resulted from spillover of Nipah virus (NiV) from fruit bats to pigs, and from pigs to humans. In total, 283 individuals were infected, causing 110 deaths. There have since been Nipah outbreaks in Bangladesh, India, and the Philippines. In total, only about 600 people have been infected in these 20 years. However, the devastating epidemic potential of NiV has placed it on the WHO list of priority diseases. In the largest outbreaks, human-to-human transmission and superspreaders were important drivers for viral spread. Family members or healthcare workers had the highest risk of infection, with prolonged exposure to the patient or exposure to body fluids being the primary causes of human-to-human transmission.

Nipah disease is characterised by acute encephalitis and acute respiratory distress syndrome. The severity and duration of disease may vary from outbreak to outbreak, depending (among other things) on the pathogenic potential and tropisms of the circulating NiV strain. The two main strains are NiV Bangladesh (NiV-B) and NiV Malaysia (NiV-M), but a variety of sub-strains have been isolated. Typically, the first symptoms of disease occur between 3 and 21 days post infection, and the case fatality rate ranges from 50 % (NiV-M) to about 75 % (NiV-B).

Fruit bats constitute the main reservoir host of NiV, as well as of other (known and unknown) emerging infectious diseases. Thus, the migration and roosting areas of bats are considered a major determinant for risk of Nipah outbreaks, and active surveillance deemed an important preventive measure. It has been shown that virus spills over into other animals and humans through the consumption of fruits that have been contaminated by bats and, in particular, palm tree sap. This may be one reason why outbreaks in Bangladesh, where the consumption of palm tree sap is part of local food culture, are so common. In later years, the number of cases in Bangladesh has dropped, which may be attributed, at least in part, to increased awareness regarding the risk of ingesting raw palm tree sap harvested overnight. For efficient surveillance and response to outbreaks, a One Health approach should be taken, where not only human transmission but also the circulation of virus in reservoir host populations is accounted for.

In addition to bats and pigs, NiV has been found to infect a number of other animals, including mice, hamsters, ferrets, cats, dogs, goats, cattle, and monkeys. Especially hamsters, ferrets, and monkeys represent important model animals for NiV research, and for the development of therapeutics and vaccines. Animal studies with live virus need to be conducted at biosafety level 4, which is a major limitation for preclinical research on NiV. Field research also requires important safety measures, and it is hoped that new assays allowing for rapid inactivation of virus will improve point of care diagnosis.

Currently, there are no licensed vaccines or therapeutics against Nipah infection. However, there are a number of promising candidates in the pipeline, and some (like the m102.4 monoclonal antibody) have already been used clinically with apparent success. One important barrier for licensure, is the conduct of controlled phase I and II clinical trials. Due to the irregular occurrence of NiV outbreaks, and the usually small number of cases, the conduct of robust trials poses a serious challenge. Furthermore, delayed onset of disease, late presentation of cases at clinical facilities, symptoms that are easily confused with other diseases, and lack of point of care rapid diagnostics all contribute to slowing down the response to a new outbreak. As a result, many treatment opportunities and trial participants may be lost. However, international and regional collaboration, development of new and improved diagnostics for field use, as well as improved surveillance and innovative trial designs, may serve to solve this. With respect to the latter, a multinational, multi-outbreak, multi-intervention clinical trial is being prepared, with regional and international collaboration on planning and implementation, and support from several of the Nipah-endemic countries.

Nipah virus is but one of a growing number of the closely related henipaviruses. While NiV is a frightening, lethal virus, other viruses that have not yet emerged may prove worse. So far, Hendra virus is perhaps the best known of the other henipaviruses, for its role in lethally infecting horses (and a few humans) through outbreaks along the Australian East coast. Traces of henipavirus infection have been found throughout a large geographic area covering Australia, South Asia, as well as sub-Saharan Africa, even in the absence of known outbreaks. Being able to predict the next outbreak would require advanced modelling based on a long list of risk factors, and an incredible amount of scientific data. However, as pointed out by CEPI's CEO, Richard Hatchett: "Unless something is done, Nipah will continue to re-emerge in smaller outbreaks, until it finds the perfect combination of circumstance and opportunity to explode again, as it did some 20 years ago". On the other hand, when the next epidemic comes, we may be facing a different pathogen altogether. Still, preparing for Nipah also means improving preparedness for other emerging infectious diseases, known or unknown.

Day 1

Opening ceremony

Co-chairs: Professor Linfa Wang, Duke-NUS / Dr. Raúl Gómez Román, CEPI

A warm welcome to everybody for joining the Nipah Virus International Conference (Nipah@20). This conference is the result of an initiative taken by the Coalition for Epidemic Preparedness Innovations (CEPI) in March 2019, and it was put together in a relatively short time. The conference has 215 participants from 24 countries, which exceeded the organisers' expectations by about 50 %! Impressively, most of the people who were invited to the conference, on relatively short notice, are now present.

As organisers, we are grateful for the contributions made by the scientific community in preparing the conference. Scientists from nine different countries have taken part in the preparations, and the conference programme is the result of their experience and input. We would also like to thank our hosts, organisers, partners, and sponsors, who have entrusted us with the responsibility and privilege of preparing the conference. On behalf of the international organising committee, we would like to wish you a productive and scientifically stimulating Nipah@20.

Message from Ministry of Health, Singapore

SMS Dr. Lam Pin Min, Senior Minister of State for Health and Transport

Nipah@20 marks the 20th anniversary of the discovery of Nipah virus (NiV). There was also a conference marking the 10th anniversary, but hopefully there will not be a need for a 30th anniversary conference – by then, an effective vaccine should ideally have been discovered. It is important to come together at a conference like this, to take stock of the latest developments in global health, and discuss how we may better address emerging infectious diseases (EIDs).

EIDs remain a threat to countries around the world, Singapore included. In addition to Nipah, Singapore has experienced recent outbreaks of Severe Acute Respiratory Syndrome (SARS), endemic Influenza (H1N1), and Zika. We are not able to predict where and when the next EID may emerge, but we are able to prepare for its emergence and mitigate its impact. Our defence against EIDs include three elements: Public health capabilities, cooperation, and commitment to research.

Strong public health capabilities and -systems are important for managing and responding to public health crises. Singapore adopts an integrated and dedicated approach to the management of EIDs, with capability to deliver clinical services as well as public health functions to detect, respond to, and contain EIDs. The National Centre for Infectious Diseases (NCID) was opened this year, to strengthen

Singapore's capabilities in public health preparedness and infectious disease management and prevention. In the event of an epidemic, the capabilities of our healthcare professionals are critical. Recognising this, Singapore continues to invest in training for healthcare professionals and public health practitioners.

The responsibility for addressing EIDs does not rest with one institution, one sector, or one country alone. This brings in the aspect of cooperation – or rather, cooperation, collaboration, and coordination. Management of zoonotic diseases, such as Nipah, underscores the need for coordination across sectors. Singapore has adopted an interdisciplinary "one health" approach, integrating human, animal, and environmental health surveillance and response. Robust animal and human surveillance, combined with infrastructure capabilities, are critical in providing early warnings to the animal and human health authorities. The Ministry of Health works closely together with a number of other agencies to develop capabilities and strengthen surveillance.

During the 1998-1999 Malaysia and Singapore Nipah outbreak, international collaboration was instrumental to the identification of pigs as an intermediate amplifying host for NiV infection. Fostering partnerships across regional and national borders is necessary to deal with infectious agents that are not constrained by such borders. In addition, the emergence of new infectious diseases stresses the importance of research investments.

CEPI was established in Davos in 2017, as a private-public partnership with the ambition to accelerate the development of vaccines against EIDs. This conference is yet another example of how different players can come together to support work and research concerning EIDs. Our hope is that the conference may lead to new partnerships and innovations that may boost the fight against Nipah virus.

Message from the Ministry of Health, Malaysia

Datuk Dr. Christopher Lee Kwok Choong, Deputy Director General of Health (Research & Technical Support)

Thank you to the organisers and Singapore for hosting this event. Nipah virus was named after the Malaysian village Sungai Nipah. To many Malaysians, the word Nipah evokes memories of the devastating zoonotic outbreak in 1998, where the pig farming industry was hit hard. A total of 283 cases were confirmed infected, resulting in 110 fatalities. The outbreak also reached Singapore, reminding us that borders are unable to stop infectious diseases.

Since the 1998 NiV outbreak, the ministry of health (MoH) has put in place a number of measures to protect the people of Malaysia from future zoonotic outbreaks. This includes strengthening of the surveillance systems in both human and animal health sectors, and in particular a structured and continuous engagement platform where information may be shared and analysed, and decisions are made collectively. The platform, corresponding to Singapore's "one health" approach, has been in place since 1999.

While the Nipah outbreak in 1998 would ideally have been a one-off event, the last 20 years have proven the opposite. Although Malaysia has not had another outbreak, NiV outbreaks have occurred a number of times in Bangladesh and India. These outbreaks have been traced to food contaminated by fruit bats, which are now known to be the natural host of NiV. Transmission from human-to-human has been confirmed in South India, primarily affecting family members or healthcare personnel. Fatality rates range from 40 to 75 %, yet interest in NiV has been more or less confined to the countries affected.

To this day, there is no cure, vaccine, or specific treatment for Nipah, and affected individuals receive only supportive care. In the 20 years that have passed, WHO estimates that there has been about 600 Nipah cases worldwide, limited to countries in Southeast Asia. There are, however, concerns regarding the devastating zoonotic potential of NiV, as stated by the World Organisation for Animal Health (OIE). The WHO considers the risk of geographical spread of Nipah to be low, yet the migration patterns and distribution of fruit bats are extensive. Evidence from Bangladesh shows that viral spillovers from bats to humans happen regularly, providing opportunities for a more highly transmissible strain to infect and adapt in humans.

For effective countermeasures to Nipah, a broader and more comprehensive approach, with investments in therapeutics, diagnostics, and vaccines as well as surveillance infrastructure, is urgently needed. Furthermore, strengthened capabilities are warranted in rapidly detecting and verifying cases, conducting detailed contact tracing, investigating spillovers, and gaining a better understanding of NiV and its mode(s) of transmission. Equally important is the need for behavioural change, and improved support to local communities for prevention and control measures. The goal is to reduce transmission, especially in the healthcare setting.

Nipah@20 is a timely opportunity for assessing the many gaps that still remain in our understanding of NiV and, hence, our ability to prevent and treat Nipah infection. Malaysia is grateful to be involved in this meeting, and looks forward to learn from the many experts gathered at the event, as well as to engage with the other stakeholders present in the interest of moving towards more effective management of NiV infection.

Message from the WHO

Dr. Michael Ryan, Executive Director, WHO Health Emergencies Programme

Nipah virus and the related henipaviruses are severe emerging diseases with the potential to impact vulnerable populations, generating public health emergencies. Many aspects of the management and treatment of the henipaviral diseases remain unknown, and they have therefore been included on the list of WHO priority diseases, as well as on the WHO research and development Blueprint for action to prevent epidemics (WHO R&D Blueprint). The WHO R&D Blueprint is a global strategy to ensure implementation of critical research findings on highly infectious diseases in a safe, effective and timely way. In collaboration with leading experts, WHO R&D Blueprint allows for fast-tracking of effective medical technologies in the context of an epidemic.

The Nipah virus has a significant epidemic potential. Its bat reservoir has been found in a number of countries, and it has the ability to amplify in livestock, which is a source of exposure to humans. Human infection results in severe neurological disease and respiratory symptoms, causing significant long-term sequelae, and high mortality.

Our current knowledge of the henipaviruses results from remarkable collaborative, long-term efforts of the scientific community, including many of the participants at Nipah@20. WHO R&D Blueprint has contributed to developing the key R&D priorities in the fight against Nipah, by building on these collaborations and bringing together experts from around the world.

During the third Nipah outbreak in India, in May 2018, the WHO R&D Blueprint's coordinated approach led to quick identification of the most promising available therapeutic agent (the monoclonal antibody m102.4), and access to the agent within 48 hours. This case study demonstrates that proactive research planning before an outbreak allows for a quicker outbreak response, allowing for prompt implementation of potentially life-saving medical interventions.

Epidemic risk can only be addressed by accelerating global preparedness, with an integration of research into all of our preparedness and response activities. This conference represents a unique opportunity to share knowledge, and to lay the groundwork for a clear and coordinated research agenda for Nipah, at the regional and national levels.

Message from NIAID

Dr. Cristina Cassetti, Deputy Director, Division of Microbiology and Infectious Diseases, NIAID, NIH

Emerging infectious diseases have been an important research priority at the U.S. National Institute of Allergy and Infectious Diseases (NIAID) for years. The aim is to gain a better understanding of these pathogens and why the emerge, and to develop countermeasures such as diagnostics, therapeutics, and vaccines. With substantial biodefence funding from U.S. Congress, the institute has been able to support Nipah research since the early 2000s, contributing to important advances such as identification of the receptor used by NiV for cellular entry and the development of animal models to be used in the evaluation of vaccines and therapeutics.

NIAID has been involved in the development of therapeutic and vaccine candidates, some of which have now been transferred to CEPI and other partners for further development. The Nipah programme of NIAID remains very active, with about 17 currently ongoing extramural programmes and a number of internal programmes focusing on the development of vaccine candidates, as well as studies on the pathogenesis of NiV using non-human primates (NHP). At Nipah@20, NIAID hopes to reconnect with old friends and partners, as well as to learn about new advances in the field of NiV research.

Welcome Address

Professor Thomas Coffman, Dean of Duke-NUS Medical School

Emerging infectious diseases constitute an important part of the research portfolio of Duke-NUS, and Professor Linfa Wang, who is the co-chair of Nipah@20, runs the Duke-NUS emerging diseases research programme. Duke-NUS is very excited to bring this conference to Singapore, partly because this was where the first Nipah outbreak happened. At the conference venue, there is a storyboard with news clippings from the first outbreak, clearly depicting the terror the disease caused when it first emerged.

Another reason why Singapore is a suitable location for such a conference, is the many travellers that come through its airport, as well as its large volume of shipping. Singapore was very significantly affected by SARS, and emerging diseases represent a topic for concern to authorities as well as the public. Nipah@20 brings together experts from all over the world, and highlights the importance of facilitating global communication between experts from different disciplines such as epidemiology, surveillance, basic science, vaccine development, and clinical treatment. Nothing can replace face-to-face discussions among experts in bringing the field forward.

Inaugural Address

Dr. Richard Hatchett, CEO of CEPI

Nipah virus and the related henipaviruses are some of the world's most frightening causes of disease. WHO has included these as one of 10 pathogens on its list of priority pathogens, since the epidemic potential of henipaviruses poses a grave risk to public health, and because there are no effective countermeasures.

It is highly appropriate that Nipah@20 convenes in Singapore, a beacon for globalism and a city that embodies the modern economic order. When Nipah first emerged in Malaysia, it rapidly spread to Singapore. In the last few decades, other frightening diseases like SARS, Ebola, and MERS have fared similarly, spreading from rural areas to densely populated cities, where the damage they can cause is amplified immensely. No city is more globally interconnected, and thus at greater risk, than Singapore.

Nipah has demonstrated its global potential, with recent outbreaks in Kerala, India – more than 3,000 kilometres (km) from Singapore, and more than 2,000 km from Bangladesh and Eastern India, where other recent outbreaks have occurred. As a result, more than 2 billion people live in areas at risk for Nipah. Unless something is done, Nipah will continue to re-emerge in smaller outbreaks, until it finds the perfect combination of circumstance and opportunity to explode again, as it did some 20 years ago.

CEPI is delighted to co-host Nipah@20 with the Duke-NUS Medical School, WHO, and NIAID. Meetings like this are important, not only because of the opportunity to share knowledge, but because of the momentum created in addressing the threat of Nipah, so that it does not become the next Ebola. In addition to being the 20th anniversary of Nipah, 2019 marks 50 years since the discovery of Lassa fever, and one of the inspirations for Nipah@20 was found in a similar meeting on Lassa fever in Abuja, Nigeria, in January 2019.

Lassa fever has haunted physicians in West Africa for half a century, occasionally flaring up in explosive outbreaks, and likely causing thousands of deaths. The burden of disease has not been known, due to poor or non-existent surveillance systems. In recent years, however, the Nigeria Centre for Disease Control, in collaboration with WHO and other international partners, has been able to draw more attention to the disease and establish Nigeria's research priorities for Lassa fever. Through the Lassa meeting in January 2019, where several hundred physicians, scientists, and public health officials participated, concerted and coordinated action against the disease was established. Subsequently, CEPI and WHO have helped Nigeria and other affected countries establish a network to support important epidemiology studies, which will lead to the design of better and more efficient clinical trials of vaccines. CEPI has also helped move the two first Lassa fever vaccines into clinical trials, with more to follow. The world has woken up to Lassa, and begun to move to take this threat off the table.

CEPI embraces the notion that the countries at greatest risk of the WHO priority diseases should be the ones to set the research agenda for these diseases. Thankfully, more than 100 scientists, public health officials, and policy makers from countries at risk of Nipah have registered for Nipah@20.

Another inspiration for this conference was the major progress the world has made against Ebola over the last year. While Ebola remains a frightening and disruptive threat, a clinical trial conducted in the Democratic Republic of the Congo for a therapeutic against Ebola was recently terminated early due to positive results. Even more recently, Merck received conditional marketing authorisation from the European Medicines Agency (EMA) for its vaccine. Within the last week before Nipah@20, GAVI announced that they will open a new funding window to establish a global emergency stockpile of Ebola vaccines. Low- and middle income countries will be able to access the vaccine free of charge, and will receive support for the operational costs of vaccination campaigns. As a result, we are approaching the day when Ebola is no longer the terrifying disease that it has been for the last 40 years.

It has taken tremendous effort by countless people to get the results seen with Ebola, but the demonstration that we can move from outbreak response to a global R&D effort that results in a fully accessible stockpile of licensed vaccine is a major victory for global health. This experience provides a roadmap for emerging infectious disease preparedness, where CEPI and the other partners present at Nipah@20 will play a critical role. Beginning today, we can do the same thing for Nipah. We can defeat Nipah, but only if we come together as a community and coordinate our efforts. The triumvirate of capabilities, cooperation, and commitment mentioned by Dr. Lam will be essential to any progress against Nipah. Fostering these was the intention with Nipah@20, and CEPI is confident that the participants will rise to the challenge.

SESSION 1 – Historic review of Nipah outbreaks

Chair: Dr. Marie-Pierre Preziosi, WHO

Overview of outbreaks

Professor John Mackenzie, PathWest & Curtin University, Perth

Outbreaks of henipaviruses, notably Hendra virus (HeV) and NiV, have affected Australia and several countries of Southeast Asia over the past two to three decades (see Figure 1). Outbreaks of HeV have been limited to the east coast of Australia, whereas NiV outbreaks have been found in Malaysia, Singapore, Bangladesh, India (West Bengal and Kerala), and the Philippines. Whereas HeV uses horses as intermediate host, NiV infects pigs. One exception was in the 2014 outbreak in the Philippines, where ten horses were found to be infected by NiV, and culled.

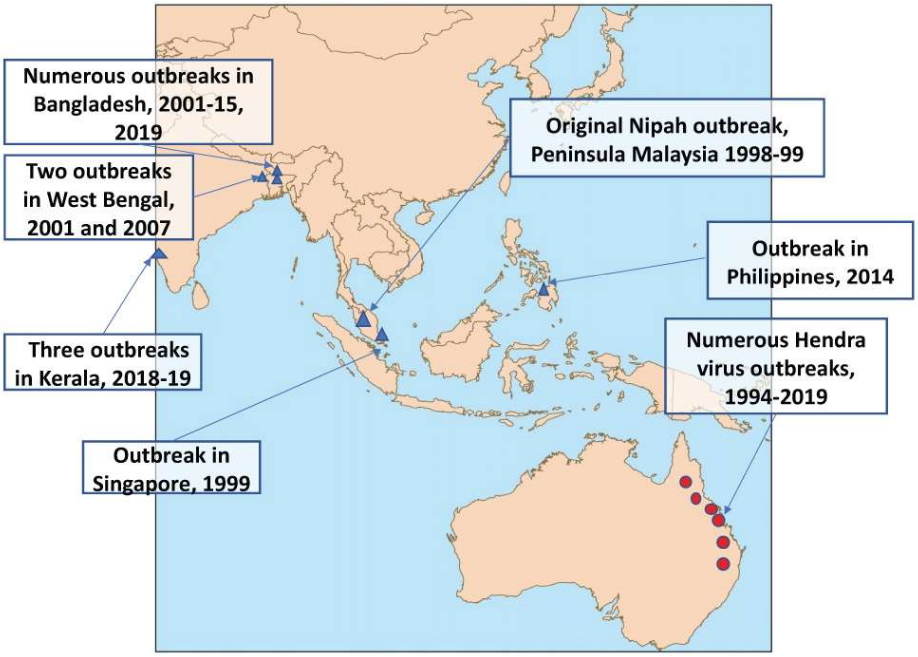


Figure 1. Map showing approximate sites of Nipah and Hendra outbreaks (Prof. John Mackenzie)

The Nipah outbreak in Malaysia and Singapore in 1998-99 was first believed to be caused by Japanese encephalitis. People were vaccinated and premises and surrounding areas fogged, but to no effect. The outbreak first started in Perak state, and later moved about 200 km south to the more intensive pig farm areas of Negeri Sembilan and Selangor, through the movement of infected pigs. During this outbreak, there was no communication between clinicians and veterinarians – an aspect that is now considered very important to the management of zoonotic diseases, as is clear from the widely used One Health approach. Other lessons learned were: 1) a previously unknown pathogen could emerge from a wildlife source at any time, in any place, and without warning, to threaten the health, well-being, and economy of a country – or even globally; 2) there is a clear need for countries to have the capability and capacity to maintain an effective alert and response system to detect and quickly react to outbreaks of international concern, and to share information about such outbreaks rapidly and transparently; 3) responding to novel disease threats of possible international concern requires global cooperation and global participation.

Australia

Dr. Hume Field, Science & Policy Advisor, EcoHealth Alliance & University of Queensland

The Brisbane suburb of Hendra was close to racing tracks, and there are a number of small training stables in the area. In August of 1994, after a trainer brought a sick horse to his stable to better care for it, there was an outbreak of acute respiratory disease, and a cascade of cases that, within a week, saw 13 horses dead, and the trainer and a stable hand in hospital. The trainer died whereas the stable hand recovered. This was an unprecedented event, all the stables were quarantined, and the movement of horses in South East Queensland was brought to a standstill.

Within a week, a novel paramyxovirus was identified by the Australian Animal Health Laboratory. When inoculated into a healthy horse, it caused the same acute, rapidly progressing, fatal infection that was observed in the Hendra stables. While initially named Equine morbillivirus, it was later found that the new virus did not fit into any existing genus. In the subsequent years, there have been nearly 100 equine cases and 7 human cases. The case fatality rate (CFR) for horses approaches 90 %, and in humans 60 %. Both respiratory and neurological presentations are recorded in horses. An effective vaccine has been available for horses for over five years, but is still not widely used due to concerns about cost and exaggerated reports of side effects. Equine cases of Hendra continue to occur almost annually.

Extensive wildlife studies have shown that species of pteropid fruit bats, or "flying foxes", are the natural reservoir of Hendra virus. Infection prevalence fluctuates in these populations, and urine is the primary route of viral excretion. Transmission to horses is likely caused by ingestion of urine-contaminated pasture, or direct inoculation of mucous membranes while horses are grazing under trees where flying foxes are feeding. All human cases have had direct contact with infected horses; direct transmission from bats to humans has not been recorded, and neither has human-to-human transmission.

The multiple similarities between HeV and NiV, and Dr. Field's role in the identification of bats as the natural host of HeV, led to his joining the Nipah outbreak veterinary response team that was assembled by the Malaysian government in early 1999. Epidemiologically, it appeared that pigs were the source of Nipah infection in humans, and the first task of the team was to perform pig necropsies at "hot" farms and collect tissue samples. They were able to isolate the same virus that caused human infection from multiple pigs, at multiple farms. The work then shifted towards gaining a better understanding of the nature of infection and transmission in pigs. It was clear that not all pigs were affected at "case" farms, and they therefore sought out "recovered" farms to serologically survey different classes of pigs, and collect convalescent serum. Parallel to these investigations, the mass culling of pigs was underway. The urgency and logistics of culling meant that the animals were typically killed and buried on the farm, which frequently involved the destruction of pig sheds and other farm infrastructure.

Two other components of the veterinary response were the investigation of infection and/or disease in non-pig species, and the origin of infection in pigs. Horses, dogs, and cats were identified as incidental hosts. As with Hendra, serological studies identified species of local bats as the likely source of infection in pigs, and the reservoir of the virus in nature. Subsequent studies have confirmed this, and further shown that henipaviruses are widespread in many bat populations, and likely have an evolutionary association with bats. While there may have been deficiencies in the early response to the Nipah outbreak in Malaysia, at this stage the Malaysian government had established collaborative and coordinated efforts to control the outbreak, in a One Health perspective.

Nipah virus outbreak in Malaysia

Dr. Chua Kaw Bing, Sr. Principal Investigator, Temasek Lifesciences Laboratory

One believes that NiV was infecting pigs and humans even prior to the 1998-99 outbreak in Malaysia, though the cases were never picked up on. There were reports, prior to the outbreak, of pigs coming down with encephalitis, which were assumed to be afflicted by "classical swine fever". Once transmitted to humans, public health authorities suspected an outbreak of Japanese encephalitis (JE), which was confirmed by what was later found to be false positive test results (found in about 20 % of the patients) for circulating JE IgM and viral particles. The Malaysian MoH launched an intensive campaign with JE vaccination and fogging of pig farms and surrounding areas to eradicate the supposed JE mosquito vector. When the outbreak persisted, in spite of the authorities' efforts, the possibility of a novel pathogen was finally considered.

The first direct observation of NiV was made on March 15, 1999, by electron microscopy of a sample section made by Dr. Bing, with the help of the U.S. CDC at Fort Collin. At U.S. CDC Atlanta, the same sample preparation later tested positive to Hendra antibodies in an indirect immunofluorescence assay, and it was thus determined that the outbreak was caused by a Hendra-like virus. Final identification of the virus was achieved by molecular characterisation (amplification of a 120 base-pair fragment of the Phosphoprotein gene by reverse transcriptase polymerase chain reaction (RT-PCR), followed by nucleotide sequencing) at U.S. CDC Atlanta. The new virus was named after Kampung Sungai Nipah, the village were it was first discovered.

Already with the first electron micrographs, it became clear that the Malaysia outbreak was not caused by JE, and Malaysian public health authorities were able to implement more appropriate control measures. Malaysia paid dearly for the inaccurate and untimely diagnosis, with 265 patients suffering from acute viral encephalitis, and 105 fatalities. The local swine industry also suffered great losses; about 1 million pigs were culled, and 800 pig farms were demolished.

The 1999 Nipah outbreak in Singapore

Professor Leo Yee Sin, Executive Director, National Centre for Infectious Diseases

The Singapore Nipah outbreak was, in fact, an integrated part of the Malaysia outbreak, yet much more limited in time and space. At the time, there were two abattoirs in Singapore, and the outbreak quickly resolved when the import of live pigs from Malaysia was banned, and the abattoirs closed. The eleven cases of the outbreak, of which there was only one fatal case, were all identified within a period of 9 days in March 1999. All of them had had close contact with live animals. In the Singapore outbreak, there was no human-to-human transmission, and all of the abattoir and healthcare workers screened after the outbreak tested negative for NiV antibodies. It was estimated that about 50 % of the infections were asymptomatic.

In spite of being limited in nature, the Nipah outbreak in Singapore provided several important lessons that have later proven useful, for example in the context of the Singapore SARS outbreak in 2003. Key lessons were to facilitate collaboration across borders, as well as to take a multidisciplinary, One Health approach to infectious disease prevention and control.

Nipah Encephalitis in Bangladesh

Professor Mahmudur Rahman, Consultant, Programme for Emerging Infections, icddr,b

Nipah was first identified as the cause of an outbreak of encephalitis in 2001 in the Meherpur district of Western Bangladesh, though the link to Nipah was discovered three years after the outbreak, in 2004. In 2006, Bangladesh established its Nipah surveillance system and laboratory. Since then, a total of 313 cases have been identified, of which 226 (72 %) have died. Looking only at the outbreaks, and eliminating independent cases, 37 outbreaks have led to 221 cases and 171 deaths, resulting in a very high CFR of 77 %. Many of the outbreaks have been small and localised, often limited to individual households and frequently resulting in 100 % mortality of cases.

Among the 64 districts of Bangladesh, 31 have been affected by Nipah. The affected districts may be said to form a "belt" concentrated in West Bangladesh. The majority of cases are children and young adults. Among the cases, about 60 % report to have consumed date palm sap. The sap is collected overnight, and bats have been found to contaminate the sap by urination.

Nipah outbreak investigations are conducted in a systematic way and with a One Health approach, involving epidemiologists, veterinarians, and anthropologists. In most cases, transmission is successfully traced back to its source, which has often been found to be the ingestion of raw date palm sap. The second most common route of transmission is human-to-human. There is a seasonality to outbreaks, with more outbreaks occurring from December to May. The median incubation period is eight days, and about 35 % of cases are secondary cases.

India

Dr. Nivedita Gupta, Scientist F, Division of Epidemiology & Communicable Diseases, ICMR

Whereas the first Nipah outbreak in Malaysia and Singapore did not involve human-to-human transmission, the outbreaks in India and Bangladesh in 2001 both did. The 2001 outbreak in Siliguri, West Bengal, India, represents the biggest Indian outbreak to date, involving 66 confirmed cases and 45 deaths (CFR: 68 %). Subsequent outbreaks have occured in Nadia, 2007 (5 confirmed cases, 5 deaths – CFR 100 %), Kozhikode, 2018 (19 confirmed cases, 17 deaths – CFR 90 %), and Ernakulum, 2019 (1 single case – CFR 0 %). Surprisingly, the two latter outbreaks were both in Kerala state, at the opposite side of the country as compared to the first two outbreaks.

During the 2001 outbreak, the Indian healthcare system was unable to diagnose Nipah, and instead mistook it for measles infection, since these are related viruses. With the help of U.S. CDC, they were able to confirm cases of NiV infection, but this took a long time. By the time of the 2007 outbreak, diagnosis had been established, with serological and molecular tests provided by the U.S. CDC. At this time biosafety level (BSL)-3 labs were available, yet early detection failed. In 2015, India had one operational BSL-4 laboratory, and capacity for bat surveillance was established. During the 2018 outbreak, early diagnosis and containment was available, though the index case was not correctly diagnosed. Finally, in the 2019 outbreak, the index case was quickly diagnosed and contained, and the outbreak stopped there.

For the two first outbreaks of 2001 and 2007, the origin of infection remains unknown. However, for the Kozhikode outbreak in 2018, *Pteropus* bats have been identified as the source. Since there is no tradition for palm sap consumption in Kerala, one does not know how transmission happens, though the close similarity of viral gene sequences of viruses isolated from cases and local bats clearly suggests that the outbreak originated with the bats.

Philippines

Dr. Fidel Malbas Jr., Research Institute for Tropical Medicine

Following a preliminary investigation into a cluster of deaths in the south of the Philippines, the Philippine government requested technical assistance from the WHO representatives to the Philippines on May 12, 2014. A multidisciplinary team investigated further, and clinical, epidemiological, and laboratory findings indicated an outbreak of a viral infection lasting from March to May. Seventeen cases were identified, displaying symptoms compatible with henipavirus infection. Eleven of the cases had acute encephalitis, and nine of them died.

Epidemiological data suggest that the primary route of human infection was through direct exposure to body fluids from infected horses. At least five cases were considered infected by direct human-to-human transmission, presumably through infected respiratory secretions or contact with other bodily fluids from sick patients. Laboratory results include serological evidence of exposure to henipavirus in recovering or fully recovered patients. Importantly, this is the first time a henipavirus has been detected in humans and domestic animals on the Philippines. The environmental source of infection has not yet been firmly established, but evidence points to the Megabats as a possible reservoir.

Recommendations for further actions, aiming to reduce transmission between bats, horses, and humans, are as follows: 1) Focus on prevention and control of infection in healthcare settings; 2) Use personal protective equipment (PPE) and ensure high levels of hygiene among healthcare workers, butchers, and people dealing with horses; 3) Avoid slaughter and consumption of sick horses; 4) Strengthen surveillance of human and animal cases; 5) Conduct further testing on clinical specimens; 6) Study the bat reservoir host; 7) Develop coordinated response plan between human and animal health agencies; 8) Ensure flow of information from the Department of Health to regional authorities in human and animal health, as well as agriculture.

Session 1 Q&A

Q: It was estimated that 50 % of Nipah cases in the 1999 Singapore outbreak were asymptomatic. Is this not unusual?

A: Thirteen asymptomatic cases were detected as part of the serological screening of abattoir workers. Although they had not had any symptoms, magnetic resonance imaging (MRI) conducted on some of the cases revealed that they had the hyper dense brain lesions typical for Nipah encephalitis. In India, serological surveys among close contacts of Nipah patients from the Kerala outbreak showed seropositivity in only two of about 300 subjects, suggesting a very low proportion of asymptomatic cases. In Bangladesh, four out of 212 subjects exposed to sap and/or bats tested positive for circulating antibodies against NiV. In the first HeV outbreak, 13 horses died, 4 had mild symptoms but were euthanised, and 3 tested positive for circulating HeV antibodies without any preceding signs of disease.

Q: Can asymptomatic cases be a source of infection?

A: There have been no reports of that, so far.

SESSION 2 – WHO Nipah R&D Roadmap

Chair: Dr. Christina Spiropoulou, CDC

WHO Nipah Research and Development Roadmap

Professor Steve Luby, Stanford University

Professor Luby presented the WHO Nipah R&D Roadmap (view draft here) as a member of the taskforce involved in preparing it. The purpose of the Roadmap was *to provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritising areas and activities for accelerating the collaborative development of* ***medical countermeasures*** *against Nipah virus infection*. The Roadmap has three big development priorities: 1) Rapid and accurate point-of-care diagnostics; 2) Safe and effective treatment and post-exposure prophylaxis; 3) Safe and effective vaccines.

In the Roadmap, three important issues were identified: 1) The market failure to create medical countermeasures; 2) The centrality of improved Nipah diagnostics; 3) Promise and barriers for Nipah therapeutics. On the positive side, the technologies required for developing effective counter-measures to Nipah are available, and NiV seems to be a fairly easy pathogen to deal with. The reason why effective countermeasures are still not in place, is a lack of incentives for private industry to develop the products: There are few people affected, typically in rural and low-income areas, and when the focus is on prevention of disease, rather than treatment of annual cases, the standard measures of cost-effectiveness are not met.

While the impact of a Nipah epidemic would be high, the probability is low: Over the past two decades, several outbreaks have happened, but sustained human-to-human transmission in a larger geographic area remains to be seen. However, prudent actions should be guided by both probability and impact. Thus, the primary justification for public investment in NiV countermeasures is not the management of the smaller outbreaks that seem to occur on a regular basis, but the prevention of a pandemic where perhaps millions of people could be affected by an infection that has a CFR of 75 %. Importantly, however, cognitive biases discourage attention to low probability events, making them more difficult to prepare for. The low probability of a NiV pandemic creates a setting where the market fails to meet societal needs. This is especially true when obtaining the necessary countermeasures involves expensive research and development, with requirements for licensure, BSL-4 work, and the use of costly animal models.

In the interest of creating a sustainable business model for the development of Nipah medical countermeasures, the Roadmap considers several supportive actions: 1) Public/philanthropic funding for R&D; 2) Clarifying regulatory pathways; 3) Assessing market size. For the latter, important insights were: 1) Increased surveillance would increase demand for diagnostics; 2) Treatments that can be used to treat several pathogens will have a stronger business case; 3) Demand for treatments and vaccines will come from healthcare and laboratory workers, in addition to patients and people at risk;

4) Rotating stockpile and mass vaccination would result in stronger business cases. Moving forward, one strategic goal of the Roadmap committee is to *identify sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV medical countermeasures*. They are currently working on a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Improved availability and use of Nipah diagnostics can provide insight on the prevalence of human infection. The range of *Pteropus* bats covers areas inhabited by 2-3 billion people, who are potentially at risk of Nipah infection. Given the spread of confirmed human Nipah infections, many human cases are likely to go unrecognised. Better diagnostics would serve to 1) improve response; 2) increase visibility to decision makers; 3) improve risk forecasting. In the low-resource settings were Nipah typically occurs, the ability to distinguish between Nipah infection and other types of encephalitis (NiV accounts for less than 5 % of cases, even in the "Nipah belt" in Bangladesh) would be essential to guide therapy. However, there are several reasons for why realising widely available Nipah diagnostics is made difficult: 1) NiV is uncommon and occurs in poor communities; 2) Without effective therapy available at the medical facility, there is no clinical justification for testing; 3) The testing of NiV comes with biosafety concerns; 4) There is limited availability of clinical samples for assessing new products. Thus, from a business case perspective, a successful diagnostic requires a successful therapeutic, and vice versa. In order to facilitate further development of NiV diagnostics, the Roadmap committee has defined the following prioritised activities: 1) Create a virtual repository of clinical reference samples for use in R&D; 2) Generate a target product profile (TPP) for NiV diagnostics; 3) Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk.

Several promising Nipah therapeutic agents are in development, of which m102.4, fusion inhibitory peptides, favirpiravir, and GS-5734 are but a few. However, patients generally present late in the course of illness, and they often present to facilities with limited capacity, commitment, and practice in diagnostics. Thus, effective treatment requires more than just making a therapeutic agent available. The Roadmap has defined the following strategic goals regarding therapeutics: 1) Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks; 2) Develop and evaluate therapeutic agents for the treatment of NiV infection and for post exposure prophylaxis to prevent NiV infection. With CEPI on board, the Roadmap committee has been less concerned with vaccine development. However, the following strategic goals have been developed for vaccines: 1) Engage national regulatory authorities and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure of a vaccine against NiV; 2) Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

These are exciting times, and people are now understanding that the low probability, high impact event of a pandemic NiV outbreak is worth preparing for. Promising technologies are in development for diagnostics, therapeutics, and vaccines against NiV. Currently, the issues that need the most attention are: 1) Development of a sound business case to support product development; 2) Enabling work within the constraints of sporadic cases / outbreaks in settings with limited resources. These are crucial for succeeding with the development of medical countermeasures against Nipah in the next 10 years.

WHO Nipah R&D Roadmap – Monitoring its implementation

Dr. Marie-Pierre Preziosi, WHO R&D Blueprint (on behalf of Dr. Claudia Nannei, WHO Monitoring & Evaluation)

The WHO R&D Blueprint efforts focus on both **research preparedness** and **research during outbreak response**. The aim of the R&D Blueprint is to fast-track the development of effective diagnostics, therapeutics, and vaccines to respond to outbreaks effectively and to prevent them. The R&D Blueprint preparedness activities are organised around three main approaches: 1) Improving coordination and fostering an enabling environment; 2) Accelerating R&D processes; 3) Developing norms and standards tailored to the epidemic context.

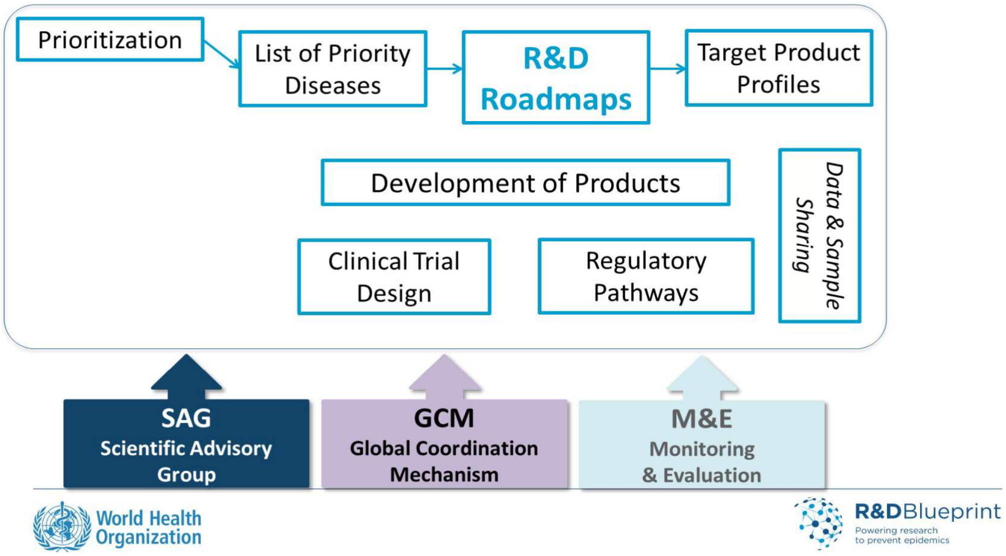


Figure 2. Overview of the WHO R&D Blueprint (WHO)

On an annual basis, WHO R&D Blueprint update their list of prioritised diseases, and for each of the diseases listed, R&D Roadmaps are prepared (see Figure 2). When relevant, TPPs are prepared to guide the development of products. Furthermore, WHO R&D Blueprint take part in developing clinical trial designs for each pathogen, and address the regulatory pathways. They are governed and receive input from a scientific advisory group (SAG), and through a global coordination mechanism (GCM). The monitoring and evaluation (M&E) is important to ensure assessment of progress and observation of any changing circumstance, thus allowing the accomplishment of strategic goals and the realisation of the vision.

The R&D Roadmap needs to be clearly and concisely defined and must, to remain relevant and useful, represent a living document that is monitored and updated regularly. The document itself is not enough – the true measure of success is whether or not the R&D Roadmap is implemented and achieves the desired outcome. To help in this, it is proposed that the R&D Roadmap sets 1) a results chain for each of the strategic goals, implementable over a limited period of time and verifiable in the form of milestones; 2) a framework for monitoring, using performance indicators; 3) regular (mid-term) reviews for assessing risks and assumptions in the critical path towards implementation.

A draft R&D Roadmap has been published for Nipah, with invitations to comment, and the final version is expected to be published shortly – on the WHO website, as well as in a scientific journal.

Session 2 Q&A

C: In the context of Nipah outbreaks, with such short duration, it is important to strengthen clinical research capacity to the extent where it is available on demand – if outbreaks are to be prevented (described in the "Money & Microbes" report from The World Bank).

A: Response from Prof. Steve Luby: Given the wide spread of NiV, it is hard to predict where the next outbreak is going to be. It will require an investment that may not be efficient, but could be resilient in terms of capturing all emerging cases and allow for data capture to serve the development of diagnostics as well as therapeutics. Definitely an important issue.

Q: Do you have any examples of products that have successfully overcome the low probability high impact context?

A: Response from Prof. Steve Luby: Outside of the U.S. and Milton Friedman's "free market", market failures are real. There is a role for the State in funding projects that lack financial interest from private investors. One example is NASA's tracking of asteroids to prevent asteroids from causing serious damage to the Earth. Solutions exist, even if the private market fails to address them, but involvement of the State is likely to be necessary.

Q: How can we bring scientists together in closer collaboration, in between meetings like this, to be able to address the different market needs more effectively?

A: Response from Prof. Steve Luby: CEPI is a good example, for having put together a large coalition of the different players needed in the development and implementation of new vaccines. They have both public and philanthropic funds available that serve to de-risk the investments of private investors. It is hard to envision a mechanism that addresses the markets in the way asked, if it doesn't share the holistic and strategic approach adopted by CEPI. An integrated approach across affected geographies, as well as across the various components of diagnostics, therapeutics, vaccines, surveillance, and emergency response, is likely to lower overall costs and make the process more efficient.

SESSION 3 – Progress and challenges in surveillance of NiV

Dr. Peter Daszak, EcoHealth

Southeast Asia is a hotspot for EIDs, as demonstrated in two recent publications. The risk of novel emerging zoonosis is high, and wildlife in the area is brimming with potentially zoonotic viruses. This is why there is such a focus on Southeast Asia and Nipah infection: While NiV is a frightening, lethal virus, other viruses that have not yet emerged may prove worse.

For us to be able to prepare for future EIDs, it is vital to discuss surveillance, and a session dedicated to surveillance at Nipah@20 is very welcome.

Nipah surveillance and outbreak response in Bangladesh

Professor Mahmudur Rahman, Programme for Emerging Infections, icddr,b

Following the first Bangladesh Nipah outbreaks in 2001, 2003, 2004, and 2005, the country established its Nipah surveillance system and laboratory in 2006, in a collaboration between Institute of Epidemiology, Disease Control and Research (IEDCR) and the U.S. CDC. So far, Bangladesh is the only country to have implemented dedicated Nipah surveillance. The surveillance system was originally established as a joint JE and Nipah surveillance system (the Acute Meningo Encephalitis Surveillance system, AMES), with 11 sites across the country. Currently, there are five active and two passive sites, with an additional 47 sub-district- and 3 district hospitals added during Nipah season. In addition to surveillance at the sites, IEDCR operates a 24/7 hotline, nationwide media monitoring, and follows up on any informal reports.

IEDCR distinguishes between probable and confirmed cases, where probable cases are linked to deaths in a Nipah-affected community around an outbreak, where sampling was not possible. In total, there have been 313 cases since the surveillance started, and 226 (72 %) deaths. Surveillance case definitions used in Bangladesh are: 1) Axillary temperature > 38.5°C AND evidence of acute brain pathology [*e.g.*, altered mental status, seizures], or 2) Axillary temperature > 38.5°C AND illness < 7 days AND severe shortness of breath [*i.e.*, dyspnoea prevents patient from walking unassisted for 10 steps] AND chest radiograph consistent with diffuse acute respiratory distress syndrome. In addition to fulfilling at least one of the two definitions, an epidemiological link is always sought after. This can be geographic location close to an outbreak, that the time of year is within the Nipah season, or recent consumption of date palm sap. A cluster of Nipah cases is defined as at least two cases within a walking distance of 30 minutes, and with clinical onset within 21 days of each other. All suspected cases are investigated, but clusters of cases receive immediate attention and are prioritised.

The surveillance procedure starts with the reporting of a suspected case or cluster of cases from one of the surveillance sites (see Figure 3), and ends with laboratory-based diagnosis. Some tests are performed by IEDCR and some by the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). The U.S. CDC, Atlanta, conducts quality controls. Outbreaks are evaluated in a One Health approach, including epidemiological investigations, animal studies, environmental contamination assessment, behavioural studies, and case control studies.

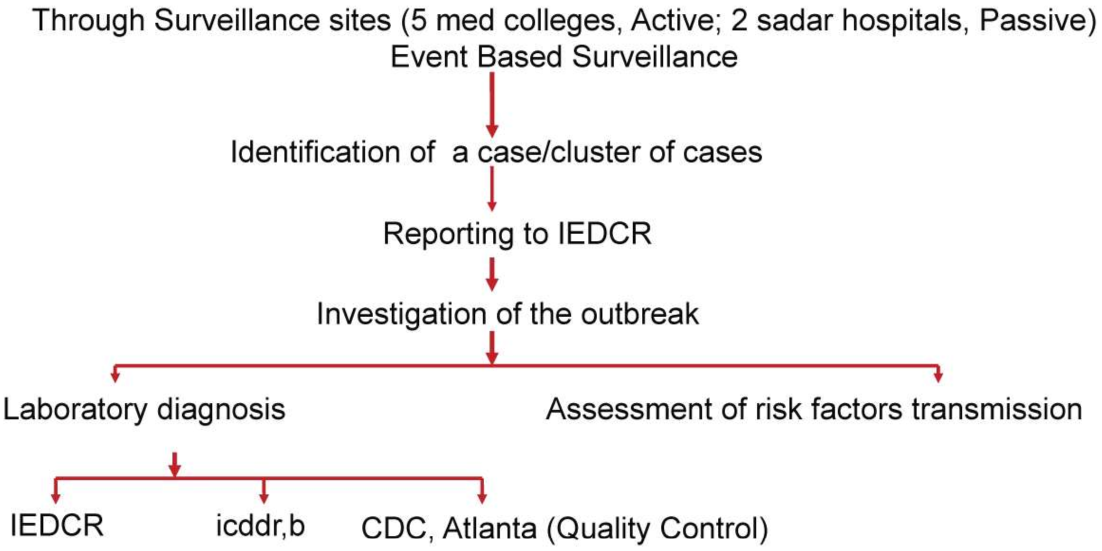


Figure 3. Nipah detection in Bangladesh (Dr. Mahmudur Rahman)

Immediately after a case has been identified, all contacts are listed and accounted for. Symptomatic contacts are subjected to a Nipah IgM test, and all contacts receive weekly follow-up for up to 21 days. All contacts are interviewed for relevant details and contact history. At the end of the follow-up, contacts are tested for Nipah IgG.

Nipah virus dynamics in bats and implications for zoonotic spillover to humans

Dr. Jon Epstein, EcoHealth Alliance

Part of understanding the probability of outbreaks, is understanding how NiV operates in its natural reservoir, and understanding the risk of spillover. Given the wide distribution of the reservoir host, the *Pteropus medius* bat, why are we not seeing more outbreaks?

We should be prepared for NiV outbreaks anywhere where the bats and virus coexist. As such, the Kerala outbreak should not have been a surprise, in spite of the long distance from other outbreaks. The outbreaks in Bangladesh and Malaysia are linked by the fact that, in both places, the source of infection was a human-provided (anthropogenic) source of food. In Malaysia it was a mango orchard planted next to a pig enclosure, in Bangladesh it was the date palm sap. The bats thrive in the company of humans, and through human harvesting of date palm sap during the winter months, they have been able to access a food source that would otherwise not have been available to them – in a time when food is otherwise scarce. As a result, humans are creating opportunities for the Nipah virus to spill over.

In a study of viral ecology, Dr. Epstein and colleagues are trying to answer two questions: 1) Is there a spatial difference in infection patterns in Bangladesh, between the "Nipah belt" and the rest of the country?; 2) Are there seasonal infection patterns? Results were collected over a period of 8 years, from 2006 to 2014, and a publication is currently in review. Although seroprevalence varied, the team found no difference in seroprevalence inside and outside of the "Nipah belt". Traces of NiV infection were found in all of the eight different bat colonies studied. In Faridpur, one bat colony was followed longitudinally, with sampling every 3 months to follow seroprevalence over time. This resulted in a cyclical pattern, where levels of circulating antibodies waxed and waned. As a rule, circulating antibodies do wane over time, and it is believed that the waxing was in response to active viral transmission in the colony. Modelling suggested that bat epidemics occurred biannually, that it was driven by adults (not births, as has been suggested), and that the population density was an important determining factor.

The Bangladeshi bats are less mobile than the Malaysian bats, but sufficient evidence exists to suggest that the different colonies are still connected, creating a metapopulation of bats where viral strains may circulate. In fact, there is no genetic distinction between different colonies across Bangladesh. There is even evidence of hybridisation with other bat species, such as *P. lylei* of Myanmar and *P. vampyrus* of Malaysia. Considering the genetic diversity of viral strains within a colony, there seems to be little variation. However, genetic variation between colonies at different geographic locations is considerable. Due to the mobility of the bats, strains typically found in one location may be transferred to another, contributing to the overall genetic diversity of NiV.

A recent study on the evolutionary history of NiV, based on all available genetic sequences, suggests that NiV originally migrated from India to Bangladesh in 1941, spread within Bangladesh, and then migrated back to India, including Kerala. The *P. medius* bat is common throughout the Indian subcontinent, and the Nipah virus travels with it. Nipah or related henipaviruses have also been found in many domesticated animals, including cattle, goats, and pigs. One mode of transmission between bats and domestic animals could be the feeding of animals with fruit bitten by bats. The mode of transmission causing the Kerala outbreak in humans is still unknown.

Considering the wide distribution of bats and NiV, improved surveillance systems are necessary to better understand where there are potential hotspots for spillovers.

Survey of Pteropus *bats during Nipah outbreak in India revealed its association*

Dr. Pragya Yadav, National Institute of Virology, ICMR

Only four human outbreaks of Nipah infection have so far occurred in India, yet the *Pteropus* bats serve as a reservoir for the virus. Cues for the ongoing risk of a new outbreak may be found in the bats, so surveys of the bats are conducted regularly. In the laboratory, samples are handled at the BSL-3 level. In the field, however, some of the security measures that are otherwise found in the lab, such as directional airflow and HEPA filtration, will be missing. Thus, field work involves careful preparations including training, health measures, and risk assessments.

Capacity for bat surveillance in India was first established in 2011, yet a permanent surveillance system still does not exist. The first report to confirm the presence of NiV RNA in *P. giganteus* in India was published in 2012, following a survey of bats in West Bengal. Dr. Yadav and her team collected samples from five different species of bats, and isolated viral RNA. In addition to NiV, they also found RNA from other viruses, some of which were isolated for the first time. None of the other viruses have so far been detected in humans, but the viral diversity observed in bats underscores their ability to serve as a natural reservoir for pathogens and, potentially, as the source of new EIDs.

During the 2018 outbreak in Kerala, NiV RNA was collected from humans and bats, and sequenced. A large proportion of the bats (23.07 %) proved to be infected with virus that was almost identical to the virus causing the outbreak (99.7-100 % sequence homology), thereby identifying *Pteropus* bats as the likely source of infection. Similarly, in response to the single transmission of NiV to a 21-year old college student in Kerala in 2019, five sites the patient had recently visited were surveyed for infected bats. Again, some of the bats were found to carry NiV, and 21 % were seropositive. The NiV strains from the 2018 and 2019 Kerala outbreaks were found to be closely related (97.9 % sequence homology) to several Bangladesh strains circulating at the time.

Effective surveillance is necessary for detection, response to, and prevention of Nipah infection. Currently, a nationwide Nipah virus survey in *Pteropus* bats is underway in India, with eight states (out of 28) and two union territories (out of nine) surveyed since January 2019.

Long-term One Health surveillance of Nipah virus in Thailand forewarns risk of outbreak

Dr. Supaporn Wacharapluesadee, Chulalongkorn University, Bangkok

Thailand is an at-risk country for a Nipah outbreak, due to the abundance of *Pteropus* bats carrying the virus, the country's proximity to Malaysia which has previously experienced an outbreak, and the large swine industry (though the pigs have so far tested negative for NiV). Since 2002, Thailand has developed its capacity for Nipah surveillance, as shown in Figure 5.

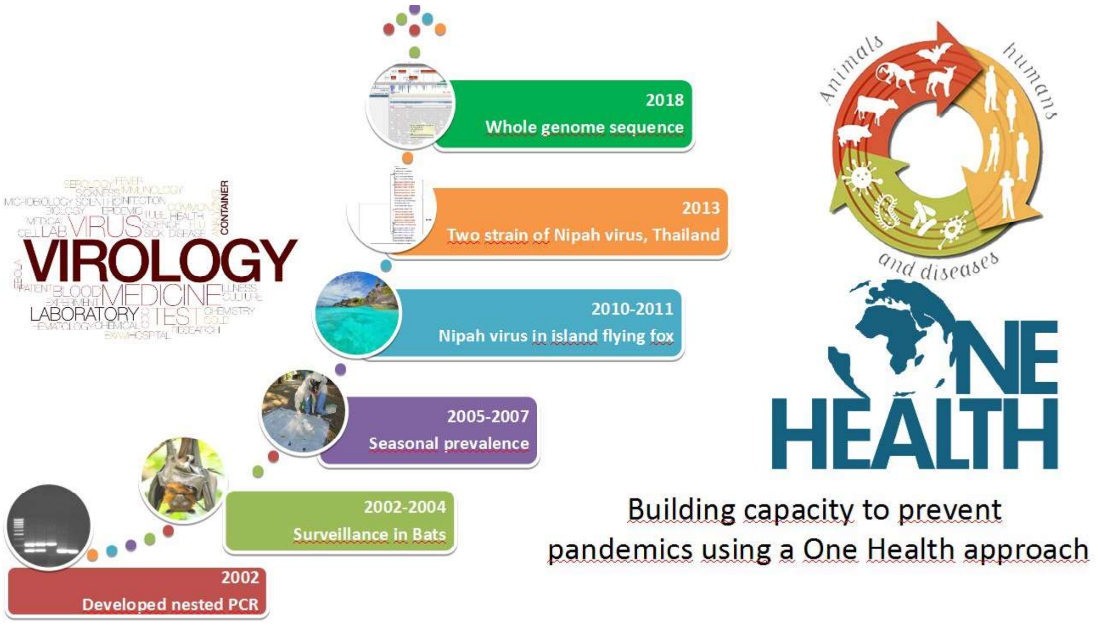


Figure 4. Thailand: Building capacity to prevent pandemics using a One Health approach (Dr. Wacharapluesadee)

In a large survey published in 2005, including six species of fruit-eating bats ($n = 1{,}041$) and six species of insect-eating bats ($n = 263$), Nipah viral RNA was found in urine and saliva from *Pteropus lylei* ($n = 76$, 9.3 %) and saliva of *Hipposideros larvatus* ($n = 1$). These results were followed by a longitudinal study at 7 sampling sites in central Thailand of the prevalence of NiV in *P. lylei* bats, to see if the viral prevalence was seasonal, as had been demonstrated in Bangladesh. Indeed, pooled urine collected on plastic sheets laid out underneath roosts were found positive for NiV RNA in the months of December through June. Later it was found that *Pteropus hypomelanus* living on the islands in South West Thailand carried Malaysian NiV, whereas the *Pteropus lylei* of central Thailand carried a NiV strain akin to the ones in Bangladesh. Different bat species → different location → different Nipah strain.

A longitudinal study in *Pteropus lylei*, lasting 17 years from 2002-2019, demonstrated the genetic stability of NiV: Among 119 NiV-positive pooled urine specimens collected throughout the study, sequence homology in a partial coding sequence of the nucleocapsid gene was 99.44-100 %. Whole genome sequencing of a NiV-positive Thailand bat revealed 99 % sequence homology to NiV isolated from a patient in Bangladesh in 2004, and 98 % sequence homology to NiV isolated from one of the patients from the 2018 outbreak in India.

In a One Health surveillance study of NiV in Thailand in 2012, bats ($n = 374$), pigs ($n = 248$), and humans ($n = 418$) were tested for NiV RNA and circulating NiV IgG. Among the bats, 2 % tested positive for NiV RNA and 10 % were seropositive. The pigs and humans were all seronegative. A public awareness campaign was conducted in relation to the study.

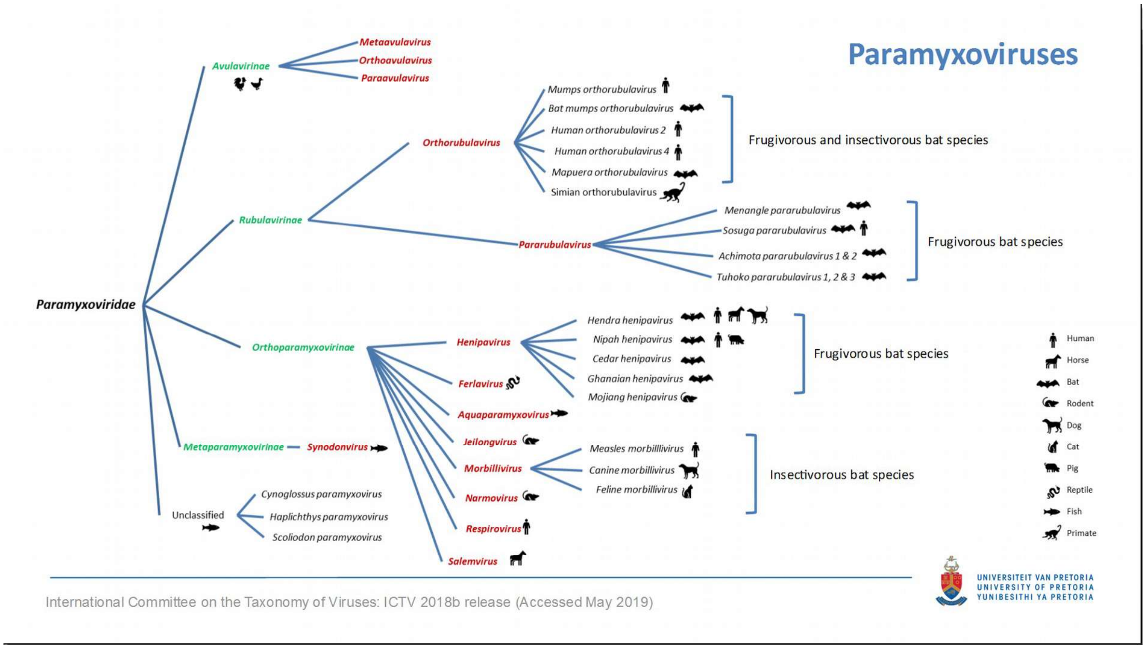
GPS tracking of *Pteropus lylei* showed that maximum linear distances between day roosts and foraging areas varied greatly between individuals, and ranged from 2.2 to 23.6 km. With this information, Dr. Prateep Duengkae has been able to draw Nipah risk maps for central Thailand, based on the estimated density and activity of bats.

Long-term One Health Surveillance of Nipah virus in Thailand forewarns risk of outbreak, for two main reasons: 1) *P. lylei* bats have tested positive at the same location for 18 years, indicating that *P. lylei* is a natural reservoir for NiV; 2) High sequence homology between NiV carried by Thai bats and the 2004 Bangladeshi patient indicates that virus circulating in Thailand is capable of infecting humans.

Henipavirus biosurveillance in South African bats –A research overview

Professor Wanda Markotter – Centre for Viral Zoonoses, University of Pretoria, South Africa

Nipah and Hendra viruses are not present in Africa, and neither are the reservoir *Pteropus* bats. However, one related virus was recently identified in Ghana and added to the henipavirus family under the name of *Ghanaian henipavirus* (see Figure 5). This virus is known to infect bats, notably fruit bats, and since fruit bats of different species are found all over Africa outside of Sahara, the virus is expected to be widespread. So far, henipavirus RNA has been detected in South Africa and several Central African countries.



International Committee on the Taxonomy of Viruses: ICTV 2018b release (Accessed May 2019)

Figure 5. Phylogeny of paramyxoviruses (ICTV, 2019)

There is very little evidence to support spillover of African henipaviruses, though one study has found antibodies to henipa-like viruses in domestic pigs in Ghana, and another found evidence of spillover into human populations. No outbreaks have been reported, and longitudinal studies are non-existent.

At a study site in Limpopo, South Africa, Prof. Markotter and her team study bats roosting in a local cave, with primary attention to the Egyptian fruit bat, *Rousettus aegyptiacus*. This is a cave-dwelling fruit bat species found throughout Africa. In pooled urine collected from the bats, a number of different viruses have been identified (by RT-PCR and next-generation sequencing [NGS]), of which some are henipaviruses and some are henipa-like viruses. Through monthly sampling over 14 months, it was found that virus excretion varied over time, with a characteristic peak in July on two consecutive years. This peak coincided with the breeding season of *R. aegyptiacus*, as well as the dry winter period (nutritional stress?) and the waning of maternal antibodies in juveniles (susceptible individuals). Other factors may also influence the observed excretion pattern.

In conclusion, the *Rousettus aegyptiacus* population in South Africa hosts a diversity of paramyxoviruses including henipaviruses. The detected viruses group closely with ones previously described from other African countries, either due to co-evolution or the forming of meta-populations. The first study reporting on the paramyxoviral excretion dynamics in *R. aegyptiacus* identified the dry winter as a high-risk period for potential paramyxovirus transmission and spillover.

Session 3 Q&A

Q: Prof. Markotter showed very nice peaks of excretion, up to 60 %. It seems this is very high in terms of nucleic acid detection?

A: Yes, in the peak seasons, 60-70 % of the collected samples can be positive. Keep in mind that these are pooled urine samples. Assuming the observed frequencies reflect the true prevalence, one explanation could be the high density of *R. aegyptiacus*, which lives in larger populations than the *Pteropus* species.

Q: Have you been able to look at other virus species through NGS?

A: Not on these samples, but we have also studied other virus families, including the other paramyxoviruses, coronaviruses, and rubulaviruses. Corona- and rubulaviruses have different excretion patterns, where coronaviruses seem to be present at all times, whereas rubulaviruses have a second peak during the pregnancy season of the bats.

Q: While Nipah and Hendra represent pressing needs of Southeast Asia, one should keep in mind the diversity in viruses found there and elsewhere. Polyclonal, neutralising antibodies made for NiV and HeV are often unable to cross-neutralise even closely related viruses, such as *Ghanaian henipavirus*, and this needs to be considered in developing therapeutics and vaccines. There is also the question of how pathogenic these related viruses can be, if they were to spill over into humans.

Q: The longitudinal data were interesting to see, as were the data illustrating differences between the different countries, concerning bats and virus shedding. Notably, there seemed to be an inverse relationship between viral shedding and the frequency of outbreaks. For example, Thailand has a high prevalence of NiV in bats, but no outbreak. How could this be?

A: A lot of the high shedding rates were from pooled urine samples from whole colonies, and these may not be comparable to individual samples. There may also be large variation between colonies, so that the low shedding documented in one colony may not reflect the overall shedding in the area, especially if the colonies are out of sync in terms of viral prevalence. Studies in Bangladesh have observed elevated shedding over a period of time around spillover events, which seems to contradict the assumption made in the question. Another factor that affects transmission rates is human behaviour, as exemplified in outbreaks caused by the drinking of date palm sap. Public awareness on safe practices is key to prevent outbreaks.

Chair: We are all extremely grateful for everything WHO, NIAID, and CEPI are doing to push the agenda for improved surveillance, in spite of criticisms from other scientists. What we have seen here, is that the Nipah vaccine initiative is likely to be only the beginning, with many more initiatives following, due to the wide diversity of circulating viruses. Imagine what spillovers we would catch if we had the same level of surveillance everywhere, as around hospitals in Thailand and Bangladesh. The challenge is to cover all high-risk interfaces, not only in Southeast Asia, but in Africa, too. Hopefully, this sort of initiative will go down in history as the beginning of the end of pandemic risk.

SESSION 4 – Progress and challenges in diagnostics

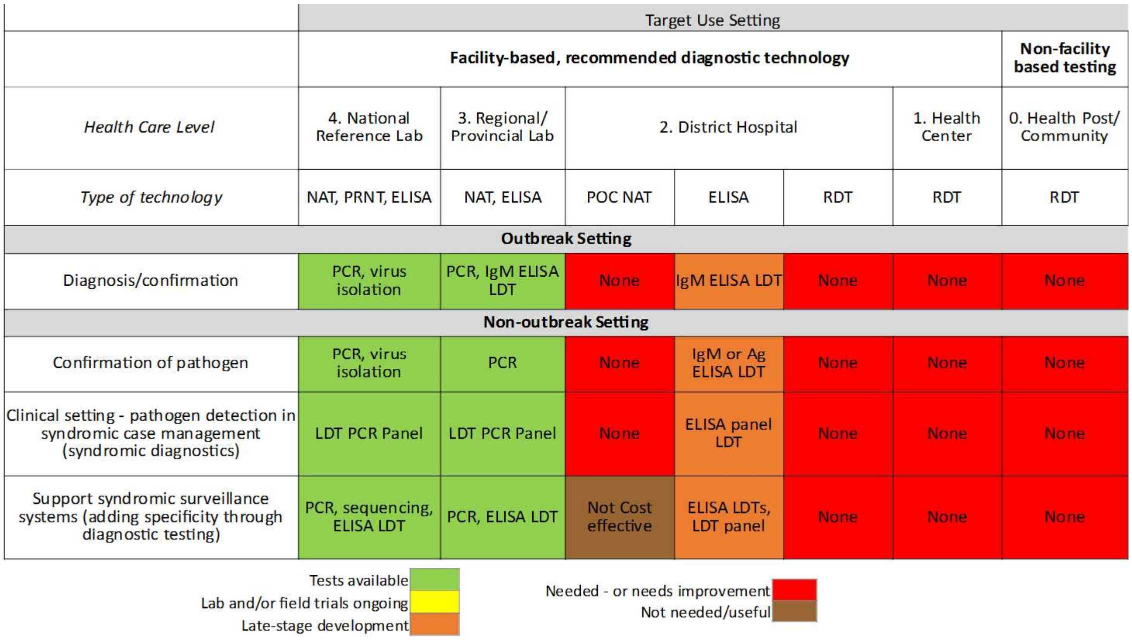
Chair: Dr. Joel Montgomery, CDC

Overview of Nipah diagnostics

Dr. Jillian Sacks, FIND

The Foundation for Innovative New Diagnostics (FIND) is a global non-profit organization driving innovation in the development and delivery of diagnostics to combat major diseases affecting the world's poorest populations. With diversified public and private funding, they partner to develop and deliver field-adapted diagnostic solutions to low- and middle income countries (LMICs). Their pandemic preparedness programme, which covers many of WHO's priority pathogens, has been crafted on the premise that diagnostics are fundamental to the identification, containment, and resolution of outbreaks. They partner with WHO and others, and perform pathogen, technology, and partner landscape assessments, to identify critical needs and gaps in diagnostics – and address these through requests for proposals (RFPs) and funding of prioritised R&D initiatives.

Concerning Nipah, a TPP has already been developed by WHO, and is currently open for comments. Some diagnostic tests are already available, such as nucleic acid test (NAT), plaque reduction neutralization test (PRNT), and enzyme-linked immunosorbent assay (ELISA), for use in centralised labs (see Figure 6). There are also tests available for veterinary diagnostics (not shown). What is still missing, is NAT, rapid diagnostic test (RDT) or panels to be used in decentralised settings. We also lack a standard, which would be required for the development of new diagnostics and to ensure proficiency of testing in existing laboratories.

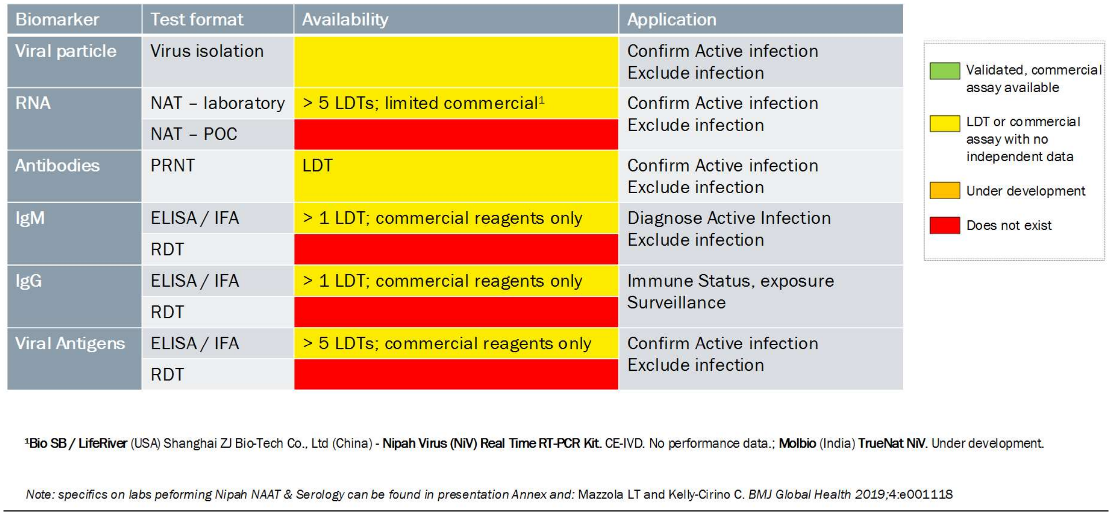


	Target Use Setting						
	Facility-based, recommended diagnostic technology						**Non-facility based testing**
Health Care Level	4. National Reference Lab	3. Regional/ Provincial Lab	2. District Hospital			1. Health Center	0. Health Post/ Community
Type of technology	NAT, PRNT, ELISA	NAT, ELISA	POC NAT	ELISA	RDT	RDT	RDT
Outbreak Setting							
Diagnosis/confirmation	PCR, virus isolation	PCR, IgM ELISA LDT	None	IgM ELISA LDT	None	None	None
Non-outbreak Setting							
Confirmation of pathogen	PCR, virus isolation	PCR	None	IgM or Ag ELISA LDT	None	None	None
Clinical setting - pathogen detection in syndromic case management (syndromic diagnostics)	LDT PCR Panel	LDT PCR Panel	None	ELISA panel LDT	None	None	None
Support syndromic surveillance systems (adding specificity through diagnostic testing)	PCR, sequencing, ELISA LDT	PCR, ELISA LDT	Not Cost effective	ELISA LDTs, LDT panel	None	None	None

Tests available 🟩
Lab and/or field trials ongoing 🟨
Late-stage development 🟧
Needed - or needs improvement 🟥
Not needed/useful 🟫

Figure 6. Nipah diagnostics heat map of available test technology for a given target use setting (FIND)

Since the symptoms of Nipah are easily confused with other conditions, it is useful to have testing panels, covering multiple pathogens, that can be used in a non-outbreak setting for syndromic case management. These are available for centralised labs, but not in a decentralised setting. Some of the tests that can be used to confirm a Nipah diagnosis, such as virus isolation and neutralisation assays, require a BSL-4 lab, which serves to drastically limit the availability of these tests. Other tests that are suitable for detecting circulating virus are based on the detection of RNA, antibodies, or viral antigens. For these, a number of laboratory-developed tests (LDTs) exist, typically intended for local use, but they are not validated or commercially available (see Figure 7). Though appropriate for select countries, LDTs are generally not easily implementable or scalable for response to new outbreaks. NATs for use at the point of care (POC) have not been developed, and neither has RDTs. FIND strongly advocates for the development of commercially available tests to ensure quality and facilitate comparison across testing sites.



Biomarker	Test format	Availability	Application
Viral particle	Virus isolation	🟨	Confirm Active infection Exclude infection
RNA	NAT – laboratory	🟨 > 5 LDTs; limited commercial[1]	Confirm Active infection Exclude infection
	NAT – POC	🟥	
Antibodies	PRNT	🟨 LDT	Confirm Active infection Exclude infection
IgM	ELISA / IFA	🟨 > 1 LDT; commercial reagents only	Diagnose Active Infection Exclude infection
	RDT	🟥	
IgG	ELISA / IFA	🟨 > 1 LDT; commercial reagents only	Immune Status, exposure Surveillance
	RDT	🟥	
Viral Antigens	ELISA / IFA	🟨 > 5 LDTs; commercial reagents only	Confirm Active infection Exclude infection
	RDT	🟥	

🟩 Validated, commercial assay available
🟨 LDT or commercial assay with no independent data
🟧 Under development
🟥 Does not exist

[1] **Bio SB / LifeRiver** (USA) Shanghai ZJ Bio-Tech Co., Ltd (China) - **Nipah Virus (NiV) Real Time RT-PCR Kit.** CE-IVD. No performance data.; **Molbio** (India) **TrueNat NiV.** Under development.

Note: specifics on labs peforming Nipah NAAT & Serology can be found in presentation Annex and: Mazzola LT and Kelly-Cirino C. *BMJ Global Health 2019;*4:e001118

Figure 7. Nipah diagnostic test availability (FIND)

There are important diagnostic gaps for rapid identification and response to Nipah outbreaks: 1) Limited availability of commercialised or standardised kits with regulatory approval; 2) Lack of an international reference standard, and a sample repository to aid in development of new diagnostics; 3) Lack of tests suitable for POC or community settings; 4) Lack of tests that can be used on minimally invasive samples, to minimise biosafety requirements. Better understanding of Nipah epidemiology and viral kinetics will facilitate the development of appropriate NiV diagnostic tests to serve both outbreak and non-outbreak intended uses.

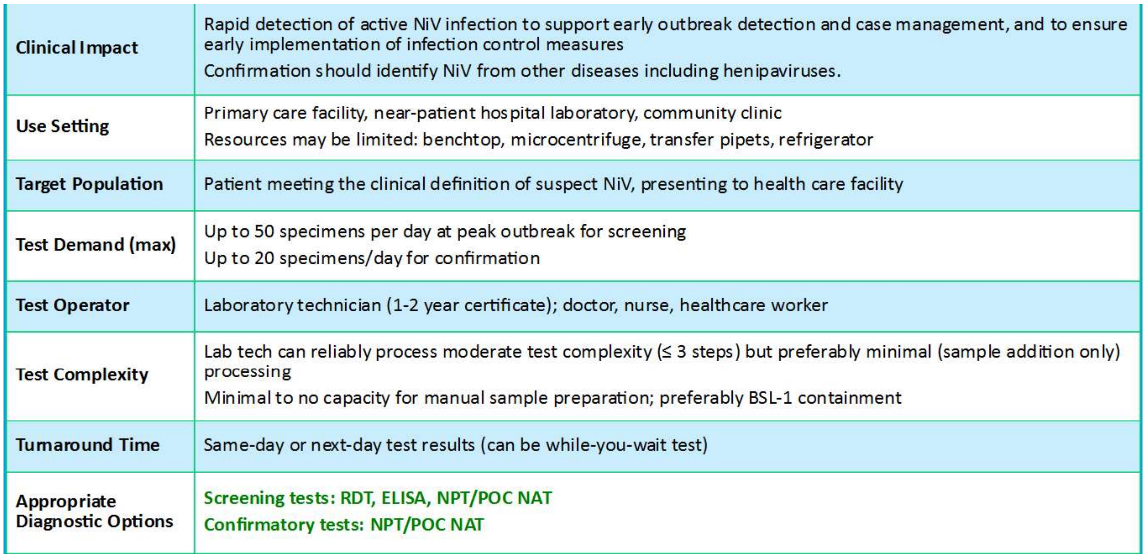
Note: A list of in-house NAT and LDT serology tests for NiV is provided in the presentation Annex and in Mazzola LT and Kelly-Cirino C. *BMJ Global Health 2019*;4:e001118.

The WHO Nipah diagnostics target product profile (TPP)

Dr. Laura Mazzola, WHO

Following the WHO R&D Blueprint, published in 2016, a 5-year framework for Nipah in the form of the Nipah R&D Roadmap was drafted in May 2018. The R&D Roadmap addresses current primary challenges, key needs, and knowledge gaps for diagnostics, therapeutics, and vaccines; sets strategic goals for all of these; and defines priority areas and activities for obtaining the goals. From the needs and knowledge gaps described in the Roadmap, use cases and TPPs for Nipah diagnostics were developed and recently published for consultation on WHO's website. In this document, key priority actions needed to drive new NiV diagnostic test development were identified, with the following two use cases in mind: 1) Screening for active NiV infection; 2) Confirmation of active NiV infection. Screening is typically done in the field, in a POC or near-patient (NPT) setting, where laboratory infrastructure is limited or non-existent. Confirmation is typically done at a centralised reference laboratory, though often without BSL-3/4 capacity. Nipah being a BSL-4 pathogen, some sort of on-site or immediate inactivation procedure would be desirable.

For the priority use case, detection of active NiV at a peripheral health centre or hospital, a set of requirements were established as shown in Figure 8. These laid the basis for the development of TPPs for rapid screening and confirmation, respectively.



Clinical Impact	Rapid detection of active NiV infection to support early outbreak detection and case management, and to ensure early implementation of infection control measures Confirmation should identify NiV from other diseases including henipaviruses.
Use Setting	Primary care facility, near-patient hospital laboratory, community clinic Resources may be limited: benchtop, microcentrifuge, transfer pipets, refrigerator
Target Population	Patient meeting the clinical definition of suspect NiV, presenting to health care facility
Test Demand (max)	Up to 50 specimens per day at peak outbreak for screening Up to 20 specimens/day for confirmation
Test Operator	Laboratory technician (1-2 year certificate); doctor, nurse, healthcare worker
Test Complexity	Lab tech can reliably process moderate test complexity (≤ 3 steps) but preferably minimal (sample addition only) processing Minimal to no capacity for manual sample preparation; preferably BSL-1 containment
Turnaround Time	Same-day or next-day test results (can be while-you-wait test)
Appropriate Diagnostic Options	**Screening tests: RDT, ELISA, NPT/POC NAT** **Confirmatory tests: NPT/POC NAT**

Figure 8. Use case: Rapid detection (screening & confirmation) for suspected NiV infection at peripheral setting during an outbreak (WHO)

The TPPs were made to describe the requirements for a rapid screening and a rapid confirmation diagnostic test, respectively, for human use in an outbreak setting. They specify minimal and optimal requirements – a newly developed test is expected to at least meet the minimal requirements, whereas being able to meet the optimal requirements will yield a better test. A **screening** test is 1) intended for the detection of NiV-specific IgM and/or NiV antigen (Ag); 2) an ELISA or RDT kit; 3) validated for at least NiV-B and NiV-M strains; 4) quicker than 4 hours to get results; 5) used on at least serum; 6) appropriate for biosafe collection and includes inactivation protocol; 7) sensitive (> 90-95 %); 8) specific (> 80-90 %); 9) minimally cross-reactive with other pathogens, may have some cross-reactivity to other henipaviruses. A **confirmation** test is 1) intended for the detection of NiV ribonucleic acids (RNA) by RT-PCR; 2) semi-automated or fully automated; 3) validated for at least NiV-B and NiV-M strains; 4) quicker than 6 hours to get results; 5) used on at least plasma and serum; 6) conducted in three or fewer steps, including inactivation (sample preparation); 7) detecting down to 1,000 copies/ml; 8) sensitive (> 95-98 %); 8) specific (> 95-98 %); 9) not cross-reactive with other pathogens, including other henipaviruses.

There are other issues related to the development of new Nipah diagnostics, besides the diagnostics themselves: 1) For clinical validation, access to specimens is critical, and specimen repositories that are representative for endemic and at-risk regions should be established; 2) International reference standards need to be developed for calibration, comparison, and quality control; 3) Criteria for test performance and use need to be established; 4) Harmonisation of regulatory requirements is desirable; 5) For biosafety, a safe and simple method of sample inactivation needs to be devised that does not interfere with diagnosis. Then there are also a number of issues related to the procurement, implementation, training, proficiency testing, and post-market surveillance of new diagnostics. Finally, there needs to be put in place appropriate geographic deployment strategies for outbreak preparedness.

By 2021, WHO hopes to have completed preclinical evaluation of at least two POC or NPT diagnostics that align with the TPP. By 2022, the aim is to complete field studies for these.

Role of early diagnosis during Nipah virus outbreak in India

Dr. Pragya Yadav – National Institute of Virology, ICMR

In the initial phase of the Kerala outbreak in 2018, the logistics of sample analysis and clinical management proved somewhat chaotic. Systems were not in place for safe handling of samples and clinical cases. Additionally, as Nipah is a frightening disease, many wanted to be tested, and the resulting rapid increase in workload put severe strains on the available diagnostic capacity. Two major infrastructural requirements were identified during the outbreak: 1) Laboratory facilities for quick diagnosis; 2) Isolation wards for confirmed and suspected cases. In spite of the difficulties, Indian healthcare workers eventually succeeded in containing the outbreak. The lessons learned from 2018, and the resulting awareness campaign, led to rapid response and the successful containment of the ensuing 2019 outbreak, which affected only one person, who survived.

There is a need for NiV diagnostics that can ensure rapid and reliable diagnosis in the event of an outbreak, to achieve early implementation of infection control measures. Affordable POC diagnostics are a top priority. The ability to detect and confirm infection on-site allows not only for proper case management, but also for early public health interventions such as contact tracing, isolation, and prophylactic measures. It is important that POC diagnostics are easy and safe to use, with minimal training.

The National Institute of Virology has developed a number of diagnostic tools to be used in India: 1) Anti-Nipah human IgM ELISA; 2) Anti-Nipah bat IgG ELISA; 3) POC assay for NiV detection. These are being validated by IEDCR, Bangladesh. A mobile diagnostic system developed in collaboration with MolBio, as private industry partner, has received approval for clinical use by the Indian Central Drugs Standard Control Organization. The system includes an automated RNA extraction machine and a real-time PCR device tailored to detecting NiV. It is portable and battery-operated, uses stabilised, disposable consumables, and requires minimal handling and, thus, training. The lysis buffer included in the kit has been shown to inactivate NiV, allowing for POC use. Suitable sample materials include throat swabs, serum/plasma, urine, whole blood, other body fluids, and tissue materials (including bat tissues).

The mobile diagnostic system is especially suited for outbreak response in remote areas, and offers easily deployable state-of-the-art technology for effective field diagnosis. However, it does not replace the need for centrally located containment laboratories, more of which are still needed in India. Effective surveillance is key to effectively managing future outbreaks of NiV.

Nipah virus diagnostics at CDC – current strategies

Dr. John Klena – Viral Special Pathogens Branch, U.S. CDC

The Viral Special Pathogens Branch (VSPB) of the U.S. CDC provides support to outbreaks around the world. They have a BSL-4 high-containment laboratory, which is used for high hazard virus diagnostics and research: They also contribute to rapid detection and control during outbreaks through epidemiology support, provision of mobile labs, and health communications. Finally, they conduct ecological investigations to detect the source of zoonotic outbreaks (most EIDs are zoonotic RNA viruses), and devise strategies for disease prevention and control. In the context of Nipah, CDC is working to improve assays for both serological detection and nucleic-acid based detection. While they do not seek commercialisation of their own assays, they are happy to support others who are working to commercialise new diagnostic tests. CDC willingly shares serological and PCR assay components with partners, but this has become increasingly difficult in recent years, due to stricter regulations on shipping of biological materials – something that is especially challenging with the shipping of positive viral controls.

The ELISA-based serological assay of CDC has been known to be difficult to work with, and some changes have been implemented over the past couple of years to improve the assay's sensitivity. One major change has been to replace the historically used goat anti-human IgM capture antibody with goat IgM F(ab')2 (Thermo Scientific A24490). This change will be implemented in the testing in Bangladesh this season. Furthermore, the use of chinese hamster ovary (CHO) cell slurry with recombinant antigen (N-protein), rather than Vero E6 cell slurry with infected cells, seems to improve the background signal, but comes with a loss of sensitivity of the NiV IgM assay. When testing survivors for IgG, serology results remain positive for more than 5 years. Still, more systematic testing of survivor serum is needed to evaluate the longevity and protection of the IgG response.

Nucleic acid testing, using real-time RT-PCR, seems very sensitive and suitable for use in the field when testing for NiV. CDC has historically run these tests against the nucleocapsid (N) protein, and are looking to improve their assay. With sequences from the 21 Nipah whole genomes sequenced by CDC so far, one new domain in the N gene has been identified that has no mismatches among the 21 different viruses. Therefore, this seems a promising target for a new and improved RT-PCR-based diagnostic test, and a set of primer and probe is currently being used to screen samples collected from Bangladesh in the seasons 2017-2018 and 2018-2019. As new, full-length genome sequences are being made available, further revisions to the test will be made.

Hendra DIVA assay for the detection of antibodies in vaccinated horses

Dr. Kim Halpin – Australian Animal Health Laboratory (AAHL)

Horses are highly susceptible to HeV infection, and will often die. However, it was infection in humans that led to the development of a vaccine against Hendra. The Equivac® HeV vaccine by Zoetis is a subunit vaccine containing the soluble G protein (sG), which is a HeV glycoprotein responsible for attachment of HeV to target cells. It was released late 2012 upon approval by the Australian Pesticides and Veterinary Medicines Authority (APVMA) for minor use, and fully registered in 2015. The vaccine requires three doses initially, and a yearly booster to remain effective.

The Hendra DIVA test is a serological test to assess whether a horse has been infected by or vaccinated against HeV. It is an ELISA-based assay, in which the plates are coated with HeV antigen (HeV sG or recombinant N protein, in separate wells) and detected by a specific mouse monoclonal antibody (mAb; anti-sG or anti-N, as appropriate). The secondary antibody is anti-mouse IgG, conjugated with the enzyme responsible for colorimetric reaction. When first incubating with serum from infected horses, binding of the mAb is blocked, resulting in a "blank" color reaction for both sG and N antigen wells. For vaccinated horses, only the sG wells will be "blank", since this is the only protein used in the vaccine. Serum from previously unexposed horses will yield colorimetric results for both sG and N wells.

The test is being validated in 1,000 unvaccinated and 1,000 vaccinated horses, so that it can be used for healthy horses that need to be transported internationally. HeV vaccination is entered in a national database in Australia, which allows for assessment of the number of vaccines given to a particular horse, and see the time elapsed since the last vaccination. In the current validation, where about 250 horses have so far been included, most horses appear to achieve protective antibody titres upon the third vaccination, as evaluated by serum neutralisation tests (SNTs). In these horses, the protective antibody titres seem to last for at least 20 months after the last vaccination. The vaccine thus appears to be effective, and the hope is that documenting efficacy of the vaccine will counter some of the negative attention the vaccine has received from certain circles in the general population.

Today, the Australian Animal Health Laboratory (AAHL) is the only OIE Henipavirus Reference Laboratory. Within the OIE network, with their priority diseases, reference laboratories are assigned to support other laboratories with training, technology transfer, and technology support. As the only reference laboratory on henipaviruses, the synergies that would otherwise be present in a larger network of henipavirus laboratories are missing. Thus, AAHL is interested in talking to other laboratories with an interest in joining an informal network of laboratories performing henipavirus diagnostics. Network activities could for example include sharing of protocols, reagents, and samples.

Serological assays for henipavirus diagnostics

Professor Anne Balkema-Buschmann – Friedrich-Loeffler-Institut (FLI)

(Presented on Day 2 of the conference)

Friedrich-Loeffler-Institut (FLI) is developing diagnostic assays for henipaviruses in Europe, far from the endemic areas, in the interest of preparing for potential epidemic and bioterrorism threats. Testing European animals that may have been exposed to henipaviruses requires previous validation of the diagnostic assays against indigenous livestock breeds. First, an indirect henipavirus ELISA assay was developed with NiV N protein expressed in *E. coli*, and the cut-off value was determined against negative samples from Canadian pigs. Furthermore, the establishment of indirect ELISAs for NiV G and HeV G, using proteins expressed in *L. tarentolae* (which closely mimics the protein glycosylation patterns seen in humans), allowed for differentiation between NiV and HeV infection: While there is still cross-reactivity between NiV and HeV in these two latter assays, the homologous virus yields a higher response. Finally, Western blot is used to confirm positive samples against plasmid-derived NiV G, using a monoclonal antibody for NiV G as positive control. The assay has now been validated for North American and European serum samples, and is available for screening and diagnostic purposes.

Working to establish a Hendra diagnostic for horse samples, an indirect ELISA was developed based on HeV G expressed in *L. tarentolae* and HeV N expressed in baculovirus-infected insect cells. Screening of 288 German negative horse sera yielded the cut-off, and assay validation was conducted in Geelong using samples with confirmed diagnosis (135 negative, 95 vaccinated, and 21 infected horse sera). The German ELISA assay compared very well with the Australian assay, having very similar cut-off values and high, comparable levels of sensitivity and specificity.

There is serological evidence of henipavirus spillover into human populations in Africa, and although the clinical relevance is unclear (there have been no detectable cases), there is a desire to establish strain-specific tools for serosurveillance. Positive sera were generated immunising fruit bats (*Rousettus aegyptiacus* and *Eidolon helvum*) hosted at the FLI with different recombinant viruses. In preparing for these infection studies, the circadian temperature baseline had already been established (body temperatures in the bats range from 34 to 41 °C depending on time of day and activity levels). Sera from vaccinated bats, when tested with ELISA or the corresponding multiplex setup (on Luminex®), again demonstrate cross-reactivity, but show the strongest response with the homologous test. FLI now continues to work on establishing multiplex assays for henipavirus differentiation in pigs, horses, and bats, as well as detecting antibodies against other relevant antigens in bats and pigs.

Establishment of an immunofluorescence assay to detect IgM antibodies to Nipah virus using HeLa cells expressing recombinant nucleoprotein

Dr. Yoshihiro Kaku – National Institute of Infectious Diseases, Tokyo

The National Institute of Infectious Diseases (NIID) started working on Nipah in response to the 2014 outbreak on the Philippines, partnering with Research Institute of Tropical Medicine (RITM), WHO, and AAHL. NIID was involved in performing serological and nucleic acid testing on the samples from three of the seventeen cases detected in the outbreak.

The ambition of NIID is to develop diagnostic methods for NiV that are available under BSL-2 conditions and use inexpensive equipment. At the time, NIID did not have an IgM detection system in place, due to the lack of positive control human serum. Instead, they embarked on developing a positive control through the immunisation of macaques (*Macaca fascicularis*), since IgM and IgG from macaques are typically cross-reactive to human IgM and IgG. The macaques were immunised with 1 mg NiV N-protein purified from baculoviruses, followed by 0.2 mg booster doses on days 15, 28, 42, and 56. Serum was collected on days 5, 7, 9, 11, 13, **15** (30 ml), 53, and **63** (whole blood), where serum from day 15 was used as positive control for IgM, and whole blood from day 63 served as positive control for IgG.

An IgM capture ELISA was designed as follows: Wells were coated with goat anti-human IgM, to which samples and positive controls containing human and macaque IgM, respectively, were added. Half of the wells were incubated with NiV-N antigen corresponding to the antigen to which the macaques had been immunised, and the other half with irrelevant antigen from baculovirus. Captured antigen was detected by rabbit anti-NiV-N serum and goat anti-rabbit IgG linked to horseradish peroxidase (HRP). The IgM assay was tested against serum collected from two immunised macaques on days 5-15, demonstrating seroconversion for one of the macaques around day 9 (which could then be used as positive control). Macaque number two failed to elicit a significant immune response to NiV-N.

An IgM indirect fluorescence antibody test (IFAT) was developed by (stably or transiently) transfecting HeLa-229 cells with plasmids containing NiV-N cDNA and blastcidin as reporter. For transient expression, the HeLa cells were trypsinised on day 4, seeded onto 14-well glass slides and fixed with acetone. For stable expression, blastcidin-expressing cells were selected, trypsinised, and mixed with mock-transfected cells in a 1:3 ratio prior to fixation, since this yields a setup better suited for distinguishing between false positive and true positive results. The IFAT was conducted by adding test serum, and subsequently Alexa Fluor 488-conjugated goat anti-human IgM, to the fixed HeLa cells. If the test serum contains anti-NiV-N IgM, the NiV-N-expressing HeLa cells will emit fluorescence.

The IgM capture ELISA proved to have a lower detection limit for anti-NiV-N IgM than the IFAT assay, yet the three patient samples received by NIID from the 2014 Philippines outbreak were all confirmed NiV positive by both assays. When testing the IgM IFAT assay against normal (uninfected) sera from The Malaysian Cohort (TMC) project, all samples were negative at a 1:100 serum dilution. The IFAT assay was also shown to be able to detect anti-NiV-N IgG, and to be cross-reactive for anti-HeV-N IgG. The IFAT antigen slides are fairly stable, and can be stored at -80 °C for several years awaiting future use. It is possible to use them directly after thawing, which makes them suitable for rapid diagnostic response in a laboratory setting.

NIID has also developed a pseudotyped VSV neutralisation assay, which is more sensitive than neutralisation assays with live NiV. The assay is suitable for both screening (in a high-throughput setting) and confirmation of infection status, and could potentially limit the need for confirmation with a second assay. NIID are happy to share the technique and reagents with other laboratories.

Session 4 Q&A

Q: Are there plans for using the mobile diagnostic system developed in India as part of prospective surveillance? Can you bring the system on a flight? What is the cost?

A: The system can be used both prospectively and in an outbreak setting. Yes, the system can be brought on the plane, but may sometimes be rejected by staff, for example due to safety concerns. The cost per test is reasonable, about 800 Indian rupies.

Q: What more can be said about the vocal groups opposing the Hendra vaccine?

A: One challenge is social media like Facebook, where people hesitant to the use of vaccines come together and share stories about horses getting sick from vaccination, and claims that the manufacturer is pushing the vaccine in regions where HeV has never been seen. A recent spillover event close to Sydney may serve to counter this view, and has caused all thoroughbred stables in the area to vaccinate their horses. A recent pro-bono legal class action has caused a number of people to come forward saying that their horse had a reaction to the vaccine. This certainly detracts from our cause of trying to save the lives of people and horses, but we have to keep offering up all the good information about the vaccine and ensure continued use.

Q: Can the HeV vaccine be used in humans?

A: The approach in Australia has been to vaccinate the horses, rather than people, and protect everyone – since the horses are the amplifying host (humans have never gotten Hendra directly from a bat). From a One Health point of view it makes sense to vaccinate the horses.

Q: How is the use of the HeV vaccine being monitored, in terms of side effects?

A: The monitoring is ensured by the APVMA, and all reports of adverse events are sent to them. The manufacturer has a role in facilitating this, and in following up on the reports. According to the vaccine company, adverse events linked to the use of vaccine have been minimal.

Q: Annual booster doses of the HeV vaccine seems a bit excessive. Is there any evidence to support this?

A: With the documentation available, 12-month boosters are still recommended. With more data, one may be able to reduce the number and frequency of boosters.

SESSION 5 – Pathogenesis and animal models

Chair: Dr. Amy Shurtleff, CEPI

Development of a nonhuman primate model for Nipah virus infection that accurately reflects human disease

Dr. Mike Holbrook – NIAID, NIH

NIAID works to develop a non-human primate model that accurately recapitulates human disease to aid in the development of medical countermeasures to Nipah virus infection. While, historically, such an animal model would have been designed to be uniformly lethal, NIAID seeks to develop a model that is more true to NiV infection in humans. Specifically, they wish to replicate the clinical hallmarks of Nipah disease: 1) Acute Respiratory Distress Syndrome (ARDS), as evidenced by "ground glass" opacities of lung computed tomography (CT) scan or X-ray; 2) late-onset encephalitis, manifesting as focal hyper-intense lesions or confluent cortical involvement on brain MRI scans; 3) widespread vasculitis, especially of highly vascular organs such as the spleen. At the NIAID integrated research facility, one has been able to study the impact of exposure to different-sized particle aerosols with NiV in animals, using clinical imaging to assess the disease progression in lungs and brain, and immunological tests to evaluate immune responses. These are the only facilities in the world to allow for advanced clinical imaging of live animals kept at BSL-4.

Currently, NiV-M and NiV-B particles about 12 µm in size are used to infect wild-caught Caribbean origin African green monkeys (AGM) at a dose of about 500 plaque-forming units (pfu) per monkey. Surprisingly, infecting with NiV-M or NiV-B results in widely different disease progression, where NiV-M causes extended disease with about 30 % survival, and NiV-B leads to rapidly progressing disease with about 50 % survival. Quantification of RNA in blood, nasal swabs, and cerebrospinal fluid (CSF) demonstrates viral shedding during the acute phase of disease, as well as penetration of NiV-M across the blood-brain barrier (BBB) into the central nervous system (CNS). Seroconversion is detected in only some of the animals, starting around day 10 post infection. Neither seroconversion nor the presence of neutralising antibodies seem to be reliably protective. While antibodies may limit respiratory disease, no effect has been found on the progression of neurological disease.

A number of different coagulation assays point to a link between acute-phase disease progression and the development of haemorrhagic disorders: 1) Reduced platelet levels, as well as increased partial thromboplastin time (PTT) and thrombocytopenia suggest onset of disseminated intravascular coagulation (DIC); 2) Elevated fibrinogen suggests severe DIC; 3) Elevated von Willebrand factor (vWF) is indictive of vasculitis. Brain imaging is conducted regularly on live, infected animals (CT throughout infection, MRI from day 15 since it requires anaesthesia), and later guides the acquisition of brain biopsies that allow for confirmation of encephalitis. Detection of circulating cytokine levels may serve to corroborate the inflammatory status of the animal, though clinically predictive patterns have not yet been identified. Lung infiltration of NiV-B has been demonstrated to severely affect lung capacity.

The AGM model results in the development of neurological disease several weeks after exposure, and requires survival of the acute respiratory phase of the disease. The animals respond differently to infection, as would be expected in the human population: Some show no clinical evidence, some are infected with clinical manifestations but survive, and some succumb to the infection. When delivered to the oropharynx and upper respiratory tract, NiV-M causes less respiratory disease than NiV-B. Haemorrhage and coagulopathy seems to be a critical aspect of Nipah disease. Neurological disease results from vasculitis (microinfarcts) or inflammation (encephalitis) rather than virus specifically targeting neural tissue, and this occurs even in the presence of NiV neutralising antibodies.

Some points to consider: 1) NiV infection may initially present as a mild to moderate respiratory infection, progressing to neurologic disease in some cases if unrecognised or untreated; 2) Serological and nucleic-acid based diagnostic tools complement each other, and should be used in combination, with whole blood and nasal swabs as suitable sources of viral RNA; 3) Therapeutic approaches focusing on vasculitis/DIC should be developed; 4) Continued development of the NHP model for NiV infection will aid in understanding the disease and in developing clinical countermeasures.

Advances in non-human primate models of henipavirus disease: refinement of routes and the importance of species selection

Dr. Robert Cross, University of Texas Medical Branch (UTMB)

In the AGM model, NiV infection by the intratracheal or intratracheal + oral route results in disease characterised by thrombocytopenia, leukopenia, neutrophilia, generalised vasculitis (lung and brain), hypoalbuminemia, pulmonary distress, and (often) neurological symptoms. NiV-B has proven more potent than NiV-M, and consistently kills the monkey in 9-10 days. Respiratory disease has a rapid onset around day 8-10, showing up on X-ray shortly before the animal needs to be euthanised. Neuronal and endothelial deposition of viral antigens causes the characteristic brain lesions. Circulating viral genome may be detected from day 7 post infection in this model.

Whereas the intratracheal route may have its uses, it is labour-intensive and cumbersome to use. Therefore, an intranasal NiV exposure AGM model has been developed using the mucosal atomization device MAD Nasal™ from LMA®. Using doses of 2,000 and 20,000 pfu, the intranasal model was uniformly lethal, with time to death of about 9-10 days. Vasculitis and viral depositions in the spleen may be one reason why a protective immune response to the infection is not established and the animals succumb. Nipah is a vascular disease, and wherever endothelial cells are present, viral replication may occur. Thus, viral RNA is found in blood as well as in most tissues examined. Cytokine profiling indicates no systemic inflammation until late in the disease.

While the AGM model is uniformly lethal, another model has been developed using cynomolgus monkeys (CM) and administration of NiV-M, NiV-B, or HeV intratrachially (5.0×10^5 pfu). This model has pathologies consistent with human infection and other animal models, yet the animal survives. While circulating virus may be detected from day 5 or 6 – with peak viremia around day 7 – spleen, lung, and brain tissues are minimally affected. One reason for this may be stronger immune responses to henipaviruses in CM, as detected by considerable titres of serum neutralising antibodies and high circulating levels of the chemokines MCP-1 and IL-6. A differential gene expression microarray analysis showed characteristic differences between AGM (fatal infection) and CM, and this is hoped to help clarify the mechanisms underlying CM survival. Furthermore, while reagents for phenotyping of immune cells are lacking for use in AGM, digital cell quantitation based on results from the microarray analysis has allowed the *in silico* identification of B cells and Th1 cells as possible key cell populations in survival of AGM infected by NiV. In contrast, mast cells and NK cells may play a detrimental role in the pathogenesis of Nipah infection.

Oronasal challenge of ferrets with henipaviruses

Dr. Glenn Marsh, CSIRO, Australian Animal Health Laboratory (AAHL)

Over the past 15 years, AAHL has developed a ferret model for Nipah infection that closely mimics the human disease. When infected with NiV-B, the ferrets experience predominantly respiratory disease, whereas with NiV-M the disease has more of a neurological character. Clinical endpoints are typically reached day 6-9. As with the non-human primate models, terminal animals experience a drastic drop in platelets (causing haemorrhaging) and circulating lymphocytes. The model has been extensively used for the assessment of clinical countermeasures like the HeV sG equine vaccine, monoclonal antibody m102.4, peptides, and repurposed medications.

Significant differences are seen between NiV-B and NiV-M, in animal models as well as in humans. NiV-M is the result of a single spillover event with bat-to-pig and pig-to-human transmission, yet no human-to-human transmission. NiV-B, on the other hand, has seen multiple spillovers over the last years, with bat-to-human and human-to-human transmission. The question is whether the epidemiological differences are a result of distinct viral properties, or if environmental and host factors also play a role. Notably, different NiV-B isolates used in animal testing may have different properties, as well. The strain used by AAHL was isolated from an outbreak in Rajbiri in 2004, where human-to-human transmission was not a major source of infection (other strains have not been available to them).

Comparing NiV-B and NiV-M in the ferret model, NiV-B was demonstrated to be more infectious by a factor of about 100 (5 pfu needed to infect two out of two animals, whereas the corresponding dose for NiV-M was 500 pfu). The pathology of infection, however, is comparable for the two strains, with onset of symptoms day 5-6 and euthanasia day 7-9. As in other models, gross pathology of NiV infection in ferrets involves visible haemorrhaging on the surface of the lungs and other organs. One difference between NiV-B and NiV-M in ferrets is the higher viral load and, hence, higher oral and nasal shedding, of virus in NiV-B infection. However, this shedding is not sufficient to cause transmission of virus to other animals through regular contact, suggesting that transmission requires direct transfer of bodily fluids, and that aerosol transmission occurs rarely if at all. This suggests that preventing close contact with infected individuals is likely to be an effective measure against human-to-human transmission.

Nipah virus pathogenesis in experimentally infected swine

Dr. Brad Pickering – Canadian Food Inspection Agency (CFIA), Winnipeg

During the first Nipah outbreak in Malaysia, close to a million pigs were culled, and the financial repercussions led to a high interest in studying NiV infection in pigs. There has since not been a single outbreak, outside of the Philippines, involving pigs as an intermediary host. Pigs remain, however, an interesting model for the study of NiV infection – especially given their potential to cause new epidemics. Although clinical signs of NiV infection in pigs include acute febrile illness with respiratory and neurological symptoms, as well as increased salivation and nasal discharge, the infection is often asymptomatic and may result in sudden death. During the Malaysia outbreak, the incidence of infection within affected farms was 100 %, with 1-5 % mortality. The disease would typically last for about 2 weeks on each farm, providing ample opportunity for spillover into humans.

A NiV-M infection model in swine used at CFIA involves the oronasal or nasal inoculation of 10^5 pfu per animal, resulting in relatively mild infection with some clinical signs such as laboured breathing and sometimes CNS involvement. Viral shedding peaks between days 3 and 5, allowing for detection in oral and nasal swabs. Through a nasal lavage it is possible to detect viral shedding as early as 2 days post infection (DPI). In spite of few symptoms, the animal's organs may be severely affected by the infection, with enlargement and bleeding. Oronasal infection typically spreads through local lymph nodes, lungs, and nose, and into the brain via cranial nerves (specific to pigs).

It is important to understand whether NiV-B may also infect pigs, as a potential mode of spillover into humans. Thus, Canada's National Centre for Foreign Animal Disease (NCFAD) has obtained a recombinant NiV-B (rNiV-B) isolate for comparisons with NiV-M *in vitro* and *in vivo*. The kinetics of virus replication was tested in three different cell lines and the two viral strains were found to be comparable in all cell lines. When used to infect piglets (who were indeed susceptible), at 2.5×10^5 pfu, rNiV-B caused oral shedding from day 2 post infection (as assessed on a community basis by cotton ropes hanging in the pen, which the pigs would chew on). One difference between NiV-M and rNiV-B is that the latter also sheds rectally at detectable levels, from 1 to 10 DPI.

Furthermore, only low-level antibody responses were induced after oronasal infection with rNiV-B, and titres of neutralising antibodies were low. Thus, detection of circulating antibodies may not be an option for diagnosis of potential NiV-B infection in swine, and emphasis should be put on active surveillance and ensuring diagnostic capability of veterinary laboratories in at-risk regions.

Session 5 Q&A

Q: The differences in pathology seen between NiV-B and NiV-M infections are intriguing. Has the same been seen in humans?

A: More data is needed to assess this.

Q: In the NiV-B AGM model, the lungs of the animals were gravely affected in the acute phase (example shown from diseased animal at day 8). Was this consistent for all animals, and did respiratory disease occur at a later stage with NiV-M?

A: It was pretty consistent in the animals infected with NiV-B that succumbed to the infection, and typically occurred around day 8-10. NiV-M infection given by inhalation of large particles did not result in respiratory disease.

Q: Were the infected ferrets displaying respiratory symptoms, such as coughing, sneezing?

A: Yes, coughing and sneezing did occur in some of the animals. In others, the neurological symptoms were more prominent.

Q: Were cohabiting ferrets able to interact physically?

A: Yes, the ferrets are typically housed in pairs, and for transmission studies three ferrets are housed in the same cage. They will often curl up in a corner of the cage, and thus enjoy close interactions. This is true even for sick animals, in general. With other pathogens than NiV, transmission is frequently seen under these conditions.

Q: Could airflow between cages in the BSL-4 facilities contribute to transmission?

A: Yes, with easily transmissible pathogens, transmission has been observed across cages due to the forced airflow of BSL-4 facilities. One tends to try and avoid this through careful positioning of the cages.

Q: Will the swine model be used for transmission studies?

A: Yes, once the plans for expanding the facilities have been completed, transmission studies will be conducted.

Q: The swine model is one of few non-fatal NiV animal models. Could this be used to investigate a potential role of "immune amnesia" in NiV infection, akin to what has been described for measles?

A: Some work is being done on the actual immune cells, including infected cells. This will be published soon.

Q: Could the lack of neutralising antibodies induced by rNiV-B be linked to the age of the animals (piglets) used?

A: Yes, age is definitely a factor regarding the development of immunity in pigs. Piglets are used in the interest of ease of handling in a BSL-4 context. The use of a recombinant strain could potentially be a factor, though not necessarily.

Q: Have you looked at blood gas oxygenation as an endpoint in the pig model?

A: No, but more chemistry and blood work will be included in future studies.

Day 2

SESSION 6 – Transmission/Case management

Chair: Dr. Pierre Formenty, WHO

Person-to-person transmission of Nipah virus

Dr. Emily Gurley, Johns Hopkins Bloomberg School of Public Health

The first studies of human-to-human transmission of NiV were published in 2001 and 2002, with inconclusive results (data from healthcare workers in Malaysia and Singapore). In 2004, the first study to conclude with human-to-human NiV transmission, with data from two outbreaks in Bangladesh, was published. The conclusion was further corroborated by studies in India and Bangladesh (published in 2006 and 2007, respectively). In the latter studies, so-called superspreaders were identified, which served as the source of infection for a large number of individuals. Importantly, the 2007 publication observed that contacts who washed their hands were at reduced risk of infection. In contrast, a publication from 2010 suggested that transmission of NiV could also happen in the absence of close contact. Moreover, a case-report from 2012 of a woman who had presented with late-onset Nipah encephalitis 11 years after the initial outbreak in Malaysia, questioned the original conclusion that the Malaysia outbreak did not involve human-to-human transmission. A 2013 report also demonstrated transmission from a dead person. With the publication in 2015 of the first henipavirus outbreak in the Philippines, one began to realise that the "emergence" of Nipah and related diseases was linked to human-to-human transmission, which causes outbreaks big enough to be detected even in the absence of active surveillance. The outbreak in Kerala in 2018 was a surprise, with Nipah emerging far from previous outbreaks, yet again the outbreak was caused by a hospitalised superspreader.

Key questions concerning human-to-human transmission are: 1) When and where does transmission occur?; 2) Which patients are more likely to transmit to someone else?; 3) Which contacts are most likely to be infected?; 4) What mechanisms are the most important drivers of transmission? Models trying to describe human-to-human transmission consider 1) the availability of infected (and infectious) individuals; 2) the availability of susceptible individuals; and 3) the ways these individuals interact. Whether a patient is infectious depends on the level of viral shedding, clinical symptoms that may help spread the virus (such as coughing and sneezing), and the infectiousness of the virus, which can vary from strain to strain. When it comes to NiV susceptibility, all humans are susceptible to infection. The nature of human interactions, which are often affected by the disease itself in terms of seeking healthcare and receiving visitors, is an important driver for transmission. Population density and duration of illness are other factors influencing the risk of transmission through contact.

Importantly, the route of transmission affects the progression of Nipah disease. Patients infected through date palm sap have more rapid onset of disease and a higher mortality (~90 % vs. ~50 %) than patients infected through human-to-human contact. The latter are also more likely to be diagnosed before death. This has broad-reaching implications for identification, containment, and resolution of outbreaks.

The basic reproduction number (R 0) of Nipah infection, defined as the average number of people one infected person will infect in a susceptible population over the course of infection, has been as low as about 0.2 during the last decade. For a larger outbreak to occur, an R 0 close to or higher than 1 is required. There is substantial variation in the viral strains spilling over from bats to humans every year, and a low R 0 indicates that the spillover viruses have been less suitable for human-to-human transmission. A case-contact study in Bangladesh (published 2019) identified male adults with breathing difficulties to be the primary source of human-to-human transmission of NiV. This is an important clue for safe case management. In total, 9 % of cases (22 out of 248) were shown to transmit NiV to contacts. Highest at risk for human-to-human transmission were family caregivers, individuals with prolonged exposure (> 12 hours) to a case, and individuals who came into contact with a patient's body fluids. All of the transmitters died from the infection, and the number of contacts (physical or fluid) went up in the later stages of the disease, increasing the risk of transmission. Notably, only 4 of the 22 transmitters were admitted to a surveillance hospital, representing a serious limitation for surveillance.

A systematic review of 133 henipavirus transmission studies (52 human and 81 animal studies) revealed that the proportions of transmitters were comparable in Bangladesh, India, and the Philippines (9 %, 10 %, and 12 %, respectively). The patterns of human-to-human transmission were consistent across countries; adult males with respiratory symptoms being the primary source of infection, and family members being primarily affected. While representing only a tenth of the individuals infected, transmitters were shown to be an important determinant for the size of an outbreak. Published data on viral shedding was only available for 37 patients, in which shedding was documented in oral/nasal swabs as well as in urine, but only during acute infection. Viral shedding in semen was documented in one Indian case, for at least 25 days after the onset of disease. Not a single human study has so far documented circulating viral loads.

Patient management & infection control practices

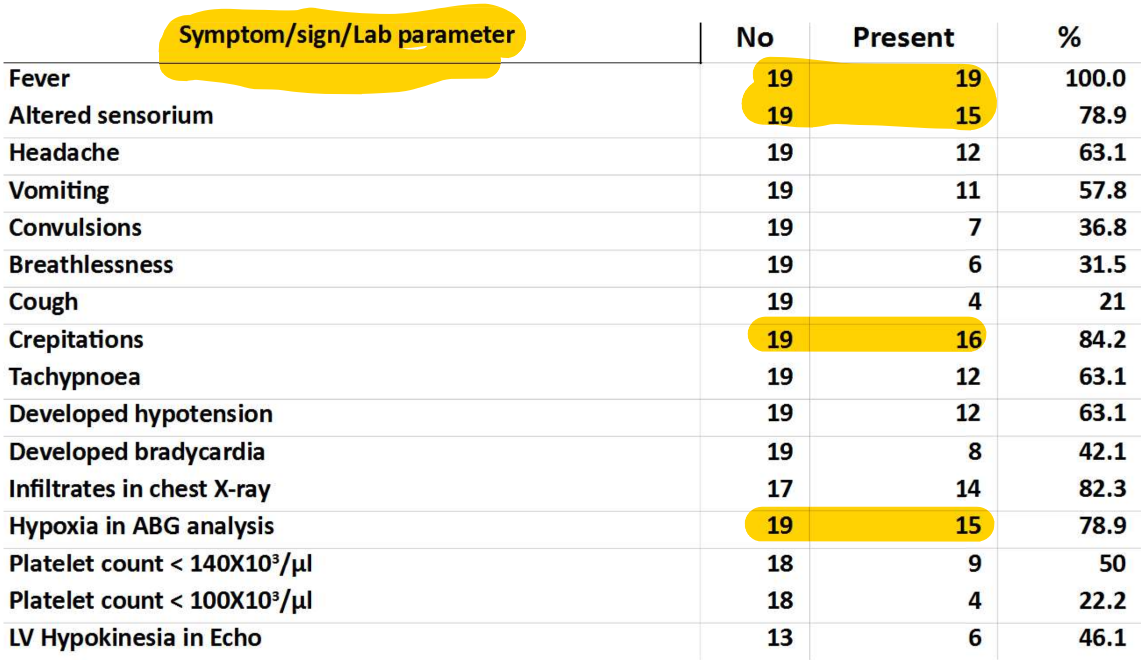
Dr. Chandni Sajeevan – Government Medical College Kerala

The Kerala outbreak in 2018 seemed to start on May 17 with a 32-year old female presenting to a private hospital with ARDS and myocarditis. The day after, lab results showed the presence of a potentially dangerous virus, and on May 20 the Indian National Institute of Virology (NIV) officially declared the NiV outbreak. Later on, it became clear that there had been another case in Kerala of viral encephalitis with ARDS on May 5, a 26-year old male. He died the same day as he arrived at the hospital. The clinical profile of NiV in the Kerala outbreak often included myocarditis in addition to encephalitis and ARDS, an interesting new observation.

The response to the outbreak was immediate, both on the hospital and public health levels. Outbreaks are not unusual in Kerala, and plans for action in the face of an outbreak already existed. Seven steps were deemed critical for controlling the NiV outbreak: 1) Maintain strict infection control practices (ICPs); 2) Ensure proper use of PPE; 3) Follow appropriate house-keeping practices; 4) Monitor staff health (no sick people at work); 5) Implement strict visitor policy; 6) Ensure safe collection and handling of samples; 7) Strict adherence to protocol for handling deceased individuals. A successful response requires preparations based on continuous learning and committing to the necessary practice changes during an outbreak.

During an outbreak, individuals presenting to the hospital with fever are triaged and, if matching the Nipah case definition, are put in isolation. Seriously ill patients are managed by the intensive care unit (ICU). *Suspected* NiV cases are individuals from an area known to be affected by an outbreak, who has encephalitis, ARDS, or myocarditis. A *probable* case is a suspected, now deceased (before diagnosis could be made) individual who had been in contact with what is assumed (or confirmed) to be an infected individual. A *confirmed* case is a suspected case with laboratory confirmation of NiV infection, by way of real-time RT-PCR.

In the 2018 Kerala outbreak, there was one index case and 18 confirmed cases (12 male, 7 female), as well as 4 probable cases (3 male, 1 female). Of the former, two patients survived, one who had typical symptoms and one with only mild disease (flu-like symptoms). All cases had fever, and in two instances the decision to isolate the patient was based solely on the fever and contact history. Altered sensorium was observed in 15 of the 19 cases at presentation (see Figure 9 for more details).



Symptom/sign/Lab parameter	No	Present	%
Fever	19	19	100.0
Altered sensorium	19	15	78.9
Headache	19	12	63.1
Vomiting	19	11	57.8
Convulsions	19	7	36.8
Breathlessness	19	6	31.5
Cough	19	4	21
Crepitations	19	16	84.2
Tachypnoea	19	12	63.1
Developed hypotension	19	12	63.1
Developed bradycardia	19	8	42.1
Infiltrates in chest X-ray	17	14	82.3
Hypoxia in ABG analysis	19	15	78.9
Platelet count < 140X10^3/µl	18	9	50
Platelet count < 100X10^3/µl	18	4	22.2
LV Hypokinesia in Echo	13	6	46.1

Figure 9: Symptoms of Nipah cases at time of presentation (Kerala, 2018; Dr. Sajeevan)

After the 2018 outbreak, a number of challenges were identified and addressed, including the need for 1) early diagnosis of suspected cases; 2) contact management; 3) uniform standard of care; 4) safe and dignified burials; 5) resolving psychosocial problems; 6) long-term follow-up of survivors. When the second spillover in Kerala happened in May 2019, response plans were implemented within 24-48 hours, perhaps as a result of the experiences from 2018. Implementation included training, setting up an isolation facility, contact tracing, and establishing a point of care laboratory.

A case-control study of henipavirus outbreak, Philippines, 2014

Dr. Paola Katrina Ching, Dr. Jose N. Rodriguez Memorial Hospital, Philippines

The 2014 henipavirus outbreak in the Philippines was the first in this country and affected two villages in the southern province of Sultan Kudarat. It was first reported as a possible case of food-borne illness from the consumption of horse meat, on April 1. Among 128 residents who had eaten horse meat, initially four individuals were reported to have gotten sick with CNS symptoms and died. Later in April it became clear that two potentially related syndromes had been identified: 1) Food poisoning; 2) Febrile illness with CNS symptoms. These were both linked to the consumption of horse meat from sick, injured, or deceased animals. The Philippines Field Epidemiology Training Program (FETP) conducted an investigation, and identified an outbreak of a human, fatal encephalitis syndrome that had resulted in 79 cases, causing 9 deaths. The causative agent was not identified, but JE, Chikungunya, and dengue were suspected. As a precautionary measure, consumption of meat from sick or dead horses was banned, and animal handlers and health care workers (HCW) were advised to follow protocols on infection control. On May 12, the Philippine Government requested WHO's assistance for further outbreak investigation.

In a joint mission starting May 22, Philippine health officials and WHO conducted a detailed outbreak investigation, with the objectives of confirming the aetiology of the outbreak and ensuring containment. Cases were now serologically confirmed, and defined as any person from Sultan Kudarat positive for henipavirus antibodies. Hence, there were 17 confirmed henipavirus cases in the outbreak (16 males, 1 female), of which 11 had acute encephalitis syndrome (AES). The rest had milder disease characterised by meningitis (n = 1) or influenza-like illness (ILI; n = 5). All of the cases had headache and fever, plus a range of other classical symptoms including difficulty of breathing, coughing, vomiting, and altered sensorium. Case onset lasted from March 10 to May 23, 2014. The incubation period ranged from 3 to 27 days (median for AES: 15 days; median for ILI/meningitis: 10 days). CFR was 83 % among cases with AES. For ten of the cases, transmission was linked to consumption of horse meat, whereas five cases had been in contact with sick humans. For two of the cases, transmission was likely to have been caused by contact with a sick horse and/or a sick human. The two AES survivors had long-term sequelae, whereas the other survivors did not experience sequelae.

In the two villages, 11 horses had died, and four cats had died after eating horse meat. It was found that horses were often tethered day and night, in forests where bats roosted. Potential food sources for flying foxes were available. Eighty percent of the horses had neurological symptoms, and four died of AES within 12 hours after initial onset of symptoms. In a case-control study, infected individuals were 20 times more likely than controls to have taken care of a sick horse, and 64 times more likely to have taken care of a sick person. NiV RNA found in the Philippine outbreak had 99 % sequence homology to NiV isolates from Malaysia. However, it was not possible to isolate the replicating virus. While flying foxes were not formally confirmed to serve as reservoir in this outbreak, it remains likely that flying foxes infected the horses, which in turn infected humans that later resulted in human-to-human transmission. Since the outbreak, measures have been taken to improve nationwide readiness and surveillance of AES, yet there have been no new cases.

Lessons learned from pathology and disease course: Malaysia

Professor Kum Thong Wong, University of Malaya

Acute henipavirus infection is a multi-organ infection caused by dual pathogenetic mechanisms; 1) vasculopathy (endothelial infection, vasculitis, thrombosis, microinfarction); and 2) parenchymal cell infection (infection of organ cells). Viremia is thought to result in vascular infection, which causes vasculitis leading to thrombosis. Thrombosis, in turn, leads to vascular occlusion and microinfarction, damaging the endothelium and allowing the virus to escape and infect the parenchymal cells of the CNS, lungs, kidneys, and other organs.

Relapsing and late-onset henipavirus infections seem to be confined to the CNS, and are not accompanied by the vasculopathy of acute-phase infection. Both NiV and HeV have been demonstrated to cause relapsing or late-onset infection, resulting in inflammation and confluent (geographical) lesions of the brain – as opposed to the necrotic plaques seen in acute infection. In late-onset henipavirus infection (seen to affect 3.4 % of cases), the patient does not experience encephalitis in the acute phase – instead, encephalitis occurs weeks, months, or years later. In relapsing henipavirus infection (observed in 7.5 % of cases), the encephalitis experienced during the acute phase recurs at a later time.

Viremia has never been documented for henipavirus infection, though it is believed that viremia is a prerequisite to account for the ability of the virus to affect multiple organs. One hypothesis explaining the differences seen between acute and relapsing/late-onset encephalitis, is that the viral foci observed in acute encephalitis are disseminated by viremia, whereas relapsing/late-onset encephalitis involves the recurrence of infection from residual foci. In some cases, the confluent lesions typical for relapsing/late-onset infection is seen very early, even in the acute phase. More clinical and animal model data (ideally in an animal model for relapsing/late-onset infection, which has not yet been developed) is necessary to address the mechanisms behind the observed differences in pathogenesis between cases. Possible factors influencing the development of relapsing/late-onset encephalitis include 1) viral dose; 2) pathogen virulence and mutations during the infection (as seen with measles, which along with NiV and HeV is a paramyxovirus); 3) host immunosuppression (also seen with measles).

Session 6 Q&A

Q: Could the reduced mortality of NiV upon human-to-human transmission indicate that the virus is attenuated upon replication in humans?

A: There is no data to support this. Another relevant factor is the dose of virus given through the different routes of transmission.

Q: Is there any evidence of "barking coughing" in superspreaders of NiV, as there has been for superspreaders of measles virus?

A: Not too much is known about the superspreaders of NiV, who often do not present themselves at hospitals, though all of the transmitters have respiratory symptoms. The superspreader from the Kerala outbreak did have a prolonged cough.

Q: What protective measures were available for HCW during the first Kerala outbreak?

A: The HCW had access to gloves and masks, but it is not clear to what extent the measures were adhered to. Two HCW got infected, of which one died (the nurse who cared for the index case, who vomited and was very ill).

Q: In the Philippine henipavirus outbreak, there were five dogs identified with antibodies against NiV. Were you able to eliminate the possibility of anyone being infected by these dogs, who had been infected?

A: All the identified cases had been in contact either with a sick horse or a sick person, as revealed through interviews. Contact with dogs did not come up as a likely source of transmission.

Q: What have been the measures implemented for bat surveillance on the Philippines, and what has been found, so far?

A: In 2014, 144 bats were sampled, of which six were positive for NiV antibodies. Whether subsequent surveys in bats have been conducted is not clear.

C: Prof. Wong mentioned a lack of data in animal models concerning variations in CNS tropism among the different NiV strains. In ferrets, there are no apparent differences between NiV-B and NiV-M. However, some differences have been observed between wild-type and W- or CW knockout animals.

A: Thanks. To get the full picture, it is recommended to do pathology on complete sections of the brain, not just fragments.

C: Autopsies have so far not been available in Bangladesh, but could be instructive for improving our understanding of CNS-involvement in Nipah disease, as well as for the development of therapeutics.

A: Yes.

Q: Are there any pathological similarities between the rare subacute sclerosing panencephalitis (SSPE) following measles infection and relapsing/late-onset encephalitis in henipavirus infection?

A: SSPE typically occurs 6-7 years after measles infection, in a slow process where the brain shrinks and the patient eventually dies. In contrast, cases with relapsing/late-onset encephalitis typically survive. Neurons are the target for both viruses, but the progress is slower in SSPE.

SESSION 7 – Epidemiological needs for clinical trials

Chair: Dr. Jakob Cramer, CEPI

Approaches to demonstrate the effectiveness of Nipah vaccines

Dr. Marie-Pierre Preziosi, WHO R&D Blueprint (on behalf of Ana María Restrepo, WHO R&D Blueprint)

There are several pathways for bringing a new vaccine to licensure: 1) *Traditional approval* (the gold standard), based on a randomised controlled trial (RCT) with clinical endpoint or accepted correlate of protection; 2) *Accelerated approval* for therapeutics with considerable benefit over existing therapies (breakthrough status, based on preliminary clinical data) that are also considered PRIority MEdicines (PRIME); 3) *Animal rule* (only U.S. Food and Drug Administration [FDA]), or the somewhat corresponding *Exceptional circumstances* (EMA), where approval is based on animal studies due to inability to conduct clinical trials for serious or life-threatening conditions; 4) *WHO emergency use listing (EUL)*, which is a time-limited approval for use during public health emergencies of international concern (PHEIC), based on basic clinical safety data and/or immunogenicity data. In addition, there is a separate procedure for immune markers, provided they are reasonably likely to predict protection against disease. As indicated, the amount of evidence required for each pathway varies, and data missing at the time of licensure must be provided in a timely fashion post licensure.

WHO R&D Blueprint works to ensure a systematic approach to accelerated development of vaccines (Vx), therapeutics (Tx), and diagnostics (Dx), including the development of a baseline situation analysis (BSA), an R&D roadmap, TPPs, as well as trial designs for therapeutics and vaccines. For Nipah, this work is well underway, with trial designs already established. The main challenge for a Nipah vaccine trial is sample size, since outbreaks occur irregularly – as is true for all EIDs. The advantage of Nipah is that outbreaks do occur relatively frequently, with some regularity for example in Bangladesh. The TPP for a NiV vaccine includes two distinct use cases: 1) Preventing disease in a non-emergency setting through vaccination of populations living in endemic areas, as well as HCW; 2) Protection of individuals at risk in the area of an ongoing outbreak. In a population, everyone should be considered for vaccination, including pregnant and lactating women. End points to be considered for a vaccine trial include laboratory-confirmed Nipah clinical illness as the primary end point, as well as death and infection as secondary end points. Individual randomisation should be done within each study site. All study participants should be screened for seropositivity before vaccination, to allow for exclusion or stratification of seropositive individuals. If included and stratified, differences between groups of individuals with or without a history of NiV infection could serve to inform the design of an immune correlate of risk and/or surrogate of protection. The development of a correlate of protection, as noted in the TPP, requires availability of validated and standardised immunogenicity assays. Both neutralising antibodies and cell-mediated immunity appear to have a role in preventing NiV infection. Correlates of protection may vary across vaccine platforms.

In the absence of RCTs, observational studies such as case-control studies can provide valuable evidence. Suitable controls could be test negative (without circulating evidence of Nipah infection) or standard, matched controls from the same communities. This requires a population with high but partial vaccine coverage (< 90 %). An alternative approach would be to design a one arm study with a prospective cohort of unvaccinated individuals. Such a design involves challenges in terms of selecting comparator, choosing statistical methods, as well as adjusting for key confounders, but could provide additional evidence. Obviously, a highly effective vaccine will yield more robust results, regardless of the design. Furthermore, one powerful approach could be to conduct studies (observational studies or RCTs) across several outbreaks with a standardised protocol and blinding until the data monitoring committee deems the results of the trial to be conclusive and motions to end the trial for safety, efficacy, or futility reasons. In such a setting, care must be taken with α–spending (the overall probability of error), blinding for analysis, and comparability of risk in clusters as more are added to the trial.

Resolving epidemiological uncertainties to inform development of Nipah countermeasures

Professor Steve Luby, Stanford University

Some epidemiologic uncertainties remain for Nipah, which must be addressed before we can progress to licensure of therapeutics and vaccines. There are about a dozen promising therapeutics in the pipeline for treating NiV infection, but to test just one of them will require a clinical RCT with 132 Nipah patients (based on the assumptions of improving the survival seen in India and Bangladesh from 25 % to 50 %, as well as having 80 % power and 95 % two-sided confidence). Over the last 18 years, India and Bangladesh have seen 377 Nipah cases. In order to reach 128 study participants, all of the cases in the past eight years would need to be included, with no dropouts. Thus, it will only be feasible to test one therapeutic or one package of therapeutics for Nipah, and the question is how to select the most promising candidate(s) for testing.

Or, perhaps, are there undetected Nipah cases that could improve the situation? In Bangladesh, most of the cases from 2007 to 2014 were detected near the surveillance hospitals, which are typically situated in urban areas where people do not consume date palm sap. Correcting for health care utilisation among patients with symptoms of meningo-encephalitis, it was estimated that 48 % of the outbreaks were left undetected. Resolving this epidemiological uncertainty, through improved surveillance and access to rapid diagnostics, could help improve the power of Nipah clinical trials. Another prerequisite for clinical testing is clinical trial capacity in endemic, under-resourced settings.

How much can we shorten the time between outbreak detection and enrolment? This will be a critical parameter in terms of identifying cases. One challenge (in Bangladesh, which probably has the best NiV surveillance system in the world) is that epidemiologists are usually called in only at the end of an outbreak, when most of the cases have already died or recovered. This precludes enrolment in a clinical trial. Multiple strategies for early detection and enrolment could be tested.

Is it possible to test Nipah vaccine efficacy in humans? Applying the ring vaccination trial design that was successfully used for testing a vaccine against Ebola, with immediate and delayed vaccination of contacts of cases, would require 516 years with the NiV incidence observed over the past 15 years. Thus, in the absence of a large NiV outbreak, this strategy will not work for Nipah. Using a cluster randomised RCT design measuring prevention of spillover infections and onwards transmission will reduce time and allow for vaccination beforehand, yet a study including the entire population of the Faridpur and Rajbari districts of Bangladesh (who see the most Nipah cases; 3 million people) would still take 81 years to accumulate a sufficient number of cases and reach conclusive results. In contrast, a case-control study with vaccination of the same population and recruitment of 10 matched controls whenever a case is identified would require only 7 years to be completed (provided 70 % vaccine coverage). Such a study is within the realm of the possible, and would yield data on the effectiveness of a vaccine (case-control efficacy estimates of other vaccines have generally aligned with RCTs). Notably, a study like this is easier to implement with a single dose vaccine, and increasing case identification would improve power.

Some years have shown higher spillover rates than others. Understanding the reasons for this could improve our ability to model future outbreaks and design successful clinical trials for therapeutics and vaccines. Variations in viral shedding from bats, temperature, and ecological stress have all been suggested to impact spillover rates. Another epidemiological uncertainty that is worth resolving, is the apparent decrease in spillovers in Bangladesh during the past few years, correlating with fewer people in Bangladesh consuming date palm sap (in accordance with guidelines from the public health authorities). Is the number of cases actually declining, and how will this affect future clinical trials? The conclusion is that it should be possible to clinically test one therapeutic regimen and one vaccine against Nipah, though the international community will need to come together to decide on which candidates to advance towards clinical evaluation.

Practical issues in advancing human testing of Nipah vaccines in Bangladesh

Dr. K Zaman, icddr,b

With its five study sites across Bangladesh, icddr,b has been involved in about 85 clinical trials, of which many have been vaccine trials (phases I-IV). The first trial involving a Nipah vaccine could conceivably be designed as a randomised phase I/II placebo-controlled trial, assessing the safety and immunogenicity of the vaccine in healthy adults and children at different doses/dosing schedules, and potentially providing some preliminary information on vaccine efficacy. Such a trial could involve about 100 participants and last for about 36 months (provided enough cases).

The Directorate General of Drug Administration (DGDA) is the national regulatory body responsible for approving new therapies for use in Bangladesh. Their objective is to ensure easy access to safe, effective, and good-quality therapeutics/vaccines at affordable prices, as well as rational and safe use of these. Moreover, they have an interest in facilitating domestic production and export, and of implementing effective surveillance systems for post-licensure follow-up. A Nipah vaccine trial at icddr,b will require approval from the icddr,b institutional review board (IRB), consisting of the Research Review Committee (RRC) and Ethical Review Committee (ERC). The study will also have to be approved by DGDA, based on the provision of 1) project summary including a summary of formerly obtained preclinical and clinical data; 2) ERC-approved study protocol; 3) IRB approval; 4) the investigator's brochure, compiling information about the vaccine from preclinical and other clinical trials; 5) informed consent form (ICF); 6) sponsor agreement; 7) good manufacturing practice (GMP) certificate for the manufacturer. Vaccine import will require the presentation of a proforma invoice, as well as the DGDA approval letter, the investigator's brochure, and the IRB approval letter.

Issues that need to be considered in relation to the development and testing of a new vaccine include: 1) Establishing preclinical and clinical study protocols; 2) Selecting study sites; 3) Implementing a Data and Safety Monitoring Board (DSMB); 4) Selecting a clinical research organisation (CRO, of which there are about 8 in Bangladesh); 5) Obtaining a clinical trial agreement (CTA) as well as material transfer agreements (MTAs); 6) Maintaining cold chain for vaccine- and sample shipping and storage; 7) Obtaining permission from the Ministry of Commerce for vaccine import. Other challenges pertaining to the conduct of a clinical trial include 1) rapid identification and enrolment of eligible participants; 2) completion of follow-up on a highly mobile study population; 3) effective management of adverse events.

Epidemiologic preparedness for Nipah vaccine/therapeutic trials

Dr. Tarun Bhatnagar, National Institute of Epidemiology, ICMR

Improving epidemiologic preparedness for Nipah trials involves 1) defining the geographic area where transmissions may occur; 2) defining the population at risk; 3) gaining a better understanding of the risk factors involved in Nipah transmission; 4) improving surveillance for rapid detection of cases; 5) understanding the natural history of disease. Important parameters for assessing the risk of Nipah transmission and guiding surveillance include 1) the distribution of fruit bats; 2) evidence of NiV infection in bats; 3) previous outbreaks of NiV; 4) high-risk behaviour in humans. Fruit bats are found all over India, and Nipah outbreaks have been seen in the North East (West Bengal) and in the South West (Kerala). Thus, a systematic, nationwide surveillance system could be necessary to be able to rapidly detect the next outbreak. With risk modelling (using information about the habitats and movements of the known NiV reservoir bats), some areas have been defined that have a higher risk of spillover, and such models may serve to guide surveillance and ensure more efficient use of available resources. A strategy for identifying Nipah outbreaks that have previously gone undetected could be the testing of archived samples from AES patients with unknown aetiology. Identifying previously unknown outbreaks would help to raise awareness about the need for improved surveillance, and could shed more light on the natural history of Nipah.

Consumption of contaminated date palm sap is a known risk factor for NiV infection, and a proposed survey plans to find the frequency of date palm sap consumption among rural residents of the six

West Bengal districts bordering Bangladesh. In addition, the number of *Pteropus medius* bat roosts near rural residents will be determined. Understanding the different behaviours that may influence the risk of Nipah transmission in different geographic regions is of importance. Outbreaks may occur in clinical settings (like in the 2001 Siliguri outbreak where 66 individuals were infected) or in family settings (like in Kerala, 2018, with 12 infected individuals). The clinical features of infection may vary from outbreak to outbreak. By the time an outbreak is detected, human-to-human transmission to secondary cases have often occurred, and it may be too late for clinical intervention with therapeutics or vaccines.

Surveillance could be facility-based or community-based, targeting different populations. Event-based surveillance for identifying Nipah cases often targets events of AES (primarily due to JE), but (in Bangladesh and India, at least) overlooks ARDS. In addition, at least two suspected cases are required to warrant further investigation. This means that many cases are likely to be left undetected. One future approach for India could be to establish sentinel sites for NiV detection in high-risk areas, and detect outbreaks through clustering of cases of AES or ARDS that occur in close geographical proximity and within a timespan of 21 days, where an algorithm serves to select samples from possible NiV cases for laboratory testing. The testing can be conducted by the > 75 laboratories in the Department of Health Research (DHR) / Indian Council of Medical Research (ICMR) Virus Research and Diagnostic Laboratory (VRDL) network.

Respiratory illness surveillance was set up in India in 2016-2018 as part of the Global Health Security Agenda (GHSA), with nine participating laboratories across the country. This system could potentially be used for identification of Nipah cases among the many patients with respiratory symptoms who are never properly diagnosed (50-80 %, depending on age group). In addition to the public healthcare system, private institutions and hospitals should be involved in the work of identifying cases and clusters, sharing the same protocols for surveillance and sample referral. Based on learnings from Bangladesh, contacts are now classified into two categories: 1) Has had contact with body fluid (blood, urine, saliva) from confirmed/probable case; 2) Has spent time in close proximity of a confirmed/probable case for ≥ 12 hrs. This allows for active surveillance and management (including isolation) of high-risk contacts, and passive surveillance of low-risk contacts. Screening contacts for NiV may identify individuals with subclinical infections: 3 of 279 close contacts from the 2018 Kerala outbreak had subclinical infection, though this has not been seen in Bangladesh.

Current epidemiological status of Nipah virus encephalitis in Malaysia in the context of vaccine/therapeutic trials

Professor Tan Chong Tin, University of Malaya

The 1998-1999 Nipah outbreak in Malaysia lasted for 8 months, spreading from the Perak district to Negeri Sembilan and Selangor districts by way of transportation of sick pigs between pig farms. The pigs were the primary source of transmission to humans, but at least one patient had been infected by his dog. Cats, horses, and goats were also infected, and could potentially have served as a source of infection to humans (it has later been shown that horses were the source of infection in the 2014 Nipah outbreak in the Philippines). Since 1999, there has not been another outbreak involving pig farms in Malaysia, partly due to increased awareness by pig farmers not to have fruit trees in close proximity to the pig sties. However, the swine industry in Asia is huge, and another outbreak may just be waiting to happen. Other livestock, such as goat, may also pose a risk. Goats represent a major Asian industry, are susceptible to NiV infection, and are herbivorous scavengers. Whether the infection of goats involves the lungs remains unknown – this could aid transmission to humans. Consumption of date palm sap is known to be a risk factor of NiV infection, and this cultural habit is found in and around Bangladesh.

The risk of direct NiV infection from bats to human is low. However, in a seroprevalence study in Malaysia, more than 10 % of the participants tested positive for antibodies against NiV. Among the 110 members of the Malaysian households affected by the 1998-1999 outbreak, 27 % had symptomatic infection and 8 % had subclinical infection with positive serology. Of the asymptomatic subjects, 16 % were found to have brain abnormalities by MRI. Relapsing encephalitis was seen in 9 % of the cases. In total, there are more than 100 Nipah survivors in Bangladesh, and the survivors experiencing relapsing disease could be suitable for participation in clinical trials for Nipah therapeutics.

Despite the absence of reported Nipah outbreaks in Malaysia for the past 20 years, transmissions may have occurred that were not detected. Cases of NiV encephalitis may have gone undetected, in Malaysia as well as in other countries of Southeast Asia. Populations at risk are those exposed to bats and other susceptible animals, as well as those consuming date palm sap. Ribavirin and other therapeutics may have a role in the management of Nipah disease. In a clinical trial with 190 patients, the 140 who received ribavirin had a 36 % reduced mortality compared to controls.

Session 7 Q&A

Q: Has the WHO R&D Blueprint considered recommending certain data formats in accumulated clinical trials to allow for aggregation of data across studies?

A: Yes, this is certainly part of the master protocol. There should be standard operating procedures (SOPs) in place to allow for similar collection and processing of data across sites.

Q: A master protocol will be extremely challenging to implement, as it involves many sites. What would you say are the ethical aspects of conducting long-term RCTs for Nipah?

A: This applies to all of the EIDs, and the best guarantee of ethical conduct is a well-designed clinical trial. A solid data monitoring committee and governance structure, with well-defined plans for study reviews (including triggers), is a prerequisite – as is the involvement of national authorities and close collaboration across sites. One good example was the testing of therapeutics against Ebola.

Q: Does WHO support each of the pathways outlined, in terms of prequalification?

A: Discussions with regulators in WHO R&D Blueprint indicate that the traditional pathway to licensure, which includes an individual RCT, is the preferred pathway. WHO believes that this is possible, also for therapies targeting EIDs.

Q: Bangladesh has a very high vaccine coverage in general. If the vaccine coverage of a NiV vaccine for clinical testing exceeds 90 %, will the clinical trial still succeed?

A: No, for a case-control study of a NiV vaccine to succeed, vaccine coverage needs to be below 90 %. However, mass vaccinating people of all ages in a clinical trial is expected to yield less coverage than the ordinary child vaccination programmes.

Q: What would happen if the NiV vaccine had 70 % vaccine efficacy instead of 90 %?

A: A poorer vaccine efficacy would require a larger trial for evaluating the vaccine, and in planning the trial you could play around with a number of factors like this. However, the main point was to discuss the feasibility of the different study designs, where the case-control design is perhaps the only one that will yield sufficient data within a feasible time frame.

C: In Bangladesh, people are willing to take a vaccine once they have seen evidence of the disease and/or effects of the vaccine.

A: There is not a simple technical response to this, but we need to understand how an outbreak is experienced by the population, in terms of fear and other emotions, and consider how this affects vaccination rates. Reaching out to the community in a good way is what will enable us to reach 70 % vaccine coverage in an outbreak setting.

C: Perhaps one should not be so pessimistic in terms of sample size, consider the success we had with bringing an Ebola vaccine to licensure. Focusing on the alternative licensure pathways, with licensure based on immunogenicity data and limited clinical data, could be more constructive.

A: This was not to pre-empt the choice of licensure pathway. Large-scale vaccination trials would provide protection, insights, and safety data, even if being part of the post-marketing follow-up.

C: Comment from U.S. FDA: Seeking licensure through the animal rule (probably more straightforward than the accelerated rule), while planning for a large-scale clinical study as part of post-marketing follow-up would be recommended.

A: That would be brilliant. There could also be a conversation between innovators and authorities on a way forward.

C: New data could change the presumptions used in sample-size modelling, so far.

A: The modelling is indicative of a potential for realising larger sample sizes, but requires field trials.

C: You never know how an outbreak develops, as seen with Ebola. It would be good to plan for alternative scenarios, where a trial spans several outbreaks given that a single one of them is not large enough to reach a conclusion. Also, experience with Ebola has taught us that improving the overall standard of care is important for acceptance in the communities affected by the outbreak and the trial. A Nipah trial should also include measures to improve the standard of care.

A: Yes, there is a need for trust in the communities, and people need to feel that when they seek healthcare they benefit from it.

Q: To FDA: If doing the alternative pathway and relying on safety data for licensure, what sample size would be required?

A: Safety data will depend on a variety of factors. There will need to be safety data from the population where a vaccine or therapeutic will be used, but these could be substantiated by global data. In general, a considerable amount of safety data is required, for licensure as well as for informing confirmatory studies post licensure.

C: Dr. Zaman's presentation on vaccine testing in Bangladesh did not mention the need for permission to use genetically modified organisms (GMOs). There may be different implications for using replication competent versus replication incompetent organisms.

A: Ok, more information about this is welcome.

Q: Phase II efficacy testing of a NiV vaccine will have to be conducted in Bangladesh or another Nipah endemic location. Are there any preferences for the location of a phase I safety trial, which could also be conducted in the country of manufacturing of the vaccine since it only involves healthy volunteers?

A: Either way works, no strong preferences.

Q: In terms of feasibility, a phase II trial would be the only opportunity for a randomised Nipah vaccine trial, though there may be ethical issues with giving placebo to at-risk individuals. Is it worth spending time on considering randomisation just for phase II?

A: Yes, randomisation is valuable since it is not known how effective the vaccine will be. Also, ethical considerations are less poignant while the efficacy of the vaccine is unknown.

Q: Were anthropologists involved in the investigations of the 2018 Kerala outbreak? If so, at what stage? What role may anthropologists have in Nipah surveillance?

A: The epidemiologists were called in late in the outbreak, after the last case had been identified. In a subsequent case-control study, an anthropologist was engaged to talk to family members and neighbours to gain a better understanding of the index case, who had been to the jungle, and his pet rabbit and dog were dead. He also enjoyed eating fruits, etc. These are valuable insights. In a comprehensive surveillance system including risk assessments, anthropological support may be included, but the plans for such a system in India have not yet been finalised.

Q: Prof. Tin, given your experience and the fact that we may only have one shot at a trial for a Nipah intervention, do you have a preference for a vaccine or a therapeutic?

A: There are pros and cons to both, therapeutics having the disadvantage that onset of therapy may be delayed, and too late to save the patient, while mass vaccination may be difficult to achieve in real life. Vaccination of targeted high-risk groups could be one way of mitigating the issue, but I would rather not conclude one way or another.

SESSION 8 – Progress and challenges in vaccine development

Chair: Melanie Saville, CEPI

Nipah vaccines: Overview

Dr. Eun-Chung Park, NIAID/NIH

Vaccines against NiV typically target glycoprotein (G) or fusion protein (F), found on the surface of the virus, which are both involved in cellular entry. Viral strains used in vaccine research include HeV, NiV-M, and NiV-B. Vaccine evaluation is conducted in a wide range of NiV-susceptible animals, including mice, hamsters, ferrets, cats, pigs, ponies, and AGM. Different vaccine platforms exist, including subunit vaccines (mostly variants of sG), nucleic acid-based vaccines, passive transfer (monoclonal or polyclonal antibodies), and numerous viral vectors. In addition to the human vaccines, there have also been made vaccines for immunisation of intermediate host animals, primarily tested in pigs.

Protection with a single dose of vaccine is very important for the ability to curb an outbreak. A number of vaccines have been tested pre-clinically with a single dose, with good results in different animal models. The degree of variability in viral sequence may be of importance to the vaccine's efficacy, since challenge with a homologous viral strain (the same NiV strain as was used in the vaccine) yields the highest level of protection.

Two important challenges in NiV vaccine development are 1) difficulty of comparing the vaccines with respect to efficacy, since results are obtained using different study protocols (dose, schedule, route of administration, route of challenge), different strains of challenge virus (NiV-B, NiV-M, HeV), and different types of animals (*e.g.*, hamster, ferret, AGM); 2) correlates of protection remain unknown. Important gaps include 1) lack of a therapeutic vaccine; 2) little is known about the T-cell response; 3) lack of vaccine targeting T-cell immunity; 4) unknown durability of protection; 5) lack of tailored adjuvants; 6) incomplete cold chain (alternatively, stability issues in the absence of cold chain).

Ending with a question to the audience: Should a bat vaccine be considered to control NiV transmission? Bats constitute the reservoir, not only of NiV but also other viruses causing disease in humans. Preventing viral shedding from bats could prevent human disease.

A subunit vaccine (HeV-sG-V) to protect against Nipah and Hendra disease

Dr. Rong Xu, Profectus BioSciences

The Profectus subunit vaccine (HeV-sG-V) is a HeV sG vaccine adjuvanted with alum. It has been marketed in Australia as a vaccine for horses (Zoetis Equivac® HeV) since 2012, and a human vaccine has now completed pre-clinical development. The pre-clinical studies that have been conducted include three rabbit immunogenicity studies, four proof-of-concept (POC) challenge studies in ferrets (one study) and AGM (three studies), and one good laboratory practice (GLP) rabbit toxicology study. Based on the data from these studies, CEPI awarded $25 million to Profectus BioSciences, Emergent BioSolutions, and PATH in May 2018, for the development of a NiV vaccine within 5 years. This work shall include 1) manufacture of the vaccine and evaluation in phase I and II clinical trials; 2) conduct of non-clinical correlate of protection studies to support licensure under the animal rule; 3) stockpiling of up to 100,000 vaccine doses for emergency use.

The vaccine substance is to be manufactured by Emergent BioSolutions, whereas the vaccine product will be manufactured by the Infectious Disease Research Institute (IDRI). GMP manufacture of vaccine substance and product for the clinical trials were completed in June and October 2019, respectively. Certificates of analysis (CoA) for the clinical trial material (CTM) to be used in the investigational new drug (IND) submission were obtained in December 2019.

Three clinical trials have been planned: 1) Phase I adult trial (U.S., $n = 192$) to assess safety and tolerability (primary endpoint), as well as immunogenicity (secondary endpoint) of the vaccine, at one and two doses of escalating dosages (10, 30, and 100 µg); 2) Phase II adult trial (endemic area, $n = 600$) to confirm safety and immunogenicity of the dose and dosing regimen selected after phase I, as well as assessing the value and timing of a booster dose; 3) Phase I/II paediatric trial (endemic area, $n = 300$) to assess safety and immunogenicity in children (2-17 years). Assays for clinical testing, including ELISA assays as well as pseudotyped vesicular stomatitis virus (VSV) neutralisation assays, for both NiV-B and NiV-M, have already been developed and verified. Interim reference standard serum from NiV-B sG + NiV-M sG immunised rhesus macaques has also been generated and characterised, as have serum pools for normal controls (NC) and low- and high-titre quality controls (QC-L and QC-H, respectively).

Profectus will now pursue the animal rule regulatory pathway, with the HeV-sG-V IND. A request for fast-track was submitted to the FDA on December 13, 2019. The phase I study is likely to commence in February 2020.

ChAdOx1 Nipah vaccine

Professor Sarah Gilbert, Jenner Institute, University of Oxford

In spite of being replication deficient, Ad used in vaccines is still able to elicit strong immune responses: Not only does the virus present the antigen, but it also elicits danger signals in the host that are powerful immune activators. This leads to a potent immune response with good immunological memory in the form of memory B- and T cells. Replication deficiency is an important safety measure to avoid dissemination in immunocompromised individuals. Using simian adenoviruses, which do not naturally infect humans, has the advantage of avoiding neutralisation of the vaccine vector upon vaccination into a patient who has already experienced human Ad infection (this has been seen for the human Ad5 vector). The antigen is expressed by double-stranded DNA fragments contained within the vector, and is not a structural part of the virion. This means that vaccines using the same strain Ad are structurally identical, streamlining the GMP production of Ad vaccines against a variety of different antigens, and eliminating the need for optimisation of each individual vaccine vector. Furthermore, the expression of antigen can be controlled by a repressor (halting expression during manufacture) and/or a mammalian promoter (inducing antigen production once injected into a human/mammalian recipient).

ChAdOx1- and ChAdOx2-vectored vaccines have been developed for a wide range of EIDs, including MERS, Lassa, Nipah, and Ebola. For each of the pathogens, a vaccine construct has already been made. The subsequent development involves 1) testing immunogenicity and capacity for inducing neutralising antibodies; 2) efficacy studies in animals; 3) obtaining funding for clinical trials; 4) phase I/II clinical testing. The Jenner Institute already has a number of vaccines in clinical development, including vaccines against pandemic flu, MERS, Zika, and Chikungunya. More than 250 volunteers have so far received a dose of ChAdOx1-vectored vaccine.

One big advantage of the ChAdOx vaccines, is that they can be thermostabilised by mixing with sugar and storing on a dry membrane. This solves the issue of maintaining a cold chain in an outbreak situation, and vaccines may be stored for months at an elevated temperature without loosing efficacy (as demonstrated for the ChAdOx1 rift valley fever [RVF] vaccine). Storing at ambient temperature will also greatly reduce the cost of stockpiling.

The ChAdOx1 NiV vaccine, expressing NiV G protein, proved to result in complete protection against NiV-B and NiV-M challenge in a study involving hamsters. A booster dose yielded higher titres of neutralising antibodies, but even one dose was protective. The vaccine was well tolerated by the hamsters, and circulating virus levels remained below the limit of detection throughout the study. The vaccine also resulted in partial protection against HeV after a single dose. Next steps for this vaccine include 1) testing in AGM 2) preparing seed stock for GMP manufacturing; 3) phase I trial in UK; 4) further studies in Bangladesh, most likely.

NiV is a pathogen for which it is really easy to demonstrate vaccine efficacy. Thus, other points are more important to consider: 1) Rapid onset of immunity; 2) One (ideally) versus two doses; 3) Duration of immunity; 4) Thermostability (long-term for stockpile, short-term for transport to clinic); 5) Safety for the whole population, including the very young, elderly, and pregnant women; 6) Plans for vaccine licensure, where immunobridging could help mitigate the issue of not being able to conduct a full-blown phase III clinical trial. In parallel to the development of vaccines, it is also important to 1) ensure the development of standardised assays (preferably cross-species); 2) make sure that clinical trial protocols are ready and available for whenever the next major Nipah outbreak occurs; 3) develop plans for vaccine licensure.

Development of measles virus-vector vaccine for Nipah virus infection

Professor Chieko Kai, University of Tokyo

Vaccines vectored by morbilliviruses such as measles virus (MV) and canine distemper virus (CDV) have been used world-wide for more than 50 years, and demonstrated excellent safety and stability, as well as good efficacy yielding life-long immunity. The MV and CDV vectors both have the capacity to express several immunogenic genes simultaneously, and are therefore attractive vector candidates for multivalent vaccines.

Using reverse genetics, the Edmonston MV strain was genetically modified to incorporate the NiV G protein and create a recombinant NiV vaccine strain (rMV-Ed-NiVG). In a golden hamster model, this vaccine (given twice, on day 0 and day 21) protected against intraperitoneal (ip) challenge with homologous rNiV (day 28), which was comparable to the results seen with rMV-HL-NiVG, a NiV G protein vector vaccine based on the wild-type MV HL strain. Other vaccine candidates using NiV G protein, but based on other MV vector strains, did not perform as well. The rMV-Ed-NiVG vaccine also protected against NiV infection in AGM, using a similar protocol.

Some concern has been raised regarding the chance of pre-existing antibodies against MV in humans affecting vaccine efficacy, but results from studies in hamster show that pre-immunisation with plain MV-Ed did not impair the ability of rMV-Ed-NiVG to elicit an antibody response. With funding from CEPI, the development of an MV-based vector vaccine against NiV will continue.

Development of a vesicular stomatitis virus-based vaccine to prevent Nipah virus disease

Dr. Gray Heppner, Crozet BioPharma LCC

The objective of Crozet BioPharma is to develop a safe, single-dose vaccine with a rapid onset of protective immunity against all Nipah strains. This work is based on experiences with the VSV Ebola vaccine, which had a rapid onset of protection of about 10 days when used in the Ebola outbreaks of West Africa and the Democratic Republic of Congo (DRC), caused by the Zaire Ebola virus (ZEBOV). The rVSV-Nipah vaccine candidate (rVSVΔG/ZEBOVgp/NiVG) is a live, attenuated recombinant VSV (rVSV) expressing NiV-B G protein, as well as glycoprotein (gp) from Ebola virus (EBOV), and is similar to Roche's now licensed rVSV-ZEBOV (Ervebo™) vaccine. The EBOV gp moiety is retained to maintain receptor-mediated viral entry of the vaccine, and support immunogenicity. A single dose of the rVSV-Nipah vaccine protects hamsters as well as AGM against NiV challenge. Furthermore, passive immunisation (in hamsters) with serum from rVSV-Nipah-immunised hamsters was protective, suggesting a role for neutralising antibodies in protection against Nipah disease.

The TPP of rVSV-Nipah include the following criteria for a successful vaccine: 1) Capable of controlling a NiV outbreak and prevent disease in high-risk groups; 2) Can be used on individuals of all ages; 3) Has a safety profile on par with WHO recommended vaccines; 4) Has > 90 % efficacy, with rapid onset of < 14 days; 5) One dose is sufficient for protection; 6) Protective for at least 6 months when used in an outbreak setting; 7) Intramuscular or subcutaneous administration; 8) Covers all circulating NiV strains (NiV-B, NiV-M); 9) Is stable for 5 years at 2-8 °C (for stockpiling); 10) Presented in 1-dose vials (liquid frozen or lyophilised); 11) Suitable for emergency use authorisation (EUA)/EUL; 12) Appropriate for WHO prequalification.

The VSV vaccine platform has certain advantages: 1) Naturally attenuated due to host range restriction (self-limited disease in horses, pigs, and cattle, but generally asymptomatic in humans); 2) Low prevalence of immunity to the vector; 3) VSV RNA does not integrate; 4) Allows for expression of large, foreign transgenes (multigenic potential); 5) Readily pseudotyped with heterologous viral glycoproteins; 6) Complete VSV G protein deletion results in attenuated phenotype; 7) VSV G protein does not elicit neutralising antibody responses targeting the vector; 8) Recombination has not been reported; 9) Strong documentation already exists for the potency of the vaccine platform (in a phase Ib trial, rVSV-ZEBOV induced rapid and long-lasting seroconversion upon a single immunisation with a wide range of vaccine doses [$n = 512$]; in the phase III trial in Guinea, 100 % efficacy was demonstrated in humans [$n = 3{,}537$]).

With funding from CEPI, pre-clinical and clinical development of the rVSV-Nipah vaccine will continue. Key activities include 1) production of CTM; 2) animal toxicology and immunogenicity/efficacy testing; 3) assay development for serology testing and virus detection/quantification; 4) defining surrogates of protection in NHPs; 5) clinical testing in phase I (U.S.) and phase II (Bangladesh) clinical trials.

F vs G Nipah vaccines: Stabilising the pre-fusion conformation of F for a fair comparison

Dr. Keith Chappell, University of Queensland

Most of the Nipah vaccines currently in development target the NiV G protein, but the F protein may also be a viable target. Before viral fusion to target cells, the F protein exists in a highly unstable pre-fusion conformation. For a fair comparison of vaccines based on NiV G versus NiV F, it is vital that the F protein is stabilised in its pre-fusion conformation. The importance of fusion proteins was first seen with respiratory syncytial virus (RSV), where antibodies targeting the pre-fusion form of the fusion protein were shown to be the ones responsible for neutralisation. These results were published in 2012.

The University of Queensland is developing a "molecular clamp" platform technology, which enables trimerisation of viral surface proteins, thereby exposing the surface protein's epitope that is primarily responsible for inducing neutralising antibodies. Trimerisation is achieved through the incorporation of a highly stable trimerisation domain into class I and III viral fusion proteins (which are trimers in their natural form). Importantly, there are a number of class I and III envelope proteins of human and veterinary importance, including MERS coronavirus (MERS-CoV), Lassa virus (LASV), and ZEBOV. The molecular clamp is a trimerisation domain based on the six-helix bundle of human immunodeficiency virus (HIV) gp41, providing very high stability and a melting temperature of about 90 °C.

The manufacture of antigen is conducted in CHO cells, which are well known to the industry, scalable, and fairly straightforward from a regulatory perspective. Protein produced in the endoplasmic reticulum naturally trimerises, are stabilised by the molecular clamp, and secreted. Monoclonal antibodies for the molecular clamp are used for purification, resulting in a high yield of soluble, pure, and stable recombinant protein. Trimerisation is verified by size exclusion chromatography and electron microscopy, and the antigens are tested against available clones of mAbs binding the neutralising epitope. With the molecular clamp platform, The University of Queensland aims to supply CEPI with highly stable reference antigens, which can be subjected to multiple freeze-thaw cycles without loosing their binding properties.

The question now is, which antigen will produce the better vaccine; sG or molecular clamp-stabilised F (mcsF)? This is addressed in the following talk.

Developing a 'One Health' Nipah virus vaccine to protect animal and public health

Dr. Simon Graham, The Pirbright Institute

Pigs played a really important role in the outbreak in Malaysia and Singapore. The damage that was done to the Malaysian pig industry in eradicating the virus is still felt today. Nipah infection in pigs results in a less severe disease, yet poses a threat to human health. In a One Health perspective, a

Nipah vaccine for pigs could reduce the severe economic consequences of an outbreak, as well as the risk for human disease.

Eight vaccine candidates for NiV were selected and compared (see Figure 10), all of which had been tested for immunogenicity in pigs, and three of which had been tested for efficacy. Six of the candidates targeted the NiV G protein, whereas two targeted the F protein. The antigens used included mRNA, protein, and viral vectors.

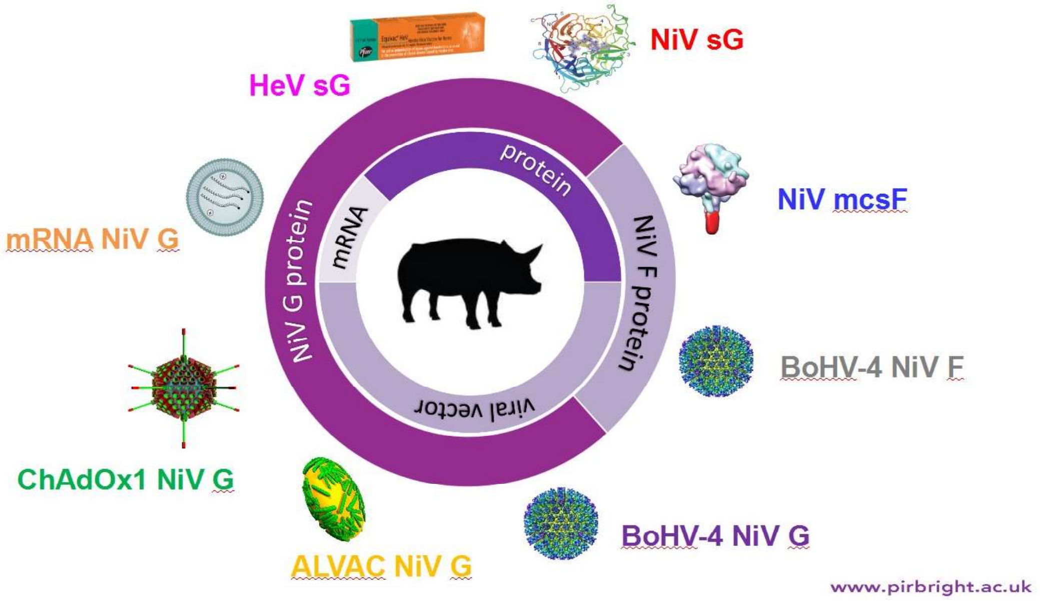


Figure 10: Nipah virus vaccine candidates (Pirbright Institute)

ALVAC-NiV G was the only one to have demonstrated protection against NiV in pigs in experimental studies. Herpes viruses (HVs) naturally stimulate very strong T-cell responses, and this property is being exploited to develop HV vector vaccines with the potential to elicit strong cell-mediated immunity. All vaccines were tested in the same regimen, with intramuscular immunisations at day 0 and day 21, and antibody titres were tested. All of the vaccines elicited NiV antigen-specific antibodies and neutralising antibodies (ALVAC-NiV G only after the second immunisation), where the NiV sG vaccine yielded the highest short-term titres. NiV sG, mcsF, and bovine HV NiV G (BoHV-NiV G) provided the highest titres of NiV cell-to-cell fusion inhibiting antibodies. The HV vector vaccines yielded the strongest T-cell responses, as measured by intracellular interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α) double-positive cluster of differentiation (CD) 4 and CD8 T cells on flow cytometry.

Efficacy was also tested, though only for three of the vaccine candidates (NiV sG, NiV mcsF, ChAdOx1 NiV G), with the same vaccination protocol. All three vaccines resulted in strongly reduced titres of circulating virus, as well as reduced levels of virus found in tissue biopsies, indicating a satisfactory level of protection. With immunogenicity and efficacy tests conducted, next steps include

1) testing single-shot efficacy; 2) selecting a prototype vaccine; 3) studying duration of immunogenicity and, finally, safety and immunogenicity in pigs under field conditions in Bangladesh and Malaysia.

The Pirbright Institute is also tracking NiV G-specific plasma B-cell responses, and using cell sorting to isolate NiV sG tetramer-labelled B cells. These, in turn, are cloned, and the resulting mAbs are tested for NiV G specificity. In an early attempt, one of the isolated mAbs was found to be cross-reactive to NiV sG and HeV sG, as well as neutralising in a pseudo-type assay.

Marketability of NiV vaccines for pigs may be challenging due to the sporadic nature of NiV outbreaks. An alternative strategy could be to include NiV antigen in a vaccine that is already widely used, creating a bivalent vaccine. Live attenuated porcine reproductive and respiratory syndrome virus (PRRSV) vaccines are extensively used in Southeast Asia, due to the impact of PRRSV on porcine reproduction. Reverse genetic engineering allows for expression of NiV G in PRRSV modified live virus (MLV), which could be an attractive vaccine for the veterinary market. The Pirbright Institute now hopes to test this bivalent vaccine in pigs.

Session 8 Q&A

Q: With a HeV antigen, as used in the HeV-sG-V Nipah vaccine, what are your thoughts for a HeV vaccine?

A: Regulatory work is in progress for use of the HeV-sG-V vaccine against HeV in Australia. The vaccine has been shown, pre-clinically, to be effective against both HeV and NiV.

Q: What is the intended vaccination schedule of the ChAdOx1 vaccine?

A: A single vaccination is sufficient in animals, and although both one and two doses will be tried in the phase I trial, the hope is that one dose will be sufficient to achieve protection.

Q: Can you explain the striking differences in vaccine efficacy seen with different MV strains?

A: This could potentially result from the different levels of attenuation of the different strains. For a lethal disease like Nipah, limiting side effects of vaccination through attenuation is not an objective, and one should focus on using the most efficacious strain. For less potent diseases, a broader range of the strains would perhaps provide sufficient protection, but for Nipah only a couple of the strains were potent enough.

Q: Is there a chance that VSV may persist or be shed from recipients?

A: In the clinical trials, saliva, urine, and blood will be tested for viremia. From the Ebola trials, we are aware of some shedding from adults and children. There is no precedent of VSV persisting.

Q: Can rVSV-Nipah cause neurovirulence?

A: Given the nature of NiV, neurovirulence studies will be conducted, and discussed further with the regulatory authorities.

Q: Has the molecular clamp approach been used for any other pathogen?

A: We aim to show that this approach works for a well characterised pathogen, with well-known correlates of protection, such as the flu virus.

Q: Is two-dose vaccination the standard approach when immunising pigs?

A: May be. We will see in a few months time if a one-dose regimen is effective.

Chair: There are a number of NiV vaccine candidates with very strong preclinical data, in a number of different animal models. This is encouraging in terms of succeeding in developing a Nipah vaccine, and it is encouraging to see that some of the vaccine candidates are about to commence clinical testing.

SESSION 9 – Progress and challenges in therapeutics development

Chair: Professor Benhur Lee, Mount Sinai

Putative Nipah therapeutics: pathophysiologic, target profile, and trial design considerations

Dr. Libby Higgs, NIAID/NIH

NIAID's involvement in the development of therapeutics for Nipah was originally inspired by the 2018 Kerala outbreak, with WHO requesting our support for the development of a concept and protocol for the use of Nipah therapeutics in the context of the outbreak, along with the WHO R&D Blueprint Therapeutics Trial Design working group, ICMR, and Kerala Officials. The protocol was prepared quickly, leveraging previous work, but was only approved as the outbreak ended. Monoclonal antibody shipped from Australia to be tested therapeutically in the outbreak was left unused. The work continued, however, and the ambition is to establish a feasible multinational, multi outbreak Nipah treatment protocol for establishing the safety and efficacy of candidate Nipah therapeutics (and, ideally post-exposure prophylaxis [PEP], but this work has not yet started), with the support of WHO, CDC, ICMR, and NIAID. Currently, an IRB-approved treatment protocol exists that is suitable for use internationally, and linked to more than 50 SOPs developed by ICMR. India and Bangladesh have accepted the protocol and are working to implement it, and other interested countries are encouraged to join. The short-lived nature of Nipah outbreaks requires the immediate implementation of the protocol from the start of an outbreak, and ongoing planning for such an event. Rapid implementation is facilitated by a stand-by multinational research capacity, as well as prepared sites in areas previously affected by an outbreak. In addition, mobile teams with specially adapted field equipment are preparing to respond to new outbreak areas.

Early identification of infected individuals is key to success with therapeutics. With delayed seroconversion (IgM at day 4), there is a need for rapid POC diagnostics (such as the mobile NAT developed by ICMR) and active surveillance of both pulmonary and CNS disease. We already have a fairly good understanding of the Nipah pathogenesis, supported by prospective natural history studies in NHP and humans, which will help inform the TPP for therapeutics. To facilitate access of therapeutics to future outbreaks, ICMR has taken the lead in ensuring free production and supply, as well as putting in place shipping agreements with manufacturers.

In designing a treatment protocol, a number of factors need to be considered: 1) What is the objective of the treatment (reducing morbidity/mortality, preventing infection); 2) What clinical phenotypes can be expected, based on viral tropisms; 3) How to account for NiV strain differences. There are currently a limited number of therapeutics with efficacy data in NHP and at least phase I study in humans, of which m102.4 and remdesivir are the most prominent candidates. A protocol for acute Nipah disease has now been established, but protocols for PEP in high-risk contacts and HCW are still missing. In the latter, the m102.4 is likely to be a good candidate.

Acute clinical Nipah disease phenotypes include vasculitis, encephalitis, pulmonary disease, and myocarditis, and represent a broader clinical spectrum than is accounted for in the current clinical case definitions. Thus, we may need to take on a broader set of case definitions in Nipah surveillance. The AGM model is not perfect, but it does emulate the clinical disease and is helpful for screening therapeutics. The late onset of viremia seen in animal models, due to the tropism of NiV for the smaller endothelial cells found in microvasculature, suggests that therapeutics should be able to penetrate organs and tissues. Also, it means that rapid diagnostics cannot depend on testing of peripheral blood.

The differences between NiV-B and NiV-M are challenging to account for in a multinational, multi outbreak protocol for clinical testing of therapeutics: Which strain should the studies be powered for, given the differences in mortality rates (75-100 % for NiV-B, 47 % for NiV-M)? With a DSNB with close oversight one can do both, stratified by outbreak and with different rules for the different pathogens: The DSNB can stop the study in the event of higher mortality rates, which allows for a smaller *n* with a successful therapeutic. Alternatively, one can do a pre-planned sample size readjustment. Both of these approaches have been tested in clinical trials against Ebola.

Delivering therapeutics to the brain for treatment of NiV encephalitis (and in general) has proven very difficult, due to the BBB. Tailored transporters are being developed, but are not available at this time. Ideal characteristics for a Nipah therapeutic are: 1) Quick onset of antiviral activity; 2) Effectiveness against both NiV-B and NiV-M; 3) Penetrates affected tissues and cells; 4) Long half-life for easier and safer clinical management; 5) Acceptable safety profile in humans. Monoclonal antibodies do not cross the BBB, and use in combination with a therapeutic that does, such as remdesivir, may be necessary. One could also consider using a cocktail of mAbs.

The idea is for the study protocol to run continuously, across outbreaks/strains and in different countries, to continuously optimise Nipah therapy as new treatments become available. The participants will be randomised to standard of care (SOC) plus placebo or SOC plus treatment at a 1:1 ratio. Once an endpoint has been reached, the study will be notified by the DSMB, and the study can continue with testing the new standard of care (if changed as a result of the study, for example by including m102.4 in the standard of care) with the next therapeutics candidate. For the study to be able to compare different study sites and settings, it is important that clinicians come together to establish a common standard of care. The issue with strain diversity is resolved through stratification on outbreak. In addition, one will stratify for neurologic involvement, based on a simple yes/no assessment. Due to the possibility of relapse of NiV infection (as has been the case for Ebola), a new treatment paradigm is considered where acute treatment is followed by maintenance therapy to prevent relapse from residual virus.

Monoclonal antibody countermeasures for pathogenic henipaviruses

Professor Christopher Broder, Uniformed Services University, Bethesda

NiV tropism is conferred by the Ephrin-B2 and Ephrin-B3 receptors expressed on the surface of endothelial cells, which bind NiV G. The sG was developed to study these receptors, and also with the intent of testing its use in vaccines. As it turned out, immunisation with sG elicits strong immune responses resulting in good titres of cross-reacting, neutralising antibodies in animal models. The m102.4 monoclonal antibody was identified through recombinant phage-display human antibody isolation, using HeV sG to fish out monoclonal Fab fragments from human antibody libraries. Among the identified Fab fragments, the m102.4 Fab was selected for its ability to detect both HeV and NiV with high affinity. Subsequently, it was light-chain shuffled, affinity maturated, put into an IgG1 format, and m102.4 IgG1 was produced in CHO cells for use in passive immunisation experiments.

Passive immunisation with NiV antibodies was first piloted in hamsters, using hamster serum and mouse mAbs. Prof. Broder and his team started testing with human antibodies in parallel, in the newly developed ferret model (the first study was published in 2009). They showed that intravenous transfer of m102.4, 10 hours after infection, provided complete protection against NiV in ferrets. Later, it was shown that m102.4 blocked the binding of HeV and NiV to the Ephrin B2/B3 receptors (and, thus, viral cell entry) through binding to the Ephrin B2/B3 binding sites of HeV and NiV G. Passive transfer of m102.4 also protected against NiV-M challenge in AGM, even when administered after the onset of disease symptoms (the animals survived). Thus, it is believed that the administration of a NiV neutralising antibody provides a critical window of relief in the about 10 days it takes the host's own adaptive immune system to mount an effective response against the virus. The recovered animals, even when receiving the antibody after the onset of disease symptoms, showed no signs of viral antigen in autopsied tissues. In contrast, when challenged with NiV-B, protection was only afforded when the antibody was administered prior to the onset of disease symptoms.

The demonstrated efficacy of m102.4 in animals has spurred compassionate use on a few occasions where people have been known to be exposed to HeV or NiV, with the first case being a mother and daughter having had close contact with a horse dying from HeV infection. None of the recipients got infected, and none of them had any adverse effects. The m102.4 CHO-K1 cell line has since been donated to Queensland Health for GMP manufacture of m102.4 and controlled clinical testing. Results from a dose escalation study in humans is now accepted for publication, and the antibody seems to have been well tolerated.

A cross-reactive neutralising mAb for NiV F has also been identified, the humanised version being known as h5B3.1. This, too, neutralises NiV-B and NiV-M, as well as HeV. Human 5B3.1 has been found to bind the pre-fusion conformation of F, blocking transition to the extended intermediate conformation, and thereby preventing viral entry. The antibody has been found to bind a highly conserved epitope of F, which explains its cross-reactivity. In the extended form of F, the epitope is broken.

Animal data and results from clinical testing suggest a role for mAbs as therapeutics agents against henipavirus infection. The ability to protect from disease even when administered post infection is key to the use of these antibodies as therapeutics or PEP. It is conceivable that new, optimised antibodies and the use of mAb cocktails can further enhance the therapeutic efficacy of such treatment.

Therapeutic remdesivir treatment protects African green monkeys from lethal Nipah virus, Bangladesh challenge

Dr. Emmie de Wit, Laboratory of Virology, NIAID, NIH

Notes from this presentation are not available.

Inhibition of Nipah virus infection by targeting viral cell attachment and entry

Dr. Branka Horvat – INSERM

The Ephrin B2/B3 receptors are highly conserved among different mammalian species, which is why the henipaviruses have such broad species tropisms. Using recombinant NiV expressing enhanced green fluorescent protein (EGFP), it was shown that among the leukocytes, NiV only infects dendritic cells (DCs), at low levels. It remains possible, however, that leukocytes could capture NiV particles on their surface and trans-infect other susceptible cells. The question is if this could be used to limit NiV infection therapeutically.

With an *in vitro* transinfection assay – which involved 1) co-culture of NiV and lymphocytes; 2) washing and treating the lymphocytes with different enzymes; and 3) co-culture of the lymphocytes with Vero cells, which are susceptible to NiV – it was determined that the NiV attachment receptor (assuming a receptor was involved) was susceptible to pronase and heparinase treatment. Proteoglycans (also known as glycosaminoglycans [GAG]), of which heparan sulphate (HS; similar to heparin, except membrane-bound instead of secreted) is one example, were deemed the most likely receptor candidate. It was found that CHO cells, which do not express the Ephrin B2/B3 receptors, were fully capable of transinfection, though that this ability was cancelled with treatment of heparinase. Furthermore, cells deficient of HS had a much smaller ability to trans-infect with NiV. Heparin was also demonstrated to bind NiV, and treatment of cells with heparin pre- or post infection resulted reduced cellular transinfection. Thus, transinfection was dependent on HS and could be inhibited by heparin in a competitive manner. *In vivo*, treatment with heparin devoid of anticoagulant activity (achieved by periodate oxidation treatment of the heparin) improved survival of hamsters infected with NiV. Thus, heparin is suggested as a potential low-cost co-treatment against henipavirus infection.

The function of the henipavirus F (fusion) protein involves a conformational change resulting in the insertion of a so-called "fusion peptide" domain of the protein into the cell membrane of the target cell. The domain next to the fusion peptide, the N-terminal Heptad repeat (HRN) domain, then interacts with the C-terminal Heptad repeat (HRC) domain to initiate the fusing of viral and target cell membranes. However, lipid-anchored peptides have been designed that prevent the viral fusion, consisting of a 35 amino-acid peptide, a polyethyleneglycol (PEG) sequence, and a lipid moiety (cholesterol or tocopherol). A β-galactosidase complementation reporter assay was conducted to measure fusion of viral particles to HEK-293 cells, and the best peptide candidates were tested in animal studies. Here, it was shown that intranasally administered lipid-tagged peptides resulted in 50 % survival of NiV-infected hamsters (compared to 0 % in controls). Similarly, in AGM, the lipid-tagged peptides resulted in 33 % survival of monkeys subjected to an excessively lethal NiV inoculum. Administration of the peptide by aerosolisation instead of parenteral injection reduced the observed development of neutralising Abs against the peptide. Now, the ambition is to develop a fusion-blocking peptide in the form of a nasal spray that can be used prophylactically against henipavirus infection.

Characterization of novel inhibitors of Nipah virus

Dr. Michael Lo, Viral Special Pathogens Branch, US CDC

Notes from this presentation are not available.

Session 9 Q&A

Q: Developing standard clinical treatment guidelines is important. Will there be any stage in the multinational, multi outbreak study where treatment is only given to some patients, based on randomisation?

A: Any patient would be given treatment. It is likely that late-stage patients do not respond to treatment, but they will still be included, so that clinicians will not question whether or not to include them.

C: With the experiences made in other viruses, such as HIV, it seems prudent to also use henipavirus mAbs in combination, rather than alone.

A: Actually, the fact that evidence of antigen is never seen after recovery of treated individuals suggests the opposite.

Q: Do the ferrets ever manifest neurologic disease, and have you been able to treat them at that stage?

A: The ferrets do exhibit neurologic disease. An experiment where ferrets with such symptoms have been subjected to therapy has not yet been conducted. The closest we have come, so far, is successfully treating NHP with fever.

Q: Have you measured mAb levels in the brain?

A: No, we have never looked for the presence of mAb in the brain. Based on the damage observed in the microvasculature, however, it is likely that the mAb has leaked into the CNS. We have given up on the work of tailoring mAbs for increased passage across the BBB.

SESSION 10 – Panel discussion: Collaboration & Synergy

Chair: Professor Linfa Wang & Dr. Raúl Gómez Román

1 Exchange of materials and SOPs, biobanking

Professor Benhur Lee, Mount Sinai

Throughout Nipah@20, we have heard people mention the difficulties they have experienced getting access to samples and standards. For example, Professor Steve Luby (WHO NiV R&D Roadmap) mentioned the limited availability of clinical samples as a major hurdle in the development of new diagnostics, and the WHO R&D Blueprint suggesting to create a virtual repository of clinical reference samples for use in R&D. Dr. Jillian Sacks (FIND) and Dr. Laura Mazzola (WHO) both pointed to the need for international reference standards and accessible sample repositories. Dr. John Klena (CDC) addressed the increased difficulty of sharing positive controls internationally. Thus, a centralisation of this function to an "approved" facility may be advantageous, for instance abiding to the Clinical Laboratory Improvement Amendments (CLIA) standards for laboratories.

Dr. Kim Halpin (AAHL), representing the only OIE henipavirus reference lab, advocated for an "informal" network of reference labs for henipaviruses. How about one that is *formal*, modelled after the WHO network of influenza laboratories? Dr. Yoshihiro Kaku (NIID) told of his generation of IgM positive controls from immunised macaques, but could this also be achieved through the sequencing and synthesis of mAbs? Their development of a pseudotyped VSV neutralisation assay allows for both screening and confirmatory test, in the absence of BSL-4. It is important that such techniques are shared internationally, like they offer to do, for more efficient development of diagnostics and therapeutics. Dr. Emily Gurley (Johns Hopkins) points to a weak point of current research on NiV; the inaccurate reporting of the strains used. Notably, in particular the NiV-B strain really constitutes a number of different strains, each with small genetic differences that may impact the pathogenicity and tropisms of the virus.

The best way forward in henipavirus R&D involves more proactive collaboration across fields and nations, and avoiding a practice of working in scientific silos. Biobanking and exchange of materials through a centralised entity will be beneficial, both in terms of access and the ease with which reagents may be shared. With a centralised function, legal and IP issues may be negotiated ahead of time, allowing for straight-forward sharing with vetted institutions across the world. The function could be assigned to an existing entity, such as BEI Resources or the National Collection of Pathogenic Viruses (NCPV), or to a new one. Finally, it is important to secure funding for all of the prioritised activities mentioned, such as standardised reagent generation, sequence curation, and so on.

2 Inter-nation collaboration

Sino-French NiV taskforce

Dr. Zhengli Shi, Wuhan Institute of Virology

A Sino-French agreement to collaborate on emerging infectious diseases was established in 2004, following the SARS outbreak. The collaboration involves three Chinese and ten French institutions, and has resulted in the building of a BSL-4 laboratory in Wuhan – the first of its kind in China. Pathogens studied at this facility include Lassa, Nipah, Hendra, Ebola, and other EIDs, with work conducted on cells and in small animals. The Wuhan facility offers repositories of biological resources, conducts R&D and biosafety training, and aims to be accredited as a WHO reference laboratory for one or more BSL-4 pathogens.

Nipah represents a priority pathogen for the Wuhan facility, due to 1) its ability to infect animals and humans; 2) its high mortality in humans; and 3) the prevalence of henipaviruses and henipa-like viruses in countries of Southeast Asia, including China. Work on Nipah is divided into six work packages: 1) NiV pathogenesis; 2) Epidemiology; 3) Development of a DC-based prophylactic mucosal vaccine; 4) Therapeutics development; 5) Diagnostics development; 6) Biostatistics analyses.

The Wuhan BSL-4 facility has an annual call around April for external organisations wanting access to the facility to test their own samples or use samples from the in-house repositories. In addition, they offer an international BSL-4 biosafety training course in an annual call around September. The 9th International Symposium on Emerging Viral Diseases will be held in Wuhan, China, on October 18-20, 2020.

Update from SEARO meeting and the International Research Collaboration Platform in India/Bangladesh (requirements for therapeutics trials)

Dr. Nivedita Gupta, Scientist F, Division of Epidemiology & Communicable Diseases, ICMR

Conducting a multi-country, multi-outbreak, multi-intervention clinical trial on NiV therapeutics requires regional collaboration. Thus, on three occasions so far in 2018 and 2019, scientists from across Southeast Asia have convened to discuss issues related to Nipah R&D. Preparations for a clinical Nipah trial, with support from NIH, NIAID, and the WHO Southeast Asia Regional Office (SEARO), were on the agenda. The ICMR has committed to conducting a trial, through 1) dedicated funding; 2) establishment of a multi-disciplinary trial team; 3) adaptation of trial protocol to the Indian setting; 4) developing necessary SOPs; 5) developing and validating indigenous POC PCR diagnostics; 6) signing an MOU with the Henry Jackson Foundation (U.S.) and the Serum Institute of India for production of m102.4; 7) adapting the trial protocol to the Bangladesh setting (where chances of recruiting a sufficient number of participants are the best).

The Regional Enabler for the Southeast Asia Research Collaboration for Health (RESEARCH) Platform was established on August 28, 2019, with ICMR as secretariat. The goal of the platform is to facilitate clinical research across the region, leveraging the unique capabilities of each country and sharing expertise. With the platform in place, it will be easier to mount rapid responses to new outbreaks, and to conduct clinical trials – not only on NiV, but also other EIDs. The platform has decided on dengue fever as the first priority disease, with more to come. Of the 11 member states in the WHO Southeast Asia Region (SEAR), 10 participated in the second meeting of the RESEARCH Platform, on December 3-4, 2019. Here, concept proposals on six tracks concerning dengue were developed: 1) Clinical management of dengue; 2) Entomology and vector control; 3) Epidemiology and vaccines; 4) Community engagement; 5) PoC diagnostics; 6) Governance.

Comments from representatives from WHO Regional Offices

SEARO & WIPRO

WHO were not available for comment at this time.

3 Challenges in vaccine safety monitoring

Need to maximise safety monitoring across programmes

Dr. Robert Chen, Brighton Collaboration

The Brighton Collaboration was formed in 2000 with the goal to build trust in the safety of vaccines via rigorous science. Unlike efficacy, safety generally cannot be measured directly, but is inferred from the relative absence of adverse events following immunisation (AEFI). Rare AEFI are easily missed in the absence of a standard case definition. Thus, the mission of the Brighton Collaboration is to develop internationally accepted and harmonised standards for monitoring vaccine safety throughout the vaccine life cycle. They do this with the help of > 750 volunteers. In its 20 years of operation, the Brighton Collaboration has delivered > 60 AEFI case definitions, tiered by 3 levels of evidence, and including guidance for the collection and reporting of vaccine safety data. These case definitions have been endorsed by key regulatory stakeholders such as FDA, EMA, and WHO.

The Brighton Collaboration's Safety Platform for Emergency vACcines (SPEAC), which is being tailored to CEPI's needs, has the following goals: 1) To provide coordinated safety data review capacity, with a fully functional pool of potential DSMB members; a liaison observer for each CEPI vaccine trial; and a meta-DSMB to integrate safety knowledge across different products, pathogens and platforms; 2) To harmonise vaccine safety monitoring during CEPI preclinical and clinical trials, with landscape analyses for potential vaccine issues; an online vaccine safety resource of standards, templates, and tools; identifying relevant vaccine safety issues for CEPI vaccine candidates; developing new adverse event case definitions for CEPI clinical trials; and completing a standard template for communicating risk/benefit data; 3) To provide a continuous improvement framework.

4 General discussion

There was no time for a general discussion.

Confidential *Draft 19.03.2020*

Authors: Members of the International Organising Committee for Nipah@20
Target audience: scientific (mSphere)
Expected length: ideally no more than 5 pages

Draft summary/outline for mSphere

The Nipah Virus International Conference of 2019 (Nipah@20) was co-hosted by the Coalition for Epidemic Preparedness Innovations (CEPI), the World Health Organization (WHO), the U.S. National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Duke-NUS Medical School (Duke-NUS).

The Conference marked the 20th anniversary of the discovery of Nipah virus. Since its first identification in Malaysia and Singapore in 1999, the understanding of Nipah disease and its pandemic potential has advanced substantially. The Conference provided a forum to review the history and key scientific findings over the last 20 years, and to understand the current challenges in developing Nipah diagnostics, therapeutics and vaccines.

To foster international collaboration in the context of epidemic preparedness, Nipah@20 brought together 218 scientists and public health professionals working in 21 different countries around the globe. Importantly, all henipavirus-affected countries (Australia, Bangladesh, India, Malaysia, the Philippines and Singapore) were represented in the Conference, with their delegations accounting for 46% of all attendees.

In terms of outcomes, the two-day Conference created a scientific evidence-based framework to: (a) inform discussions between global health stakeholders participating in CEPI's Joint Coordination Group (JCG) on December 11, 2019, (b) discuss the creation of a Nipah-focused regulatory working group to facilitate data sharing and joint review of Nipah vaccine candidates, and (c) identify further multidisciplinary actions needed to respond to the pandemic threat posed by Nipah virus.

Highlights from the Conference are summarized in **Table 1.** The page numbers refer to the Proceedings from the Conference, which are publicly available at (**CEPI Communications** working on the branded document and website for citation).

Table 1. Highlights from Nipah Virus International Conference

	Session	Highlighted Topics in Proceedings	Page number in Proceedings
1	Historic Review of Nipah Outbreaks		
2	WHO Nipah R&D Roadmap	Policy and implementation issues: 1) Without effective therapy available at a medical facility, there is no clinical justification/incentive for testing/surveillance. 2) Development of a sound business case for product development is needed to move medical countermeasures forward.	21-22
3	Progress and challenges in surveillance of Nipah virus	1) ICMR has found several new viruses among bats and is exploring EID potential. Pathogen X implications and possible candidates for test runs of rapid response. 2) A Ghanian henipavirus sequence has been isolated in S. Africa.	27, 29

	Session	**Highlighted Topics in Proceedings**	**Page number in Proceedings**
		3) A lot of work on bat surveillance, but unclear how to translate into impact.	
4	Progress and challenges in diagnostics	1) International reference standards needed for calibration, comparison, quality controls. 2) A mobile diagnostic system (MolBio) is being used in India & Bangladesh and could have applications in epi, clinical trials, and outbreak response. ~11USD per test. 3) FLI ELISAs use sG expressed in *Leishmania tarentolae.* Could be alternative for sourcing protein for assays.	34, 35, 38
5	Pathogenesis and animal models	1) NIH working on **aerosol** challenge model in AGMs. Importantly, no apparent role for antibodies in protection against challenge! 500 pfu used. 2) UTMB using an ***intranasal*** atomization device. 2,000 – 20,000 pfu uniformly lethal in AGMs. 500,000 ***intratracheal*** in cynos, not uniformly lethal. 3) CSIRO working on ***intranasal*** challenge in ferrets. Shedding not sufficient to cause transmission, arguing against aerosol route. No animal model is yet exploring myocarditis – a key clinical attribute of the disease observed in India & Bangladesh.	41-47
6	Transmission/Case management	1) Several key clinical observations that are currently not being contemplated in animal model development, for example, myocarditis and hypoxia in ABG analysis. Would be easy to implement in animal models. 2) Relapse and late-onset henipavirus infections observed in Malaysian patients several years after the acute infection – unclear if there is viremia during relapse. No animal model for relapsing disease.	48-49, 51
7	Epidemiological needs for clinical trials	1) In the absence of randomised controlled trials (RCTs), observational studies such as case-control studies can provide valuable evidence, according to WHO. 2) A case-control study with vaccination in Faridpur and Rajbari (Bangladesh) and recruitment of	54-55, 56, 57

	Session	**Highlighted Topics in Proceedings**	**Page number in Proceedings**
		10 matched controls whenever a case is identified would require only 7 years to be completed (provided 70 % vaccine coverage), according to S. Luby. 3) DGDA, the regulatory authority in Bangladesh, has several requirements outlined on top of pg. 56. 4) 3 of 279 close contacts from the 2018 Kerala outbreak had subclinical infection. Similarly, 8 % of Nipah-infected Malaysian patients had subclinical infection with positive serology. Could be important to analyse immune responses in subclinical infections, to try to emulate this type of immunity with a vaccine.	
8	Progress and challenges in vaccine development	Someone asked if the PHV/Crozet VSV-based vaccine caused neurovirulence. G. Hepner replied neurovirulence studies are planned and will be discussed with authorities.	67-68
9	Progress and challenges in therapeutics development	1) Monoclonal antibody shipped from Australia to be tested therapeutically in the outbreak was left unused. 2) To facilitate access of therapeutics to future outbreaks, ICMR has taken the lead in ensuring free production and supply, as well as putting in place shipping agreements with manufacturers. Interesting equitable access example. 3) Other CMCs: antibodies to sG and F, though abs don't cross the brain-blood barrier; heparin as low-cost method; lipid-anchored peptides to prevent viral fusion. Working on a nasal spray with these compounds to be used prophylactically vs NiV. Keep an eye on non-vaccine MCM portfolio.	69, 73
10	Collaboration and Synergy	1) A Sino-French agreement to collaborate on emerging infectious diseases was established in 2004, following the SARS outbreak. *BSL4 labs were established in Wuhan as a result of SARS.* 2) (Before the COVID-19 days), the Wuhan BSL-4 facility has an annual call around April for external organisations wanting	75-76

	Session	**Highlighted Topics in Proceedings**	**Page number in Proceedings**
		access to the facility to test their own samples or use samples from the in-house repositories. 3) The Regional Enabler for the Southeast Asia Research Collaboration for Health (RESEARCH) Platform was established on August 28, 2019, with ICMR as secretariat. The goal of the platform is to facilitate clinical research across the region, leveraging the unique capabilities of each country and sharing expertise. With the platform in place, it will be easier to mount rapid responses to new outbreaks, and to conduct clinical trials – not only on NiV, but also other EIDs.	

Opinion/Commentary – (open for comments from IOC)

From: Halpin, Kim (AAHL, Geelong AAHL) on behalf of Halpin, Kim (AAHL, Geelong AAHL) <Kim.Halpin@csiro.au>
To: Raul Gomez Roman; benassiv; Broder, Christopher; CCassetti@niaid.nih.gov; De wit, Emmie (NIH/NIAID) [E]; rgangakhedkar.hq@icmr.gov.in; Dr. Nivedita Gupta; Emily Gurley; Higgs, Elizabeth (NIH/NIAID) [E]; Lee, Benhur; PREZIOSI, Marie-pierre; Mahmudur Rahman; Ravindran Thayan
Cc: LinFa Wang Linfa; Jodie Rogers; Ana Paula De Almeida Aranha
Subject: RE: Proceedings from Nipah Virus International Conference
Date: Monday, March 30, 2020 12:30:25 AM
Attachments: Summary and Outline for mSphere_v1_KH.docx
image001.png
image002.png
image003.png

Hi there

I'm not sure if others have contributed to the *mSphere* article as requested by Raul (email below), but please find my contribution.
Others are welcome to build on this, if they have time. It would be great to see a few more comments to round out the summary of this amazing (and somewhat prophetic!) conference.
I think Benhur wanted this by 30th March – so I'm cutting this a bit fine – sorry.

Thanks everyone
Kind regards
Kim

From: Raul Gomez Roman <raul.gomezroman@cepi.net>
Sent: Thursday, 19 March 2020 9:57 PM
To: benassiv <benassiv@who.int>; Broder, Christopher <christopher.broder@usuhs.edu>; CCassetti@niaid.nih.gov; De wit, Emmie (NIH/NIAID) [E] <emmie.dewit@nih.gov>; rgangakhedkar.hq@icmr.gov.in; Dr. Nivedita Gupta <guptanivedita.hq@icmr.gov.in>; Emily Gurley <egurley1@jhu.edu>; Halpin, Kim (AAHL, Geelong AAHL) <Kim.Halpin@csiro.au>; Higgs, Elizabeth (NIH/NIAID) [E] <ehiggs@niaid.nih.gov>; Lee, Benhur <benhur.lee@mssm.edu>; PREZIOSI, Marie-pierre <preziosim@who.int>; Mahmudur Rahman <rahman.mahmudur@icddrb.org>; Ravindran Thayan <rthayan@gmail.com>
Cc: LinFa Wang Linfa <linfa.wang@duke-nus.edu.sg>; Jodie Rogers <Jodie.rogers@cepi.net>; Ana Paula De Almeida Aranha <AP.AlmeidaAranha@cepi.net>
Subject: [WARNING : MESSAGE ENCRYPTED]Proceedings from Nipah Virus International Conference

Dear International Organizing Committee (IOC) members for the Nipah Virus International Conference,

Please find a ***draft*** (pdf) with the Proceedings from the Nipah Virus International Conference, which took place in Singapore last December. The password for this document is [redacted]. Please consider this document under 'embargo' until cleared by the the respective Communications departments from CEPI and its Conference organizing partners (WHO, NIAID, Duke-NUS).

We hope this email finds you well. We apologise for not sharing the Proceedings with you sooner, but we hope you will understand all of us have been swamped with the present COVID-19 situation. Although we do appreciate the Nipah Conference may be perceived as less of a global priority now comparted to COVID-19, we believe the Proceedings contain relevant insights that should be shared with the global community in a pandemic situation.

Our plan is to upload a refined and cleared version of the Proceedings into a suitable online platform for distribution with our partners, ideally within 4 months of the Conference having taken place (i.e. before April 11th).

In addition, and thanks to Benhur Lee, the Editor-in-Chief for *mSphere* has also agreed to publish a *summary* version of this and we have started working on that. Please also find the *word* document with a *draft* summary of Conference. We would like to invite you to co-author this summary and edit/add comments to this **by Monday, March 30**. If you do not wish to co-author the summary, please let us know.

Again, we do recognise that this may be perceived as less of a priority now, but we hope the insights from the Conference can shed light in some of the scientific issues being discussed now. We would like to thank you again for your valuable contributions to this Conference, and we look forward to your participation in this process.

Best wishes,
Raúl

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Elsevier Editorial System(tm) for The Lancet Infectious Diseases
Manuscript Draft

Manuscript Number: THELANCETID-D-19-01274R1

Title: Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of Henipaviruses in healthy adults: a randomised, first-in-human phase 1 study.

Article Type: Article (Clinical Trials)

Keywords: Hendra virus, Nipah Virus, randomised clinical trial, henipavirus, phase 1

Corresponding Author: Professor Elliott Geoffrey Playford, MBBS

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Manuscript Region of Origin: AUSTRALIA

Abstract: Background
The monoclonal antibody (mAb) m102·4 is a potent fully human antibody that neutralizes Hendra and Nipah viruses in vitro and in vivo. We investigated its safety, tolerability, pharmacokinetics, and immunogenicity in healthy adults.
Methods
In this double-blind, placebo-controlled, single-centre, dose escalation phase 1 trial of m102·4, we randomised healthy adults aged 18-50 years with a body-mass index of 18·0—35·0 kg/m2 to one of five cohorts. A sentinel pair for each cohort was randomised to either m102·4 or placebo. The remaining participants in each cohort were randomised 5:1 to receive m102·4 or placebo. Cohorts 1 to 4 received a single intravenous infusion of m102·4 at doses of 1, 3, 10, and 20 mg/kg respectively, and were monitored for 113 days. Cohort 5 received two infusions of 20 mg/kg, 72 hours apart, with monitoring for 123 days. The primary objective was safety and tolerability; secondary objectives were pharmacokinetics and immunogenicity. Analyses were completed according to protocol. The study was registered on the Australian New Zealand Clinical Trials Registry, registration number ACTRN12615000395538.
Findings
Between March 27, 2015 and June 16, 2016, 40 of 77 healthy screened adults were enrolled in the study; eight were assigned to each cohort (six received m102·4, and two received placebo). Eighty-six treatment-emergent adverse events were reported, with similar rates between placebo and treatment groups. The most common treatment-related event was headache (12 of 30 [40%] in the combined m102·4 group, and 3 of 10 [30%] in the pooled placebo group). There were no deaths or severe adverse

events leading to study discontinuation. Pharmacokinetics based on those receiving m102·4 (n=30) were linear, with a mean half-life of 16·5—27·6 days across Cohorts 1-4. The elimination kinetics of those receiving repeated dosing (Cohort 5) were comparable to single-dose recipients. Anti-m102·4 antibodies were not detected at any time-point during the study.

Interpretation

m102·4 single and repeated dosing were well tolerated, displayed linear pharmacokinetics, and showed no evidence of an immunogenic response. This study will inform future dosing regimens for m102·4 to achieve prolonged exposure for systemic efficacy to prevent and treat henipavirus infections.

Funding

Queensland Department of Health, the National Health and Medical Research Council and the National Hendra Virus Research Program.

October 16th 2019

Manuscript reference number: THELANCETID-D-19-01274

Dear Dr. De Ambrogi

We are most grateful to the Reviewers for their positive and constructive comments of our manuscript. We have addressed these comments and edited our manuscript accordingly. As requested we have submitted two versions of the manuscript, one that indicates the changes made in track changes, and the revised manuscript with all changes incorporated.

Overall the conclusions of the paper remain unchanged and we hope we have now met all requirements.

A separate document detailing our point-by-point response to each reviewer has been uploaded separately with the revised manuscript.

Thank you for your further consideration of our manuscript, and we look forward to your response.

Sincerely

A/Prof. Elliott Geoffrey Playford MB,BS; MMed; PhD; FRACP; FRCPA
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Editor's comments

1- Why three years have passed between the conclusion of the trial and the submission of the paper? I have sent you an email with this question but I did not receive any reply. It is very important for us to have an answer because this is something that can influence the final decision.

- *Author response:* The trial reported in this paper was organised and funded under an atypical arrangement, i.e. this was not funded and sponsored under the auspices of a pharmaceutical company, rather by a state health department in Australia. This was the first clinical trial our Department had sponsored, and as such it meant we had to contract some of the expertise required, as well as develop clinical trials processes and approvals where none existed. Study reports and reporting had to pass through a more complex approval process than would normally be the case, and unfortunately were sometimes held up in this chain awaiting approval from busy people with competing priorities. Given the novelty of our findings, and their relevance to current henipavirus epidemiology, we believe the paper is still current and appropriate for publication in Lancet Infectious Diseases.

2- Is there any plan to move forward with the development of m102•4 in other clinical studies? I realize the difficulty to test the antibody in clinical cases given the relative rarity of the infections but it would be useful to have at least a line in the paper mentioning what is planned next.

- *Author response:* The primary objective of the reported clinical study of m102.4 was to assess its safety and, hence, to support its use as post-exposure prophylaxis in persons in Australia who have been exposed to Hendra virus (usually through contact with infected horses). Having achieved this objective, we will continue to undertake ongoing observational surveillance of the effectiveness of risk assessment (i.e. as to the decision to offer m102.4 post-exposure prophylaxis) and of post-exposure prophylaxis in such contacts.

  Although we anticipate that clinical cases of human Hendra virus infection to be rare (given the rapid and extensive nature of public health contact tracing following any equine cases in Australia), we would plan to utilise m102.4 in a therapeutic context should a human clinical infection eventuate. Observational clinical and pharmacokinetic evaluation would then be undertaken.

3- Even if the study is a dose escalating trial, I cannot find any mention of the dose that would be chosen for future studies/clinical practice. Could you clarify this point in the text, please?

- *Author response:* All humans offered post-exposure m102.4 in Australia have been dosed at ~20mg/kg as a single dose (as outlined in Supplementary Table 2). Given the safety data from the reported phase 1 trial, together with previously reported animal challenge studies, this dosing regimen remains that recommended in Australia for post-exposure prophylaxis. Regarding dose recommendations for established infection, we feel that, based upon the two African green monkey studies (refs 23 and 24), a regimen that involves two doses of 20mg/kg separated by 48 hours would be appropriate.
- *Manuscript amendments:*
  - We have therefore amended the final sentence in the third last paragraph in the Discussion to now read "The safety profile of both single and repeat dosing of m102·4, and the standard elimination pharmacokinetic profile presented in this study, underpins the current standard of care to offer *a single dose of 20mg/kg of m102·4* to humans with high levels of exposure to HeV and NiV.

    - Further, a new final sentence to this paragraph has been added that reads “It also supports its potential use for patients with established clinical manifestations of henipavirus infection; based upon African green monkey challenge studies (refs 23 and 24), two doses separated by 48 hours would seem appropriate.”

4- Two reviewers asked for more clarity about the use of the antibody and I tend to agree. Would it be used for post-exposure prophylaxis or also for therapy if the infection is confirmed? Please clarify this point in the text.

- *Author response:* As outlined above (Editor’s comments point 2), the major anticipated need for m102.4 is for post-exposure prophylaxis, although clearly, should a human clinical infection eventuate, it would also be offered.
- *Manuscript amendments:*
    - We have amended the final sentence in the Summary/Interpretation to read “This study will inform future dosing regimens for m102.4 to achieve prolonged exposure for systemic efficacy to prevent and treat henipavirus infections.”
    - We have also added the sentence described above (point #3) regarding its potential use in a therapeutic setting for established infections to the third last paragraph in the discussion.

5- It is essential that you ensure that your paper (abstract and main text) conforms as much as possible to the necessary reporting guidelines (CONSORT for randomised trials); you can find a list of these guidelines with relevant flow-charts and checklists here: http://www.equator-network.org/

- *Author response:* We have conformed as much as possible to CONSORT reporting guidelines for randomised trials.

6- Please use these formatting guidelines for RCTs as a reference when you revise the article: http://www.thelancet.com/laninf/information-for-authors/formatting-guidelines Please be aware that the Methods should go after the Introduction.

- *Author response:* We believe that we have conformed to the formatting guidelines for RCTs as outlined in the above link. Currently as submitted, the Methods section follows the Introduction on page 8

7- The word limit for the Summary is 350 words so please be aware of this limit when you revise the paper.

- *Author response and manuscript amendment:* We have revised the Summary to 350 words.

8- Please give absolute numbers (numerator and denominator) not just percentages for the main results in the abstract and in the main paper (in the text, tables, or figures).

- *Author response and manuscript amendment:* We have made the requested adjustments providing both numerator and denominator with percentages in brackets in the Summary and main paper.

9- Please indicate the date of start of the trial in the Summary.

- *Author response and manuscript amendment:* The first participant was screened on 27th March 2015. We have revised the statement in the Summary regarding enrolment to

"Between March 27, 2015 and June 16, 2016, 40 of 77 healthy screened adults were enrolled in the study."

10- Please be aware that the maximum number of references is 30 (please keep the most relevant and recent).

- *Author response:* We have adhered to the maximum 30 cited references

11- Please be aware that the word count for a trial should not be over 4500 words.

- *Author response:* The revised manuscript is within the word count limit of 4500 words

12- Please ensure that you provide your figures in an editable format and as separate files they should not be part of the text). For trial profiles a word file made of editable text boxes is the preferred format. For any statistical images (histograms, survival or time-to-even curves, line graphs, scatter graphs, forest plots, etc) you should provide editable vector files (ie, the original artwork generated by the statistical package used to make the image). Our preferred formats for these files are .ai, .eps, or .pdf. We cannot guarantee accurate reproduction of images without these files.

- *Author response:* The figure(s) have been provided as requested.

13- Be aware that the maximum number of non-text items (figures and tables) in the main article is six. You have five non-text items at the moment so if you add more than one table/figure, something will need to be moved to the Appendix (that should be a single PDF file with page numbers). The flow chart and the table with the baseline characteristics need to be in the main article. In the text, any reference to the Appendix (including tables and figures) must be something like "Appendix, page X".

- *Author response:* We have not added any further non-text items. We have added the relevant page numbers where the Appendix has been referenced.

14- Please check that all authors' names and affiliations are correct.

- *Author response:* All authors' names and affiliations are correct

15- All authors should complete and sign the author statement form and upload the signed copy (just check if you have sent already all forms). The form can be downloaded from the page (http://www.thelancet.com/for-authors/forms#author-sigs) from the fourth line of "Authors and contributions". The corresponding author must countersign manually the forms at the bottom of the page and send us the scanned version. Electronic signatures are accepted.

- *Author response:* Signed author contribution forms were uploaded with the original submission.

16- All authors are required to provide a Conflict of Interest Statement and should complete a standard form, which is available at http://www.thelancet.com/for-authors/forms#icmje-coi. This form can be uploaded with the manuscript at submission. (Just check if you have already sent all forms)

- *Author response:* Completed Conflict of Interest Statements from all authors were uploaded at submission.

17- Please also include written consent of any cited individual(s) noted in acknowledgments or cited as personal communications.

- *Author response:* Individuals cited in Acknowledgments have provided written consent which have been uploaded with the resubmission. No personal communications were cited in the manuscript.

Reviewers' comments:

Reviewer #1: The present double-blind, placebo-controlled, single-centre, phase 1 RCT was performed to examine the safety and tolerability of single and double intravenous doses of the human monoclonal antibody m102.4. The trial participants were recruited at one centre, in Queensland, Australia, between April 2015 and June 2016. The authors concluded that the treatment and repeated dosing is safe and well tolerated by healthy adult volunteers. The manuscript is very clearly written including the study design and statistical analysis. There was no formal sample size calculation, which is not surprising considering this was a phase 1 trial. I only have a couple of minor comments that the authors may wish to consider:

How were participants allocated to the cohorts and how were the sentinel pair selected? Were cohorts completed by after the other in that the first 8 volunteers allocated to cohort 1 and then cohort 2 and so on? Were the first two volunteers in each cohort allocated as the sentinel pair?

- *Author Response:* The two sentinel individuals were randomized to m102.4 or placebo a minimum of 14 days before the remaining six participants for all cohorts. This allowed review of safety and tolerability data by the Principal Investigator and Medical Monitor. The decision to progress to the next higher dose was made after safety and tolerability data was reviewed for each dose group. Repeat dosing (Cohort 5) did not commence until all pharmacokinetic data was reviewed for Cohorts 1-4 (see Appendix pgs 3 & 19).
- *Manuscript amendments:* We have added the following statement just before the last sentence of the second to last paragraph of Randomisation and Masking sub-section: "*The decision to progress to the next higher dose was made after safety and tolerability data was reviewed for each dose group. Repeat dosing (Cohort 5) did not commence until all pharmacokinetic data was reviewed for Cohorts 1-4 (see Appendix pgs 3 & 19).*"
-

Reviewer #2: This study analyzes the use of a human monoclonal antibody in a rand. The antibody was overall well tolerated up to 20 mg per kg with one to two inoculation. The antibody showed a reasonable halflife time and preserved its neutralizing ability. There are no concerns with the presented data or the design of the study.

However, this study is a toxicity study which does not provide a lot more info as previously was available. I feel this is a critical study but would be better suited for a more specialized journal because of the interest of a very restricted panel of readers.

- *Author response:* We thank Reviewer #2 for these comments. We would, however, disagree that this manuscript is more suited to a more specialized journal. Henipaviruses are an important and serious emerging zoonotic infectious threat. The paradigm of using a human monoclonal antibody with demonstrated animal model efficacy for the post-challenge prevention or treatment of infection, together with human clinical safety data (as reported in this manuscript) is applicable to other serious emerging viral infections. This approach is particularly justifiable for infections where there are no other efficacious treatments and where human efficacy data cannot be ethically obtained from later phase clinical trials.

Reviewer #3: The present manuscript by Playford et al describes the results of a phase 1 clinical trial evaluating the safety, tolerability, pharmacokinetics, and immunogenicity of m102.4, a humanized monoclonal antibody targeting the henipavirus glycoprotein. The henipaviruses Nipah and Hendra are emerging human pathogenic paramyxoviruses that can cause severe and fatal disease in patients. Hendra and Nipah virus have the potential to cause larger outbreaks in Southeast Asia and Nipah virus is listed as a priority pathogen on the WHO blueprint list. Currently, no approved vaccines or therapeutics are available for human use. m102.4 has previously been shown to be effective in preventing henipavirus induced lethal disease in the ferret and non-human primate models. Furthermore, it has already been used on compassionate grounds following exposure to Hendra virus (13 cases) and Nipah virus (1 case).

This manuscript describes results form a first in human clinical phase 1 dose-escalation study. The study outline is fairly standard for a phase 1 clinical trial. Four different doses were administered once via the intravenous route. A fifth group received the highest concentration of 20 mg/kg twice, three days apart. Overall, the administration was well received and only minor side effects, such as headache and slightly elevated ALT values were recorded. The mean half-life of m102.4 ranged from 16 to 27 days. Importantly, serum samples were able to neutralize both, Hendra and Nipah viruses in a neutralization assay for at least 8 days after administration. These data indicate that repeated dosing of m102.4 three days apart was safe and well tolerated.

Overall, the presented data are straight forward and of importance for the further development of this monoclonal antibody for future use in humans. There are few minor comments that should be addressed:

Line 57: Therapeutic application could be mentioned in addition to prophylactic treatment.

- *Author response and manuscript amendment:* see response to Editor’s comments (point #4)

Lines 150 to 159: The two strains for NiV should already be mentioned in the Introduction.

- *Author response:* We have updated the third paragraph of the Introduction which deals with NiV to include the two strains of NiV
- *Manuscript amendments:* The following statements have been added just before the second last sentence of the third paragraph of the Introduction: *“At least two strains of NiV have been identified in genetic analysis; NiV_M associated with the 1998 Malaysian outbreak, and NiV_B that has caused repeated outbreaks in Bangladesh and northeastern India.”*

Line 155: Should read "consumption of raw date palm sap".

- *Author response and manuscript amendment:* We have added the word “raw” to this sentence

Lines 299 to 300: Specify the HeV isolate and which NiV strain have been used for the neutralization assays.

- *Author response:* The HeV isolate was Hendra virus/Australia/Horse/2008/Redlands, and the NiV strain was NiV/Bangladesh/human/2004/Rajbari, R1

- *Manuscript Amendment:* This sentence has now been amended to read “Samples of HeV (HeV/Australia/Horse/2008/Redlands) and NiV (NiV/Bangladesh/human/2004/Rajbari, R1) were diluted to contain 100 $TCID_{50}$ of virus and added to the required wells.”

Lines 415 to 417: Please list reference for this statement.

- *Author response:* Broder et al (Ref # 4), listed at the end of the subsequent sentence, comprehensively reviews immunisation strategies and was intended to cover both statements. To avoid exceeding current limit of 30 references, we have not added any new references, but have now added Ref # 4 at the end of the statement at Lines 415 to 417.

Line 427: Could specify the various time points for m102.4 administration.

- *Author response:* We have added the time points to this statement.

- *Manuscript amendment:* We have deleted “at various time points post-infection” and added “beginning at 1, 3, or 5 days post-infection, and again 2 days later.”

Lines 432 to 436: It might be worth adding a brief statement regarding long-term neurological complications. These are typically not addressed in animal studies, because studies are terminated after 3 to 4 weeks. A recent study evaluating the efficacy of remdesivir in the non-human primate model for Nipah virus showed that one out of 4 surviving animals had viral RNA in brain tissue after termination of the study at day 92 post infection.

- *Author response:* We thank the Reviewer for making this point. We have already briefly mentioned the occurrence of long-term and delayed neurological complications from both Hendra and Nipah viruses (see last sentence of first paragraph in Introduction). We would be willing to make this point again in the Discussion if strongly desired, although to adhere to the required word count, we have currently left the manuscript unamended.

Line 439: Supplementary Table 2 does not contain information for the one case in the US that received m102.4.

- *Author response:* We have added the US NiV case to Supplementary Table 2. In addition, in reviewing the data in Supplementary Table, we corrected the Exposure data for Patient # 2 which should read “HeV infected horse”, NOT “HeV-infected needle-stick injury”.

Page 15 of the clinical protocol indicates that the first patient who received m102.4 already displayed progressed clinical disease and did not survive. However, in the main text of the manuscript references to patients who received m102.4 were described to have all survived. This might require some clarification.

- *Author response:* We apologise for the confusion. The patient (the first human to have ever received m102.4) was, as stated, in the terminal phase of established Hendra virus encephalitis. A small (100mg; ~1.5mg/kg) dose of the product was available and was administered on compassionate grounds. It was not expected that this would be efficacious, however, given the absence of other therapeutic options, it was given. Hence, we felt that including this patient’s details in the current manuscript together with those that received a more likely efficacious dose in a post-exposure setting (i.e. not in a setting with pre-terminal established infection) would introduce confusion.

Table 2 lists that overall 45% of the participants had infections and infestations. This is not discussed in the Results.

- *Manuscript amendment:* The following has been added to the 2nd paragraph of the Results: "*The most frequently reported events were infections and infestations, classed as mild to moderate; 13 were reported in 11 of 24 single-dose participants (45.8%), two of six (33%) repeat dose participants, and five of 10 (50%) in the pooled placebo group.*"

Reviewer #4: This clinical trial is an important step towards control of a global pandemic potential of henipavirus infection. This monoclonal antibody should be available to the affected population soon.

Minor comments are as follow:

1. NiV infected all age group people. Almost half of the cases identified in Bangladesh till date is beyond age group 18-50 years. Need discussion on this point.

- *Author response:* We thank Reviewer #4 for this comment and note that age appears to be associated with prognosis of NiV (Goh et al NEJM 2000 & Banerjee et al 2019 review article). However, in the interest of adhering to CONSORT reporting requirement, we did not feel that discussion on this point was within the scope of this report, but we agree that this warrants further investigation in an appropriately designed study.

2. In the introduction section, Page 6, line 153, the way it is written seems like, India also experience NiV outbreak almost every year, which is not true. Only Bangladesh is experiencing Nipah outbreak almost yearly. Please rephrase.

- *Author response and manuscript amendment:* We have removed the word "India" and restricted the statement to Bangladesh.

3. Page 6, line 156, "consumption of date palm sap that had been contaminated by fruit bats" is not correct. Because, though few infrared camera studies in Bangladesh have identified bat visits in shaved part of date palm tree, there is no direct evidence that the date palm sap that the cases drank was bat contaminated. Therefore, the authors might rephrase as "consumption of date palm sap that might had been contaminated by fruit bats".

- *Author response and manuscript amendment:* We have rephrased the statement to "consumption of raw date palm sap that may have been contaminated by fruit bats"

4. Page 6, line 158, I strongly feel that, to describe the gravity of the problem, authors should state corpse of human transmission of NiV has been reported from Bangladesh in 2010 (Ref: Sazzad HM, Hossain MJ, Gurley ES, Ameen KM, Parveen S, Islam MS, et al. Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. Emerg Infect Dis. 2013;19(2):210-7). This phenomenon was evident in other highly fatal pathogens like Ebola.

- *Author response and manuscript amendment:* We thank Reviewer #4 for the recommended reference and have included the following statement on page 6 with the recommended reference: "Corpse-to-human transmission of NiV has also been documented in a case series report of sporadic cases in Bangladesh in 2010."

5. Though the use of monoclonal antibody is targeted as a postexposure prophylaxis, the NiV cases in the community will be both primary and secondary cases. Primary cases usually take around one

week following onset of illness to tertiary surveillance hospitals. Secondary cases might be a potential candidate for monoclonal antibody as a post exposure prophylaxis. The authors may discuss about the timeline/ natural history of henipaviurs infection and scope of administering the monoclonal antibody in the field.

- *Author response and manuscript amendment:* We understand the point that Reviewer #4 is making. We believe that the amendments that we have made (see Editor’s comments points #3 and #4) addresses – in general – this point (i.e. that m102.4 may have a role in treating established henipavirus infections in addition to its role as post-exposure prophylaxis). In the interests of space, we did not feel that discussing this aspect in greater detail was justified.

Title

Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of Henipaviruses in healthy adults: a randomised, first-in-human phase 1 study.

Authors

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Summary

Background

The monoclonal antibody (mAb) m102·4 is a potent fully human antibody that neutralizes Hendra and Nipah viruses *in vitro* and *in vivo*. We investigated its safety, tolerability, pharmacokinetics, and immunogenicity in healthy adults.

Methods

In this double-blind, placebo-controlled, single-centre, dose escalation phase 1 trial of m102·4, we randomised healthy adults aged 18–50 years with a body-mass index of 18·0–35·0 kg/m^2 to one of five cohorts. A sentinel pair for each cohort was randomised to either m102·4 or placebo. The remaining participants in each cohort were randomised 5:1 to receive m102·4 or placebo. Cohorts 1 to 4 received a single intravenous infusion of m102·4 at doses of 1, 3, 10, and 20 mg/kg respectively, and were monitored for 113 days. Cohort 5 received two infusions of 20 mg/kg, 72 hours apart, with monitoring for 123 days. The primary objective was safety and tolerability; secondary objectives were pharmacokinetics and immunogenicity. Analyses were completed according to protocol. The study was registered on the Australian New Zealand Clinical Trials Registry, registration number ACTRN12615000395538.

Findings

Between March 27, 2015 and June 16, 2016, 40 of 77 healthy screened adults were enrolled in the study; eight were assigned to each cohort (six received m102·4, and two received placebo). Eighty-six treatment-emergent adverse events were reported, with similar rates between placebo and treatment groups. The most common treatment-related event was headache (12 of 30 [40%] in the combined m102·4 group, and 3 of 10 [30%] in the pooled placebo group). There were no deaths or severe adverse events leading to study discontinuation. Pharmacokinetics based on those receiving m102·4 (n=30) were linear, with a mean half-life of 16·5–27·6 days across Cohorts 1–4. The elimination kinetics of those receiving repeated dosing (Cohort 5) were comparable to single-dose recipients. Anti-m102·4 antibodies were not detected at any time-point during the study.

Interpretation

m102·4 single and repeated dosing were well tolerated, displayed linear pharmacokinetics, and showed no evidence of an immunogenic response. This study will inform future dosing regimens for m102·4 to achieve prolonged exposure for systemic efficacy to prevent and treat henipavirus infections.

Funding

Queensland Department of Health, the National Health and Medical Research Council and the National Hendra Virus Research Program.

Research in Context

Evidence before this study

We searched PubMed on May 31, 2019 for studies using the following terms: (“Hendra” OR “Nipah”) AND (“monoclonal antibody” OR “monoclonal antibodies”) without language restrictions. We restricted article type to clinical trials and species to human. There were no articles describing clinical trials of monoclonal antibodies designed for human prophylaxis. The human monoclonal antibody m102·4 binds to the same/similar region of the G glycoprotein of Hendra and Nipah viruses as the ephrin receptors. The m102·4 monoclonal antibody demonstrated protection from infection, and was effective when administered following experimental challenge with Hendra and/or Nipah viruses in ferret and non-human primate models.

The m102·4 has been available since 2010 for compassionate therapy following high-risk Hendra virus exposures. It has been used on 14 occasions (13 in Queensland, Australia and one in the United States). The safety and tolerability of the m102·4 has not been systematically assessed.

Added value of this study

This is the first study to assess the safety, tolerability, pharmacokinetics, and immunogenicity of the m102·4 in healthy adults using a variety of single dosing regimens (1 mg/kg, 3 mg/kg, 10 mg/kg, and 20 mg/kg) and a repeat dosing regimen of 20 mg/kg separated by 72 hours. The four single and one repeat dosing regimens of the m102·4 were generally safe and well tolerated, and had a half-life of 16·5–27·6 days with no observed immunogenicity associated with the m102·4.

Implications of all the available evidence

The findings of this study support the use of the m102·4 in humans following high-risk henipavirus exposure. Two doses of 20 mg/kg separated by 72 hours were as well tolerated as a single dose of 20 mg/kg.

Introduction

Nipah virus (NiV) and Hendra virus (HeV) are enveloped, single-stranded negative sense RNA viruses and the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae.*[1] Bats of the genus *Pteropus* appear to be the major natural reservoir hosts for henipaviruses, from which all bat isolates of HeV and NiV have been derived.[2,3] HeV and NiV are two emerged zoonotic, transboundary agents with uniquely broad host tropism capable of infecting at least 18 animal species across six orders of mammals, including their natural bat hosts.[4,5] HeV and NiV are highly pathogenic, and cause wide-spread infection with severe disease in multiple organ systems in at least 11 mammalian species. Fatality rates range from 40–100%.[6] In humans, HeV and NiV infection results in a systemic and often fatal respiratory and/or neurological disease.[7,8] Both HeV and NiV can also cause a relapsing of encephalitis following recovery from an acute infection; this appears to result from a recrudescence of virus replication in the central nervous system.[9]

HeV was first recognized in 1994 during an outbreak of fatal cases of a severe respiratory disease in horses and humans which occurred in the Brisbane suburb of Hendra, Australia.[10] In all, 14 horses and their trainer succumbed to infection together with the non-fatal infection of seven additional horses and a stablehand. To date, HeV has appeared in eastern Australia on 61 occasions, causing fatalities in four of seven human cases, death or euthanasia of more than 100 horses, and euthanasia of two HeV antibody positive dogs.[4,11]

A large outbreak of encephalitis in 1998 among pig farmers in Peninsular Malaysia led to the identification of NiV, a Hendra-like virus isolated from the cerebrospinal fluid of two patients.[12] Outbreaks of human cases of NiV infection have been recorded almost annually since 2001 in Bangladesh. Between 1998 and 2018, a total of 637 cases of human NiV infection with 373 fatalities were reported in South and Southeast Asia. The mode of NiV transmission was direct human contact with infected pigs, consumption of raw date palm sap that may have been contaminated by fruit bats, or human-to-human transmission.[13] Corpse-to-human transmission of NiV has also been documented in a case series report of sporadic cases in Bangladesh in 2010. [14] At least two strains of NiV have been identified in genetic analysis; NiV_M associated with the 1998 Malaysian outbreak, and NiV_B that has caused repeated outbreaks in Bangladesh and northeastern India.[15] There is considerable genetic heterogeneity in human isolates of NiV, particularly from the Bangladesh outbreaks.

This feature of NiV, in combination with transmission modes that include human-to-human, raises concerns of a pandemic potential.[16]

There are no approved vaccines or therapeutics to prevent or treat disease in humans. NiV and HeV are currently listed among WHO priority diseases for which a research and development blueprint of action to prevent epidemics has been developed.[17] Both are also emerging viruses selected for the human vaccine development initiative funded by the Coalition for Epidemic Preparedness Innovations.[18]

HeV and NiV particles possess surface projections composed of the viral transmembrane anchored fusion (F) and attachment (G) glycoproteins, which are the mediators of virus attachment and host cell infection. They are also the major antigenic targets of virus-neutralising antibodies. Both the HeV and NiV G glycoproteins bind to the host cell membrane proteins ephrin-B2 and ephrin-B3, which are highly conserved across known susceptible hosts, as reviewed by Broder and colleagues.[4] HeV- and NiV-neutralising human antibody fragments reactive to the G glycoproteins of both HeV and NiV have previously been isolated using recombinant antibody technology.[19] One monoclonal antibody (mAb), m102, possessed strong cross-reactive and neutralising activity against HeV and NiV, and was subsequently affinity matured to m102·4 , formatted as an IgG1 mAb, and produced in a CHO-K1 cell line.[20] The m102·4 epitope localises to the ephrin receptor binding site on the G glycoprotein and binds in a similar fashion as the ephrin molecule.[21] In a post-exposure NiV challenge experiment in the ferret model, a single dose of m102·4 administered by intravenous (IV) infusion ten hours after a lethal virus challenge was shown to provide complete protection.[22] The m102·4 was also shown to be effective against both NiV and HeV infection in nonhuman primates in studies designed to reflect a potential real-world infection exposure scenario,[15,23,24] highlighting clear potential for future approved human use.

In 2010, the m102·4 cell line was provided to the Queensland Government, (Queensland Health, Australia), to produce the m102·4 for compassionate emergency use in the event of high-risk human HeV exposure. Following an unprecedented number of equine HeV notifications (22 horses on 18 properties) in Queensland and New South Wales in 2011,[11] planning commenced in 2012 for the first phase 1 clinical trial of the m102·4 in human participants.[25] This trial aimed to evaluate the safety, tolerability, immunogenicity, and pharmacokinetics of single and multiple IV administrations of m102·4 in healthy participants, and was completed in 2016. Here we report the results of this safety trial.

Methods

Study design and Participants

We performed a single-centre, randomised, double blind, placebo-controlled, dose escalation phase 1 study to assess the safety, tolerability, pharmacokinetics, and immunogenicity of m102·4 in healthy adults at Q-Pharm Pty Ltd (Queensland, Australia). Ethics approval was obtained from the study site's Human Research Ethics Committee at the QIMR Berghofer Medical Research Institute before participant enrolment commenced. The study was conducted under the Therapeutic Goods Administration (TGA) Clinical Trial Notification Scheme (CTN) and in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and the National Health and Medical Research (NHMRC) statement on ethical conduct in human research.

Healthy males and females between 18 and 50 years of age, with a body mass index of 18·0 to ≤35·0 kg/m^2 and weight ≥50 kg to ≤100 kg, normal values for blood haematology, coagulation profiles, clinical chemistry, and urinalysis results, were eligible for inclusion. Exclusion criteria included close, unprotected exposure to sick horses with an unexplained illness within 4 weeks; known exposure to potential HeV infected horses; positive tests for HIV or hepatitis B or C viruses; treatment with an investigational drug or biologic agent within 60 days; sexually active males with a partner capable of becoming pregnant; and females capable of becoming pregnant in the absence of an effective method of birth control. Use of prescription drugs within 14 days or five half-lives prior to mAb administration, and pregnancy or breastfeeding were also exclusion criteria. Additional details of inclusion and exclusion criteria are outlined in the trial protocol (appendix pg 42).

All participants understood the study requirements and treatment procedures, and were willing to comply with all study restrictions. Participants provided written informed consent before any study-specific tests or procedures were performed.

Randomisation and masking

Participants were given a sequential screening number at their screening visit. Eligible participants were assigned to one of five cohorts, each with eight participants. In each cohort, six participants were randomised to receive m102·4 , and two were randomised to receive placebo in accordance with the randomisation plan prepared and maintained by Clinical Network Services, (Queensland, Australia), the contract research organisation.

In each cohort, two individuals in a sentinel pair were randomised in a double blinded manner to receive active and placebo in a 1:1 ratio. Sentinel pairs were dosed seven days prior to the other participants in that cohort in order to detect any serious adverse advents (SAEs) in accordance with the European Medical Agency first-in-man guidelines.[26] The remaining participants in each cohort received either m102·4 or placebo (0·9% saline) in a 5:1 ratio following review of safety data by the Investigator and the Medical Monitor. The decision to progress to the next higher dose was made after safety and tolerability data was reviewed for each dose group. Repeat dosing (Cohort 5) did not commence until all pharmacokinetic data was reviewed for Cohorts 1-4 (see Appendix pg 3 & 19). As this was a single site trial with eight participants allocated to five cohorts, no block randomisation was performed.

To maintain the blinding of the study, the randomisation plan was available only to the un-blinded pharmacist. Treatment allocation was double blinded, and m102·4 and placebo were identical in appearance and volume for each participant in each cohort.

Procedures

The m102·4 drug substance for the phase 1 study was manufactured at the National Biologics Facility (NBF) located at the University of Queensland (Queensland, Australia) using a recombinant Chinese Hamster Ovary (CHO) cell line. A parent cell line was obtained from the National Institutes of Health, (Bethesda, MD, USA) and then subcloned and adapted to a serum- and protein-free commercially available medium. Cells were expanded from a qualified master cell bank using a conventional seed-train, followed by a fed-batch bioreactor process. The clarified harvest containing secreted m102·4 was subjected to a two-step purification process including MabSelect SuRe™ LX protein A and mixed mode Capto™adhere chromatography. The process included two viral inactivation/removal steps, a low pH hold, and nanofiltration (Viresolve® Pro Modus) respectively. The m102·4 was formulated at a concentration of 10 mg/mL in phosphate buffered saline pH 7·3 and aseptically dispensed into 10 mL vials at Sypharma Pty Ltd (Victoria, Australia). The final product complied with specifications commonly accepted by regulatory agencies for identity, purity, potency, and safety.

Prior to clinical studies, Good Laboratory Practice (GLP) compliant, repeat dose toxicology studies were conducted in the Sprague-Dawley rat at vivoPharm Pty Ltd (Victoria, Australia). The m102·4 was administered once per week for four weeks at doses of 0, 10, 30, and 100 mg/kg. The antibody was well tolerated and the no-observed-adverse-effect-level (NOAEL) was determined to be 100 mg/kg, the highest dose tested.

An enzyme-linked immunosorbent assay (ELISA) was developed by the NBF, then validated and used to measure the concentration of m102·4 in human serum samples at vivoPharm Pty Ltd. Briefly, serum m102·4 bound to Hendra virus soluble G protein coated on a plate, was detected with a custom rabbit anti-m102·4 horseradish peroxidase conjugate followed by the addition of 3,3',5,5'-Tetramethylbenzidine substrate. The validated assay met criteria in the relevant US guideline[27], and had a working range of 1·7–163·2 ng/mL, which corresponded to the lower and upper limits of quantitation, respectively. All samples were diluted within this working range, quantitated via standard curve interpolation, and pharmacokinetic parameters were calculated using noncompartmental techniques.

An anti-drug antibody ELISA was developed and validated to determine the presence of anti-m102·4 antibodies in human serum samples. A bridging assay design was employed where m102·4, previously coupled either with biotin or horseradish peroxidase, was mixed with serum or positive control (a custom anti-m102·4 rabbit polyclonal antibody) to form a complex which was captured onto a streptavidin coated plate. The assay procedure included an acidification step to dissociate drug-antibody complexes. The validated assay met criteria consistent with the US guidelines,[27] and had a sensitivity of 100 ng/mL. Human serum samples were considered positive if both the screening cut-point (determined statistically based on a 95% upper confidence limit from negative normal donor sera) and specificity confirmation cut-point (where excess m102·4 was added) were exceeded.

Based on animal models of Henipavirus challenge,[1,22-24,28] a target dose of 20 mg/kg was selected as the maximum dose to be tested. Treatment recipients in the five cohorts were dosed with four concentrations of m102·4. Cohorts 1 and 2 received single IV doses of 1 mg/kg and 3 mg/kg, respectively, or placebo, infused in a total volume of 100 mL of 0·9% saline. Cohorts 3 and 4 received single IV infusions of 10 mg/kg and 20 mg/kg, respectively, or placebo, in a total volume of 500 mL of 0·9% saline, while Cohort 5 received two IV infusions of 20 mg/kg, or placebo, in 500 mL of 0·9% saline 72 hours apart. All infusions were given over a 1-hour period using a peristaltic infusion pump.

Participants in Cohorts 1–4 were domiciled for a total period of 3·5 days for initial safety and tolerability monitoring, and to facilitate blood sampling for pharmacokinetics, virus neutralisation, and immunogenicity analysis. Participants received the m102·4 mAb or placebo on day 1, and follow-up assessments were scheduled on days 4, 6, 8, 15, 29, 43, 57, 85, and 113. Participants in Cohort 5 were domiciled for a total period of 6·5 days receiving a single dose of m102·4 or placebo on days 1 and 4. Follow up visits to the clinical site were scheduled on days 7, 9, 11, 18, 25, 32, 39, 53, 67, 95, and 123. For all cohorts, the final safety assessment was scheduled to occur within a maximum period of 16 weeks after the last infusion.

Dose escalation from one cohort to the next followed review and approval of safety data for at least six participants per group. In addition, blinded pharmacokinetic data for Cohort 1 was reviewed to confirm estimation of m102·4 elimination half-life. Initiation of dosing in Cohort 5 followed review of available unblinded pharmacokinetic data for Cohorts 1–4.

A virus neutralisation assay was designed and performed by the Australian Animal Health Laboratory (AAHL) located at the Commonwealth Scientific and Industrial Research Organisation (CSIRO, Victoria, Australia). Briefly, sera from five Cohort 4 participants (20 mg/kg, a clinically relevant dose) were tested against HeV and NiV to assess the ability of circulating m102·4 to neutralise each virus in samples at four different time points post-infusion (12hr, 24hr, Day 4, and Day 8). Samples were diluted to achieve an end-point for neutralisation. Control plates were set up using m102·4-spiked media + 10% normal human serum to achieve final concentrations ranging from 50 to 0·4 µg/mL. Samples of HeV (HeV/Australia/Horse/2008/Redlands) and NiV (NiV/Bangladesh/human/2004/Rajbari, R1) were diluted to contain 100 $TCID_{50}$ of virus and added to the required wells. Serum, cell and virus controls were included. Vero cell suspension was added to every well at a concentration of $4x10^5$ cells/mL. Plates were incubated at 37°C in a humid atmosphere containing 5% CO_2 for 45–60 min. Cells were examined after 3 days under an inverted microscope for cytopathic effect.

Outcomes

The primary objective of the phase 1 trial was to assess the safety and tolerability of single and multiple IV doses of m102·4 in healthy human participants; the second objective was to determine the pharmacokinetic and immunogenicity of single and multiple doses; and the third objective was to explore the pharmacodynamics of m102·4 against HeV and NiV.

The safety and tolerability of m102·4 across all cohorts was evaluated according to the following specific assessments: physical examination, clinical laboratory tests, vital signs, electrocardiogram, signs and symptoms of tolerability, infusion site reactions, and adverse events (AEs). All AEs and treatment-emergent AEs (TEAEs) were reported for participants from dosing until the end of the study. Definitions of AEs and TEAEs can be found in the trial protocol (appendix pg 50–52).

Calculated pharmacokinetic values included maximum observed drug concentration (C_{max}), time to maximum observed drug concentration (T_{max}), area under the drug concentration-time curve ($AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$), elimination half-life ($t_{½}$), clearance (CL), and volume of distribution (V_z). We also took blood samples pre-dose and at nominal post-dose time-points for measurement of m102·4 HeV and NiV virus neutralisation, and for assessment of anti- m102·4 antibodies.

Statistical analysis

Statistical analysis comprised summary descriptive statistics of the five dose groups and placebo group. Given the exploratory nature of the study, the sample size was not based on power calculations, but was consistent with the typical sample size used for similar studies. Descriptive statistics were performed with SAS version 9·4. Pharmacokinetic data were analysed with Phoenix WinNonlinSoftware version 6·3, with pharmacokinetic listings, tables, and figures generated using SAS Software version 9·4. The study is registered on the Australian New Zealand Clinical Trials Registry, registration number ACTRN12615000395538.

Role of the funding source

The funders of the study had no role in data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between 15 April 2015 and 18 February 2016, 40 participants were allocated into five cohorts as described (figure 1). All enrolled participants received their full assigned treatments and completed the study according to schedule apart from two participants in cohort 4 who failed to attend their day 43 and day 85 visits, respectively. Demographic characteristics did not vary between treatment groups (table 1).

Overall the dosage of m102·4 appeared safe and well tolerated. No deaths, life threatening events, serious adverse events, or adverse events leading to study discontinuation occurred. Across all cohorts, a total of 37 of 40 (93%) participants had at least one TEAE, with a total of 86 TEAEs reported (table 2). The occurrence of TEAEs was similar across all active treatment and placebo groups. The most frequently reported events were infections and infestations, classed as mild to moderate; 13 were reported in 11 of 24 single-dose participants (45.8%), two of six (33%) repeat dose participants, and five of 10 (50%) in the pooled placebo group. A possible association between dose and headache was noted, occurring in eight of 12 (67%) participants dosed in Cohorts 4 and 5 compared with four of 18 (22%) participants dosed in Cohorts 1–3, and three of 10 (30%) participants in the pooled placebo group. All TEAEs were resolved upon follow-up without specific intervention.

Of all TEAEs, 31 in 20 participants were considered treatment-related, occurring in three of six (50%) participants dosed in Cohort 1, two of six (33%) participants dosed in Cohorts 2, 3, and 5, five of six (83%) participants dosed in Cohort 4, and five of 10 (50%) participants in the pooled placebo group. The most common treatment-related TEAEs were headaches (10 participants: one each in Cohorts 1–3, four dosed in Cohort 4, two dosed in Cohort 5, and one in the pooled placebo group), intravenous catheter site bruising (three participants: two dosed in Cohort 3 and one in the pooled placebo group), and elevated alanine aminotransferase (ALT) levels (three participants: two in Cohort 5 and one in placebo). Of the 20 treatment-related TEAEs, 15 were rated as mild and five as moderate. Moderate TEAEs included two headaches; one each in Cohorts 4 and 5, one intravenous catheter site bruising in Cohort 3, lethargy in a placebo participant, and reduced appetite in Cohort 3. There were no severe TEAEs (Table 2).

No clinically significant changes in haematological or coagulation parameters were observed in any participant. Three participants had elevated ALT levels, two dosed in Cohort 5 and one in the pooled placebo group; Cohort 5 participants had elevated ALT levels at day 4 and 11, respectively; both findings following m102·4 administration were considered to be mild and treatment-related. ALT levels returned to normal after day 11 without intervention, and were not considered to be clinically significant (Supplementary Table 1). No other clinically significant abnormalities in biochemical parameters, urinalysis, vital signs, or ECG findings were observed. One clinically significant abnormal physical examination finding was reported in one participant dosed in Cohort 3; pityriasis rosea was noted on the arms, legs, and abdomen on day 113 but was not assessed as treatment-related. No pregnancies were reported during the study period.

All participants had negative anti-drug antibody results at days 1, 29 (Cohorts 1–4) or day 39 (Cohort 5) and day 113 (Cohorts 1–4) or day 123 (Cohort 5). Following a single dose of m102·4, the median T_{max} for Cohort 1, 2, 3 and 4 was 8, 4, 2·5, and 4·1 hours respectively. C_{max} and $AUC_{0\text{-last}}$ increased linearly with dose, with mean C_{max} and $AUC_{0\text{-last}}$ values for Cohort 4 being 692 µg/mL and 131,648 h·µg/mL, respectively (table 3 and figure 2). Concentration over time profiles demonstrated an initial rapid peak followed by a bi-exponential decline consistent with multi-compartmental distribution kinetics. The median $t_{1/2}$ was similar for Cohorts 2–4 (between 397 and 467 hours) but higher for Cohort 1 (663 hours). Relatively low inter-individual variability of pharmacokinetic parameters was observed. Among the repeat dose cohort (Cohort 5), the T_{max} and C_{max} for the first and second dose were 6 hours and 2·5 hours, respectively and 834 µg/mL and 1077 µg/mL, respectively.

Virus-neutralisation activity for both HeV and NiV was exhibited for all samples and time points tested. The viral neutralising titres ranged from 1/8 to ≥ 1/64 across all samples, corresponding to m102·4 concentrations of 2–10 µg/mL as measured by the pharmacokinetic ELISA. In 4 out of 5 participant samples, the Day 8 neutralising titre was within 1 or 2 doubling dilutions of the 12-hour sample from the same participant. For the 5th participant sample, the Day 8 neutralising titre was within 3 doubling dilutions of the 12-hour sample for HeV, and 1 doubling dilution for NiV. These results were considered to be within experimental variation, and therefore it was concluded that the m102·4 remained active for at least 8 days following mAb administration.

Discussion

In this phase 1 study we assessed the safety and tolerability of single and multiple IV doses of m102·4 in healthy human adults. All 40 participants completed the trial, with safety data indicating that m102·4 was safe and well tolerated. The maximum dose of 20 mg/kg was in-line with the human equivalent dose conversion (based on body surface area) at the NOAEL of 100 mg/kg, assigned from the 4 x weekly dosing rat toxicology study. Therefore the maximum clinical dosing of 2 x 20 mg/kg m102·4 was supported by the nonclinical data, and is a dose hypothesised to be pharmacologically relevant based on animal challenge data (see below).

TEAE reports were similar between placebo and active treatment groups. There was an increase in treatment-related headaches recorded among participants receiving single dose m102·4 compared to those administered placebo (66·7% vs 10%); there was no increase in treatment-related headaches among those with repeat dosing. Dose-related changes in ALT of three participants were noted; however these were mild abnormalities and were not considered clinically significant. There were no deaths or severe adverse events leading to withdrawals or premature discontinuations during the study. Pharmacokinetics based on those receiving active treatment (n=30) were linear, with the mean half-life ranging from 16·5 to 27·6 days across Cohorts 1–4. There was a small to moderate accumulation of mAb in those receiving repeat dose (Cohort 5), with elimination kinetics that were comparable to those receiving single dose regimens.

A variety of active immunisation strategies have been previously explored for HeV and NiV infection involving various recombinant viral vector-based vaccines in animal challenge models.[4] These studies demonstrated the possibility of inducing strong neutralising antibody response that could afford protection in livestock or an emergency-use scenario.[4] Among the most notable studies was the identification of the m102·4, the first fully-human monoclonal antibody that cross-neutralised *in vivo* all HeV and NiV isolates, including those from the Australian 1994 HeV outbreak and the Malaysian and Bangladesh NiV outbreaks. A single dose of m102·4 administered to ferrets 10 hours after lethal virus challenge was shown to be fully protective with no evidence of toxicity.[20,22]

In subsequent studies designed to closely reflect real-world human exposure, non-human primates were challenged with lethal doses of HeV and NiV by an intratracheal route, then infused twice with m102·4 mAb (15 mg/kg), beginning at 1, 3, or 5 days post-infection, and again 2 days later. Untreated control animals succumbed to disease eight days post-infection, while those in the treatment groups recovered by day 16.[23,24] Also tissue samples collected from treated animals had no evidence of infectious HeV or NiV at the termination of the study, demonstrating the potential of m102·4 to treat HeV and NiV infections in human and animal populations. A potential challenge to developing antiviral therapies for RNA viruses is their heterogeneity and mutational instability. However, the m102·4 recognises epitopes that are highly conserved among virus variants, making it unlikely that the virus will escape neutralisation due to mutation activity, and in the context of therapeutic dosing, in an immunocompetent individual.

Prior to this trial, a total of 14 individuals received prophylactic high-dose m102·4 on compassionate grounds following high level exposure to HeV (13 in Australia) or NiV (one in the United States; Supplementary Table 2). Individuals ranged from 8–59 years of age. No individual demonstrated clinical or laboratory evidence of infection prior to, or following m102·4 administration. The m102·4 administration was generally well tolerated; two individuals developed infusion-related febrile reactions to early production lots of antibody.

Because of the available data and findings on m102·4, including those presented here, the recent outbreak of NiV in Kerala, India, encouraged a number of organisations, including the Indian Council of Medical Research (ICMR), the Henry M. Jackson Foundation (HJF), the WHO, and the US National Institute of Allergy and Infectious Diseases (NIAID) and US National Institutes of Health (NIH) to work towards the development of an NiV therapeutics protocol for the use of m102·4 in the event of another outbreak of NiV in India or Bangladesh. To date, treatment for NiV has been limited to supportive care and management of acute encephalitis syndrome, as well as standard infection control practices to limit person-to-person transmission.[29] One pharmacologic option that was used for post-exposure prophylaxis during the Malaysian NiV outbreak of 1999 was the anti-viral Ribavirin. While this was associated with a significant reduction in mortality due to acute encephalitis, treatment allocation was not randomised, and the lack of clinical trial safety data beyond short-term emergency administration limits its use in outbreak circumstances.[30] The safety profile of both single and repeat dosing of m102·4, and the standard elimination pharmacokinetic profile presented in this study, underpins the current standard of care to offer a single dose of 20 mg/kg of m102·4 to humans with high levels of exposure to HeV and NiV. It also supports its potential use for patients with established clinical manifestations of henipavirus infection; based upon African green monkey challenge studies,[23,24] two doses separated by 48 hours would seem appropriate.

The main limitation of our study is the small number of participants, which is not uncommon in phase 1 studies. Also pharmacokinetic parameters were based on active treatment cohorts only consisting of 30 individuals. Future studies will be needed to ascertain the efficacy of m102·4 for treatment and prophylaxis against different viral strains of NiV and HeV virus, particularly among populations living in settings where there is the potential for an outbreak. Also, while there is no evidence of escape mutants to m102·4, the potential for this cannot be ruled out with RNA viruses. It may be necessary to consider a cocktail of mAbs with additional targets to combat the likelihood of diminished efficacy of m102·4.

In summary, m102·4 single and repeated dosing of up to two doses, three days apart appears to be safe and well tolerated when administered to healthy volunteers. Given that a 57% mortality rate has been observed from the seven known cases of human infection with HeV, and that m102·4 has demonstrated protective efficacy in non-human challenge studies, m102·4 is the most promising therapeutic opportunity to date for addressing this unmet medical need. This study will inform future dosing regimens for m102·4 to achieve systemic efficacy against HeV and NiV as post-exposure prophylaxis.

Contributors

EGP was the lead investigator involved in study design, data collection and analysis, and interpretation; TM was responsible for pre-clinical and clinical monoclonal antibody production; PPG and SMM were responsible for study design; MG was responsible for antibody quality review and data analysis; MLJ, KLH, BH, EH and KH were responsible for antibody manufacture and project management; CdB was involved in analytical and stability testing of m102·4 for clinical trial, and animal toxicology studies; RK implemented virus neutralization studies; L-FW and ILS designed and analysed virus neutralization studies;

PG and SE were involved in clinical trial study design, conduct, supervision and data interpretation; MEG was the clinical trial coordinator for patient screening procedures; SBL, HC, DES and KDL were involved in the planning and implementation phase of the clinical trial. SBL also reviewed and interpreted safety data from the trial; CCB supplied preclinical materials including the master cell bank and data on monoclonal antibody, and contributed to study design and data analysis; MGS negotiated the transfer of preclinical materials and data on mAb from the USA to Australia; DSD contributed to trial design and supplied the cell line for production of antibody for trial. All authors reviewed and approved the final manuscript.

Declaration of interests

CCB is a US federal employee; and CCB and DSD are co-inventors on US patent Nos. 7,988,971 and 8,313,746 “Human monoclonal antibodies against Hendra and Nipah viruses”; assignees are The United States of America as represented by the Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. (Bethesda, MD). All other authors declare no competing interests.

Data Sharing

Data generated in this study will be shared as deidentified data. Study protocol, statistical analysis plan, and informed consent forms are available with this publication. Any additional data may be made available upon request to the corresponding author following review by the protocol team.

Acknowledgements

We thank the participants who generously gave their time to participate in this study, and without whom the study would not have been possible. We acknowledge Roz Glazebrook and Wendy Morotti (Queensland Health) for initial coordination and preparation of trial related activity, and support with maintaining the study; Terry Hurst (Q-Pharm Pty Limited) for initial preparation and planning of initial grant funding of this phase 1 clinical trial; Gary Crameri (CSIRO Australian Animal Health Laboratory) for technical assistance with virus neutralization studies. We thank Clinic Manager Sharon Rankine and the Q-Pharm clinic team. We thank Drs Sonya Bennett (Executive Director) and Jeannette Young (Chief Health Officer) of Queensland Health, for ongoing support for this project, and securing a significant portion of the funding for this trial. The manufacture of m102·4 drug substance was performed at the National Biologics Facility (NBF), at the University of Queensland. The NBF is supported by Therapeutic Innovation Australia (TIA), which is supported by the Australian Government through the National Collaborative Research Infrastructure Strategy (NCRIS) program. The authors thank Dr Sharon Johnatty of SugarApple Communications for providing medical writing support, which was funded by Queensland Health, Brisbane, Australia, in accordance with Good Publication Practice (GPP3) guidelines (https://www.ismpp.org/gpp3).

References

1. Wang L-F, Mackenzie JS, Broder CC. Henipaviruses. In: Knipe DM, Howley PM, eds. Fields Virology. Philadelphia: Lippincott Williams & Wilkins; 2013: 1070-85.
2. Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol* 2000; **81**(Pt 8): 1927-32.
3. Chua KB, Koh CL, Hooi PS, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 2002; **4**(2): 145-51.
4. Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. *Vaccine* 2016; **34**(30): 3525-34.
5. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* 2012; **359**: 153-77.
6. Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* 2013; **100**(1): 8-13.
7. Wong KT, Ong KC. Pathology of acute henipavirus infection in humans and animals. *Patholog Res Int* 2011; **2011**: 567248.
8. Playford EG, McCall B, Smith G, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis* 2010; **16**(2): 219-23.
9. Wong KT, Tan CT. Clinical and pathological manifestations of human henipavirus infection. *Curr Top Microbiol Immunol* 2012; **359**: 95-104.
10. Murray K, Rogers R, Selvey L, et al. A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* 1995; **1**(1): 31-3.
11. International Society for Infectious Diseases. HENDRA VIRUS - AUSTRALIA: (NEW SOUTH WALES) HORSE. June 2019. Archive Number: 20190621.6531307. http://www.promedmail.org/post/6531307 (accessed June 2019).
12. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* 1999; **354**(9186): 1257-9.
13. Chattu VK, Kumar R, Kumary S, Kajal F, David JK. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. *J Family Med Prim Care* 2018; **7**(2): 275-83.
14. Sazzad HM, Hossain MJ, Gurley ES, et al. Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. *Emerg Infect Dis* 2013; **19**(2): 210-7.
15. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Scientific reports* 2016; **6**: 30916.
16. Luby SP. The pandemic potential of Nipah virus. *Antiviral research* 2013; **100**(1): 38-43.
17. World Health Organization. WHO publishes list of top emerging diseases likely to cause major epidemics. 10 December 2015. https://www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/; (accessed June 2019).
18. Rottingen JA, Gouglas D, Feinberg M, et al. New Vaccines against Epidemic Infectious Diseases. *N Engl J Med* 2017; **376**(7): 610-3.
19. Zhu Z, Dimitrov AS, Bossart KN, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J Virol* 2006; **80**(2): 891-9.
20. Zhu Z, Bossart KN, Bishop KA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J Infect Dis* 2008; **197**(6): 846-53.
21. Xu K, Rockx B, Xie Y, et al. Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog* 2013; **9**(10): e1003684.

22. Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS Pathog* 2009; **5**(10): e1000642.

23. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Sci Transl Med* 2011; **3**(105): 105ra3.

24. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med* 2014; **6**(242): 242ra82.

25. Queensland Government. World-first Hendra treatment one step closer. Oct 31 2013. http://statements.qld.gov.au/Statement/2013/10/31/worldfirst-hendra-treatment-one-step-closer: Queensland Government; (accessed June 2019).

26. EMEA/CHMP/SWP. Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. EMEA/CHMP/SWP/28367/07 Rev.1. July 2017. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-strategies-identify-mitigate-risks-first-human-early-clinical-trials-investigational_en.pdf: European Medicines Agency; (accessed June 2019). p. 1-12.

27. US Food and Drug Administration. Guidance for Industry: Bioanalytical Method of Validation. May 2001. http://www.labcompliance.de/documents/FDA/FDA-Others/Laboratory/f-507-bioanalytical-4252fnl.pdf; (accessed June 2019).

28. Luby SP, Broder CC. Paramyxoviruses: Henipaviruses. In: Kaslow RA, Stanberry LR, Le Duc JW, eds. Viral Infections of Humans, Epidemiology and Control. New York: Springer Science+Business Media; 2014: 519-36.

29. Centers for Disease Control and Prevention. Treatment. Nipah Virus (NiV). https://www.cdc.gov/vhf/nipah/treatment/index.html; (accessed on 16 July, 2019).

30. Banerjee S, Gupta N, Kodan P, et al. Nipah virus disease: A rare and intractable disease. *Intractable Rare Dis Res* 2019; **8**(1): 1-8.

Tables

Table 1: Participant baseline characteristics

	Cohort 1 (1 mg/kg) (n=6)	Cohort 2 (3 mg/kg) (n=6)	Cohort 3 (10 mg/kg) (n=6)	Cohort 4 (20 mg/kg) (n=6)	Cohort 5 (2 x 20 mg/kg) (n=6)	Pooled placebo (n=10)	Overall (n=40)
Sex							
Male	1 (17%)	4 (67%)	5 (83%)	3 (50%)	4 (70%)	7 (70%)	24 (60%)
Female	5 (83%)	2 (33%)	1 (17%)	3 (50%)	2 (30%)	3 (30%)	16 (40%)
Age, years							
Mean (SD)	29·8 (9·1)	30·5 (5·1)	24·7 (3·9)	29·7 (10·9)	27·7 (5·5)	26·6 (5·0)	28·0 (6·7)
Range	18–42	23–36	21–32	20–44	21–35	20–37	18–44
Weight, kg							
Mean (SD)	71·5 (15·2)	84·9 (14·1)	74·7 (10·8)	67·7 (7·3)	80·5 (12·1)	79·3 (8·0)	76·7 (11·9)
Range	51·3–95·7	65·2–98·0	60·4–86·4	58·2–77·0	67·1–98·5	71·8–95·6	51·3–98·5
BMI, kg/m^2							
Mean (SD)	24·6 (5·3)	26·6 (5·3)	24·0 (4·0)	23·1 (2·5)	26·0 (4·4)	26·3 (3·4)	25·2 (3·9)
Range	19·5–33·9	21·8–31·3	19·9–29·5	19·7–26·2	20·3–32·9	22·6–32·7	19·5–33·9
Race, n (%)							
White	5 (83%)	5 (83%)	5 (83%)	6 (100%)	6 (100%)	9 (90·0%)	36 (90·0%)
Other	1 (17%)	1 (17%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	3 (7·5%)
Pacific Islander	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	1 (2·5%)

Data are n (%), mean (SD), range (minimum – maximum); BMI=body-mass index; Race='Other' were Eurasian, white/Asian/Aboriginal or South American Indigenous

Table 2: Summary of adverse events

	Cohort 1 (1 mg/kg) (n=6)	**Cohort 2 (3 mg/kg) (n=6)**	**Cohort 3 (10 mg/kg) (n=6)**	**Cohort 4 (20 mg/kg) (n=6)**	**Cohort 5 (20 mg/kg) (n=6)**	**Pooled placebo (n=10)**	**Overall (n=40)**
Participants with ≥1 TEAEs	5 (83%) [12]	5 (83%) [10]	6 (100%) [14]	6 (100%) [13]	6 (100%) [11]	9 (90%) [26]	37 (93%) [86]
Participants with serious TEAEs	0	0	0	0	0	0	0
Participants with any TEAE leading to discontinuation of study drug	0	0	0	0	0	0	0
Gastrointestinal disorders	1 (17%) [1]	0	2 (3%) [3]	1 (17%) [3]	1 (17%) [1]	1 (10%) [1]	6 (15%) [9]
General disorders and administration site conditions	1 (17%) [1]	2 (3%) [3]	3 (50%) [3]	0	1 (17%) [1]	4 (40%) [7]	11 (28%) [15]
Infections and infestations	4 (67%) [5]	2 (33%) [2]	2 (33%) [2]	3 (50%) [4]	2 (33%) [2]	5 (50%) [6]	18 (45%) [21]
Investigations	0	0	0	0	2 (33%) [2]	1 (10%) [1]	3 (8%) [3]
Metabolism and nutrition disorders	0	0	1 (17%) [1]	0	0	0	1 (3%) [1]
Musculoskeletal and connective tissue disorders	0	0	0	0	0	2 (20%) [3]	2 (5%) [3]
Nervous system disorders (headaches)	1 (17%) [1]	1 (17%) [1]	2 (33%) [2]	4 (67%) [6]	4 (67%) [4]	3 (30%) [4]	15 (38%) [18]
Psychiatric disorders	0	1 (17%) [1]	0	0	0	1 (10%) [1]	2 (5%) [2]
Renal and urinary disorders	0	0	0	0	0	1 (10%) [1]	1 (3%) [1]
Respiratory, thoracic and mediastinal disorders	2 (33%) [2]	1 (17%) [1]	0	0	0	2 (20%) [2]	5 (13%) [5]
Skin and subcutaneous tissue disorders	2 (33%) [2]	1 (17%) [2]	2 (33%) [2]	0	1 (17%) [1]	0	6 (15%) [7]
Surgical and medical procedures	0	0	1 (17%) [1]	0	0	0	1 (3%) [1]

Data are displayed as number of participants (% of participants within cohort) [number of TEAEs]

Table 3: Pharmacokinetic parameters for each cohort (8 treated participants per cohort) of healthy adults treated with m102·4

	C_{max} (µg/mL)		T_{max} (h)	$AUC_{0\text{-}last}$ (h·µg/mL)		$t_{1/2}$ (h)	CL (mL/h/kg)		V_z (mL/kg)	
	Mean (SD)	**Coefficient of Variation**	**Median (range)**	**Mean (SD)**	**Coefficient of Variation**	**Median (range)**	**Mean (SD)**	**Coefficient of Variation**	**Mean (SD)**	**Coefficient of Variation**
Cohort 1 (1 mg/kg); n=6	29·9 (5·8)	19·5%	8 (4·0–12·0)	5,528·7 (953·0)	17·2%	663·3 (474·3–735·1)	0·18 (0·03)	16·4%	167·3 (54·1)	32·3%
Cohort 2 (3 mg/kg); n=6	130·4 (38·9)	29·8%	4 (4·0–8·0)	21874·2 (4942·0)	22·6%	466·3 (382·8–522·3)	0·14 (0·03)	21·3%	95·2 (29·5)	31·0%
Cohort 3 (10 mg/kg); n=6	357·2 (72·7)	20·3%	2·5 (1·0–12·1)	79102·2 (15061·2)	19·0%	397 (333·9–491·8)	0·13 (0·03)	20·6%	75·1 (11·6)	15·4%
Cohort 4 (20 mg/kg); n=6	692 (104·7)	15·1%	4·1 (1·0–8·0)	131648·3 (18344·0)	13·9%	466·7 (351·0–889·6)	0·15 (0·02)	15·5%	112·6 (36·8)	32·7%
Cohort 5 (20 mg/kg): first dose (day 1); n=6	834·3 (150·6)	18·0%	6·0 (1·0–12·1)	33082·4 (6222·7)	18·8%					
Cohort 5 (20 mg/kg): second dose (day 4); n=6	1076·7 (187·7)	17·4%	2·5 (1·0–8·0)	337,574·6 (50058·1)	14·8%	472 (385·6–592·0)	0·06 (0·001)	14·6%	40·5 (6·6)	16·3%

Pharmacokinetic data for mAb m102·4 serum concentrations were calculated using all participants receiving m102·4 (n=30). C_{max} =maximum observed serum concentration (mean, SD and coefficients of variation). T_{max} = time to C_{max} (hours, median and range) $AUC_{0\text{-}last}$ =Area under the m102·4 concentration curve from pre-dose to last measurable concentration (mean, SD and coefficients of variation), $t_{1/2}$ =elimination half-life (hours, median and range), CL = clearance (mean, SD and coefficients of variation), V_z = volume of distribution (mean, SD and coefficients of variation).

Figure Legends

Figure 1: Study Profile

* Withdrawals were due to time constraints (n=10), personal or family illness (n=4), withdrew no reason given (n=9).

Figure 2: Mean log-scaled concentrations of total m102·4 concentrations (µg/mL) in serum from healthy adults (8 treated participants per cohort), by nominal time and treatment group, as determined by ELISA

Title

Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of Henipaviruses in healthy adults: a randomised, first-in-human phase 1 study.

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Summary

Background

~~The henipaviruses, Hendra virus and Nipah virus, are emerging zoonotic pathogens of bat origins that have been associated with poor clinical outcomes in humans.~~ The monoclonal antibody (mAb) m102·4 is a potent fully human antibody that neutralizes Hendra and Nipah viruses *in vitro* and *in vivo*. ~~Clinical trials in humans represent the next stage in the utilization of this antibody in the prophylactic treatment and prevention of deaths from henipavirus infections~~We investigated its safety, tolerability, pharmacokinetics, and immunogenicity in healthy adults.~~.~~

Methods

In this ~~We performed a randomised,~~ double-blind, placebo-controlled, single-centre, dose escalation phase 1 trial of~~study using the human monoclonal antibody~~ m102·4, we randomised h~~. H~~ealthy adults aged 18–50 years with a body-mass index of 18·0–~~– to ≤~~35·0 kg/m^2 ~~were assigned~~ to one of five cohorts. A sentinel pair for each cohort was randomised to~~in a 1:1 ratio to receive~~ either m102·4 ~~active treatment~~ or placebo. The remaining participants in each cohort were randomised 5:1 to receive m102·4 or placebo. Cohorts 1 to 4 received a single intravenous infusion of m102·4 at doses of 1, 3, 10, and 20 mg/kg respectively, and were monitored for 113 days. Cohort 5 received two infusions of 20 mg/kg, 72 hours apart, with monitoring for 123 days. The primary objective was~~ to assess the~~ safety and tolerability; secondary objectives were~~ of single and multiple intravenous doses of m102·4 mAb. We also assessed~~ pharmacokinetics and~~,~~ immunogenicity~~, and virus neutralisation~~. Analyses were completed according to protocol. The study was registered on the Australian New Zealand Clinical Trials Registry, registration number ACTRN12615000395538.

Findings

Between March 27~~April 15~~, 2015 and June 16, 2016, 40 of 77 healthy screened adults were enrolled in~~ were screened and 40 participants were enrolled in~~ the study; eight were assigned to each cohort (six ~~randomised to~~ receive~~-~~d m102·4, and~~ two randomised to~~ two received placebo~~ per cohort~~). Eighty-six~~A total of 86~~ treatment-emergent adverse events were reported,~~ by the 40 participants~~ with similar rates between placebo and ~~active treatment~~ treatment groups. The most common treatment-related event was headache (12 of 30 [40%] in the combined m102·4 group, and 3 of 10 [30%] in the pooled placebo group). ~~Thirty one events reported in 20 participants (50%) receiving m102·4 were considered treatment related, and six events were reported in five participants (50%) receiving placebo. A possible trend of treatment related headaches with m102·4 was noted; this was reported in 66.7% of participants administered single dose m102·4 compared with 10% of those administered placebo. There was no increase in treatment related headaches among those with repeat dosing.~~ There were no deaths or severe adverse events leading to study~~withdrawals or premature~~ discontinuation~~s during the study~~. Pharmacokinetics based on those receiving m102·4 ~~active treatment~~ (n=30) were linear, with a~~the~~ mean half-life of ~~ranging from~~ 16·5–~~ to~~ 27·6 days across Cohorts 1–4. ~~There was small to moderate accumulation of monoclonal antibody in those receiving a repeat dose (Cohort 5), with~~ The elimination kinetics of those receiving repeated dosing (Cohort 5) ~~that~~ were comparable to ~~those receiving a~~ single-~~-~~dose recipients. Anti-m102·4 antibodies were not detected at any time-point during the study.~~ All serum samples at all time points exhibited virus-neutralisation activity when tested *in vitro* for both HeV and NiV.~~

Interpretation

m102·4 single and repeated dosing ~~of up to two doses separated by 3 days~~ were ~~safe and~~ well tolerated, displayed ~~when administered to healthy adult volunteers. A~~ linear~~standard~~ pharmacokinetics~~ profile was observed with linear elimination kinetics~~, and showed ~~. There was~~ no evidence of an immunogenic response~~ to m102·4. Exploratory virus neutralisation studies confirmed that m102·4 remained active for at least 8 days following administration of a clinically relevant dose.~~ . This study will inform future dosing regimens for m102·4 to achieve prolonged exposure for systemic efficacy to prevent and treat~~ against~~ henipavirus infections~~es~~.

Funding

Queensland Department of Health, the National Health and Medical Research Council and the National Hendra Virus Research Program.

Research in Context

Evidence before this study

We searched PubMed on May 31, 2019 for studies using the following terms: (“Hendra” OR “Nipah”) AND (“monoclonal antibody” OR “monoclonal antibodies”) without language restrictions. We restricted article type to clinical trials and species to human. There were no articles describing clinical trials of monoclonal antibodies designed for human prophylaxis. The human monoclonal antibody m102·4 binds to the same/similar region of the G glycoprotein of Hendra and Nipah viruses as the ephrin receptors. The m102·4 monoclonal antibody demonstrated protection from infection, and was effective when administered following experimental challenge with Hendra and/or Nipah viruses in ferret and non-human primate models.

The m102·4 has been available since 2010 for compassionate therapy following high-risk Hendra virus exposures. It has been used on 14 occasions (13 in Queensland, Australia and one in the United States). The safety and tolerability of the m102·4 has not been systematically assessed.

Added value of this study

This is the first study to assess the safety, tolerability, pharmacokinetics, and immunogenicity of the m102·4 in healthy adults using a variety of single dosing regimens (1 mg/kg, 3 mg/kg, 10 mg/kg, and 20 mg/kg) and a repeat dosing regimen of 20 mg/kg separated by 72 hours. The four single and one repeat dosing regimens of the m102·4 were generally safe and well tolerated, and had a half-life of 16·5–27·6 days with no observed immunogenicity associated with the m102·4.

Implications of all the available evidence

The findings of this study support the use of the m102·4 in humans following high-risk henipavirus exposure. Two doses of 20 mg/kg separated by 72 hours were as well tolerated as a single dose of 20 mg/kg.

Introduction

Nipah virus (NiV) and Hendra virus (HeV) are enveloped, single-stranded negative sense RNA viruses and the prototype members of the genus *Henipavirus* in the family *Paramyxoviridae.*[1] Bats of the genus *Pteropus* appear to be the major natural reservoir hosts for henipaviruses, from which all bat isolates of HeV and NiV have been derived.[2,3] HeV and NiV are two emerged zoonotic, transboundary agents with uniquely broad host tropism capable of infecting at least 18 animal species across six orders of mammals, including their natural bat hosts.[4,5] HeV and NiV are highly pathogenic, and cause wide-spread infection with severe disease in multiple organ systems in at least 11 mammalian species. Fatality rates range from 40–100%.[6] In humans, HeV and NiV infection results in a systemic and often fatal respiratory and/or neurological disease.[7,8] Both HeV and NiV can also cause a relapsing of encephalitis following recovery from an acute infection; this appears to result from a recrudescence of virus replication in the central nervous system.[9]

HeV was first recognized in 1994 during an outbreak of fatal cases of a severe respiratory disease in horses and humans which occurred in the Brisbane suburb of Hendra, Australia.[10] In all, 14 horses and their trainer succumbed to infection together with the non-fatal infection of seven additional horses and a stablehand. To date, HeV has appeared in eastern Australia on 61 occasions, causing fatalities in four of seven human cases, death or euthanasia of more than 100 horses, and euthanasia of two HeV antibody positive dogs.[4,11]

A large outbreak of encephalitis in 1998 among pig farmers in Peninsular Malaysia led to the identification of NiV, a Hendra-like virus isolated from the cerebrospinal fluid of two patients.[12] Outbreaks of human cases of NiV infection have been recorded almost annually since 2001 in Bangladesh~~ and India~~. Between 1998 and 2018, a total of 637 cases of human NiV infection with 373 fatalities were reported in South and Southeast Asia. The mode of NiV transmission was direct human contact with infected pigs, consumption of raw date palm sap that may have ~~had~~ been contaminated by fruit bats, or human-to-human transmission.[13] Corpse-to-human transmission of NiV has also been documented in a case series report of sporadic cases in Bangladesh in 2010.[14] At least two strains of NiV have been identified in genetic analysis; NiV_M associated with the 1998 Malaysian outbreak, and NiV_B that has caused repeated outbreaks in Bangladesh and northeastern India.[15] There is considerable genetic heterogeneity in human isolates of NiV, particularly from the

Bangladesh outbreaks. This feature of NiV, in combination with transmission modes that include human-to-human, raises concerns of a pandemic potential.[16]

There are no approved vaccines or therapeutics to prevent or treat disease in humans. NiV and HeV are currently listed among WHO priority diseases for which a research and development blueprint of action to prevent epidemics has been developed.[17] Both are also emerging viruses selected for the human vaccine development initiative funded by the Coalition for Epidemic Preparedness Innovations~~ (CEPI)~~.[18]~~[18,19]~~

HeV and NiV particles possess surface projections composed of the viral transmembrane anchored fusion (F) and attachment (G) glycoproteins, which are the mediators of virus attachment and host cell infection. They are also the major antigenic targets of virus-neutralising antibodies. Both the HeV and NiV G glycoproteins bind to the host cell membrane proteins ephrin-B2 and ephrin-B3, which are highly conserved across known susceptible hosts, as reviewed by Broder and colleagues.[4] HeV- and NiV-neutralising human antibody fragments reactive to the G glycoproteins of both HeV and NiV have previously been isolated using recombinant antibody technology.[19] One monoclonal antibody (mAb), m102, possessed strong cross-reactive and neutralising activity against HeV and NiV, and was subsequently affinity matured to m102·4 , formatted as an IgG1 mAb, and produced in a CHO-K1 cell line.[20] The m102·4 epitope localises to the ephrin receptor binding site on the G glycoprotein and binds in a similar fashion as the ephrin molecule.[21] In a post-exposure NiV challenge experiment in the ferret model, a single dose of m102·4 administered by intravenous (IV) infusion ten hours after a lethal virus challenge was shown to provide complete protection.[22] The m102·4 was also shown to be effective against both NiV and HeV infection in nonhuman primates in studies designed to reflect a potential real-world infection exposure scenario,[15,23,24] highlighting clear potential for future approved human use.

In 2010, the m102·4 cell line was provided to the Queensland Government, (Queensland Health, Australia), to produce the m102·4 for compassionate emergency use in the event of high-risk human HeV exposure. Following an unprecedented number of equine HeV notifications (22 horses on 18 properties) in Queensland and New South Wales in 2011,[11] planning commenced in 2012 for the first phase 1 clinical trial of the m102·4 in human participants.[25] This trial aimed to evaluate the safety, tolerability, immunogenicity, and pharmacokinetics of single and multiple IV administrations of m102·4 in healthy participants, and was completed in 2016. Here we report the results of this safety trial.

Methods

Study design and Participants

We performed a single-centre, randomised, double blind, placebo-controlled, dose escalation phase 1 study to assess the safety, tolerability, pharmacokinetics, and immunogenicity of m102·4 in healthy adults at Q-Pharm Pty Ltd (Queensland, Australia). Ethics approval was obtained from the study site's Human Research Ethics Committee at the QIMR Berghofer Medical Research Institute before participant enrolment commenced. The study was conducted under the Therapeutic Goods Administration (TGA) Clinical Trial Notification Scheme (CTN) and in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and the National Health and Medical Research (NHMRC) statement on ethical conduct in human research.

Healthy males and females between 18 and 50 years of age, with a body mass index of 18·0 to ≤35·0 kg/m^2 and weight ≥50 kg to ≤100 kg, normal values for blood haematology, coagulation profiles, clinical chemistry, and urinalysis results, were eligible for inclusion. Exclusion criteria included close, unprotected exposure to sick horses with an unexplained illness within 4 weeks; known exposure to potential HeV infected horses; positive tests for HIV or hepatitis B or C viruses; treatment with an investigational drug or biologic agent within 60 days; sexually active males with a partner capable of becoming pregnant; and females capable of becoming pregnant in the absence of an effective method of birth control. Use of prescription drugs within 14 days or five half-lives prior to mAb administration, and pregnancy or breastfeeding were also exclusion criteria. Additional details of inclusion and exclusion criteria are outlined in the trial protocol (appendix pg 42). All participants understood the study requirements and treatment procedures, and were willing to comply with all study restrictions. Participants provided written informed consent before any study-specific tests or procedures were performed.

Randomisation and masking

Participants were given a sequential screening number at their screening visit. Eligible participants were assigned to one of five cohorts, each with eight participants. In each cohort, six participants were randomised to receive m102·4 , and two were randomised to receive placebo in accordance with the randomisation plan prepared and maintained by Clinical Network Services, (Queensland, Australia), the contract research organisation.

In each cohort, two individuals in a sentinel pair were randomised in a double blinded manner to receive active and placebo in a 1:1 ratio. Sentinel pairs were dosed seven days prior to the other participants in that cohort in order to detect any serious adverse advents (SAEs) in accordance with the European Medical Agency first-in-man guidelines.[26] The remaining participants in each cohort received either m102·4 or placebo (0·9% saline) in a 5:1 ratio following review of safety data by the Investigator and the Medical Monitor. The decision to progress to the next higher dose was made after safety and tolerability data was reviewed for each dose group. Repeat dosing (Cohort 5) did not commence until all pharmacokinetic data was reviewed for Cohorts 1-4 (see Appendix pg 3 & 19). As this was a single site trial with eight participants allocated to five cohorts, no block randomisation was performed.

To maintain the blinding of the study, the randomisation plan was available only to the un-blinded pharmacist. Treatment allocation was double blinded, and m102·4 and placebo were identical in appearance and volume for each participant in each cohort.

Procedures

The m102·4 drug substance for the phase 1 study was manufactured at the National Biologics Facility (NBF) located at the University of Queensland (Queensland, Australia) using a recombinant Chinese Hamster Ovary (CHO) cell line. A parent cell line was obtained from the National Institutes of Health, (Bethesda, MD, USA) and then subcloned and adapted to a serum- and protein-free commercially available medium. Cells were expanded from a qualified master cell bank using a conventional seed-train, followed by a fed-batch bioreactor process. The clarified harvest containing secreted m102·4 was subjected to a two-step purification process including MabSelect SuRe^{TM} LX protein A and mixed mode Capto^{TM}adhere chromatography. The process included two viral inactivation/removal steps, a low pH hold, and nanofiltration (Viresolve® Pro Modus) respectively. The m102·4 was formulated at a concentration of 10 mg/mL in phosphate buffered saline pH 7·3 and aseptically dispensed into 10 mL vials at Sypharma Pty Ltd (Victoria, Australia). The final product complied with specifications commonly accepted by regulatory agencies for identity, purity, potency, and safety.

Prior to clinical studies, Good Laboratory Practice (GLP) compliant, repeat dose toxicology studies were conducted in the Sprague-Dawley rat at vivoPharm Pty Ltd (Victoria, Australia). The m102·4 was administered once per week for four weeks at doses of 0, 10, 30, and 100 mg/kg. The antibody was well tolerated and the no-observed-adverse-effect-level (NOAEL) was determined to be 100 mg/kg, the highest dose tested.

An enzyme-linked immunosorbent assay (ELISA) was developed by the NBF, then validated and used to measure the concentration of m102·4 in human serum samples at vivoPharm Pty Ltd. Briefly, serum m102·4 bound to Hendra virus soluble G protein coated on a plate, was detected with a custom rabbit anti-m102·4 horseradish peroxidase conjugate followed by the addition of 3,3',5,5'-Tetramethylbenzidine substrate. The validated assay met criteria in the relevant US guideline[27], and had a working range of 1·7–163·2 ng/mL, which corresponded to the lower and upper limits of quantitation, respectively. All samples were diluted within this working range, quantitated via standard curve interpolation, and pharmacokinetic parameters were calculated using noncompartmental techniques.

An anti-drug antibody ELISA was developed and validated to determine the presence of anti-m102·4 antibodies in human serum samples. A bridging assay design was employed where m102·4, previously coupled either with biotin or horseradish peroxidase, was mixed with serum or positive control (a custom anti-m102·4 rabbit polyclonal antibody) to form a complex which was captured onto a streptavidin coated plate. The assay procedure included an acidification step to dissociate drug-antibody complexes. The validated assay met criteria consistent with the US guidelines,[27] and had a sensitivity of 100 ng/mL. Human serum samples were considered positive if both the screening cut-point (determined statistically based on a 95% upper confidence limit from negative normal donor sera) and specificity confirmation cut-point (where excess m102·4 was added) were exceeded.

Based on animal models of Henipavirus challenge,[1,22-24,28] a target dose of 20 mg/kg was selected as the maximum dose to be tested. Treatment recipients in the five cohorts were dosed with four concentrations of m102·4. Cohorts 1 and 2 received single IV doses of 1 mg/kg and 3 mg/kg, respectively, or placebo, infused in a total volume of 100 mL of 0·9% saline. Cohorts 3 and 4 received single IV infusions of 10 mg/kg and 20 mg/kg, respectively, or placebo, in a total volume of 500 mL of 0·9% saline, while Cohort 5 received two IV infusions of 20 mg/kg, or placebo, in 500 mL of 0·9% saline 72 hours apart. All infusions were given over a 1-hour period using a peristaltic infusion pump.

Participants in Cohorts 1–4 were domiciled for a total period of 3·5 days for initial safety and tolerability monitoring, and to facilitate blood sampling for pharmacokinetics, virus neutralisation, and immunogenicity analysis. Participants received the m102·4 mAb or placebo on day 1, and follow-up assessments were scheduled on days 4, 6, 8, 15, 29, 43, 57, 85, and 113. Participants in Cohort 5 were domiciled for a total period of 6·5 days receiving a single dose of m102·4 or placebo on days 1 and 4. Follow up visits to the clinical site were scheduled on days 7, 9, 11, 18, 25, 32, 39, 53, 67, 95, and 123. For all cohorts, the final safety assessment was scheduled to occur within a maximum period of 16 weeks after the last infusion.

Dose escalation from one cohort to the next followed review and approval of safety data for at least six participants per group. In addition, blinded pharmacokinetic data for Cohort 1 was reviewed to confirm estimation of m102·4 elimination half-life. Initiation of dosing in Cohort 5 followed review of available unblinded pharmacokinetic data for Cohorts 1–4.

A virus neutralisation assay was designed and performed by the Australian Animal Health Laboratory (AAHL) located at the Commonwealth Scientific and Industrial Research Organisation (CSIRO, Victoria, Australia). Briefly, sera from five Cohort 4 participants (20 mg/kg, a clinically relevant dose) were tested against HeV and NiV to assess the ability of circulating m102·4 to neutralise each virus in samples at four different time points post-infusion (12hr, 24hr, Day 4, and Day 8). Samples were diluted to achieve an end-point for neutralisation. Control plates were set up using m102·4-spiked media + 10% normal human serum to achieve final concentrations ranging from 50 to 0·4 µg/mL. Samples of HeV (HeV/Australia/Horse/2008/Redlands) and NiV (NiV/Bangladesh/human/2004/Rajbari, R1) were diluted to contain 100 $TCID_{50}$ of virus and added to the required wells. Serum, cell and virus controls were included. Vero cell suspension was added to every well at a concentration of $4x10^5$ cells/mL. Plates were incubated at 37°C in a humid atmosphere containing 5% CO_2 for 45–60 min. Cells were examined after 3 days under an inverted microscope for cytopathic effect.

Outcomes

The primary objective of the phase 1 trial was to assess the safety and tolerability of single and multiple IV doses of m102·4 in healthy human participants; the second objective was to determine the pharmacokinetic and immunogenicity of single and multiple doses; and the third objective was to explore the pharmacodynamics of m102·4 against HeV and NiV.

The safety and tolerability of m102·4 across all cohorts was evaluated according to the following specific assessments: physical examination, clinical laboratory tests, vital signs, electrocardiogram, signs and symptoms of tolerability, infusion site reactions, and adverse events (AEs). All AEs and treatment-emergent AEs (TEAEs) were reported for participants from dosing until the end of the study. Definitions of AEs and TEAEs can be found in the trial protocol (appendix pg 50–52).

Calculated pharmacokinetic values included maximum observed drug concentration (C_{max}), time to maximum observed drug concentration (T_{max}), area under the drug concentration-time curve ($AUC_{0\text{-last}}$ and $AUC_{0\text{-inf}}$), elimination half-life ($t_{½}$), clearance (CL), and volume of distribution (V_z). We also took blood samples pre-dose and at nominal post-dose time-points for measurement of m102·4 HeV and NiV virus neutralisation, and for assessment of anti- m102·4 antibodies.

Statistical analysis

Statistical analysis comprised summary descriptive statistics of the five dose groups and placebo group. Given the exploratory nature of the study, the sample size was not based on power calculations, but was consistent with the typical sample size used for similar studies. Descriptive statistics were performed with SAS version 9·4. Pharmacokinetic data were analysed with Phoenix WinNonlinSoftware version 6·3, with pharmacokinetic listings, tables, and figures generated using SAS Software version 9·4. The study is registered on the Australian New Zealand Clinical Trials Registry, registration number ACTRN12615000395538.

Role of the funding source

The funders of the study had no role in data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between 15 April 2015 and 18 February 2016, 40 participants were allocated into five cohorts as described (figure 1). All enrolled participants received their full assigned treatments and completed the study according to schedule apart from two participants in cohort 4 who failed to attend their day 43 and day 85 visits, respectively. Demographic characteristics did not vary between treatment groups (table 1).

Overall the dosage of m102·4 appeared safe and well tolerated. No deaths, life threatening events, serious adverse events, or adverse events leading to study discontinuation occurred. Across all cohorts, a total of 37 of 40 (93%) participants had at least one TEAE, with a total of 86 TEAEs reported (table 2). The occurrence of TEAEs was similar across all active treatment and placebo groups.~~ ~~ The most frequently reported events were infections and infestations, classed as mild to moderate; 13 were reported in 11 of 24 single-dose participants (45.8%), two of six (33%) repeat dose participants, and five of 10 (50%) in the pooled placebo group. A possible association between dose and headache was noted, occurring in eight of 12 (67%)~~ of~~ participants dosed in Cohorts 4 and 5 compared with four of 18 (22~~5~~%)~~ of~~ participants dosed in Cohorts 1–3, and three of 10 (30%) participants in the~~ and~~ pooled placebo group. All TEAEs were resolved upon follow-up without specific intervention.

Of all TEAEs, 31 in 20 participants were considered treatment-related, occurring in three of six (50%) ~~of ~~participants dosed in Cohort 1, two of six (33%) participants dosed in Cohorts 2, 3, and 5, five of six (83%) participants dosed in Cohort 4, and five of 10 (50%) participants in ~~of ~~the pooled placebo group. The most common treatment-related TEAEs were headaches (10 participants: one each in Cohorts 1–3, four dosed in Cohort 4, two dosed in Cohort 5, and one in the pooled placebo group), intravenous catheter site bruising (three participants: two dosed in Cohort 3 and one in the pooled placebo group), and elevated alanine aminotransferase (ALT) levels (three participants: two in Cohort 5 and one in placebo). Of the 20 treatment-related TEAEs, 15 were rated as mild and five as moderate. Moderate TEAEs included two headaches; one each in Cohorts 4 and 5, one intravenous catheter site bruising in Cohort 3, lethargy in a placebo participant, and reduced appetite in Cohort 3. There were no severe TEAEs (Table 2).

No clinically significant changes in haematological or coagulation parameters were observed in any participant. Three participants had elevated ALT levels, two dosed in Cohort 5 and one in the pooled placebo group; Cohort 5 participants had elevated ALT levels at day 4 and 11, respectively; both findings following m102·4 administration were considered to be mild and treatment-related. ALT levels returned to normal after day 11 without intervention, and were not considered to be clinically significant (Supplementary Table 1~~2~~). No other clinically significant abnormalities in biochemical parameters, urinalysis, vital signs, or ECG findings were observed. One clinically significant abnormal physical examination finding was reported in one participant dosed in Cohort 3; pityriasis rosea was noted on the arms, legs, and abdomen on day 113 but was not assessed as treatment-related. No pregnancies were reported during the study period.

All participants had negative anti-drug antibody results at days 1, 29 (Cohorts 1–4) or day 39 (Cohort 5) and day 113 (Cohorts 1–4) or day 123 (Cohort 5). Following a single dose of m102·4, the median T_{max} for Cohort 1, 2, 3 and 4 was 8, 4, 2·5, and 4·1 hours respectively. C_{max} and $AUC_{0\text{-}last}$ increased linearly with dose, with mean C_{max} and $AUC_{0\text{-}last}$ values for Cohort 4 being 692 µg/mL and 131,648 h·µg/mL, respectively (table 3 and figure 2). Concentration over time profiles demonstrated an initial rapid peak followed by a bi-exponential decline consistent with multi-compartmental distribution kinetics. The median $t_{1/2}$ was similar for Cohorts 2–4 (between 397 and 467 hours) but higher for Cohort 1 (663 hours). Relatively low inter-individual variability of pharmacokinetic parameters was observed. Among the repeat dose cohort (Cohort 5), the T_{max} and C_{max} for the first and second dose were 6 hours and 2·5 hours, respectively and 834 µg/mL and 1077 µg/mL, respectively.

Virus-neutralisation activity for both HeV and NiV was exhibited for all samples and time points tested. The viral neutralising titres ranged from 1/8 to ≥ 1/64 across all samples, corresponding to m102·4 concentrations of 2–10 µg/mL as measured by the pharmacokinetic ELISA. In 4 out of 5 participant samples, the Day 8 neutralising titre was within 1 or 2 doubling dilutions of the 12-hour sample from the same participant. For the 5th participant sample, the Day 8 neutralising titre was within 3 doubling dilutions of the 12-hour sample for HeV, and 1 doubling dilution for NiV. These results were considered to be within experimental variation, and therefore it was concluded that the m102·4 remained active for at least 8 days following mAb administration.

Discussion

In this phase 1 study we assessed the safety and tolerability of single and multiple IV doses of m102·4 in healthy human adults. All 40 participants completed the trial, with safety data indicating that m102·4 was safe and well tolerated. The maximum dose of 20 mg/kg was in-line with the human equivalent dose conversion (based on body surface area) at the NOAEL of 100 mg/kg, assigned from the 4 x weekly dosing rat toxicology study. Therefore the maximum clinical dosing of 2 x 20 mg/kg m102·4 was supported by the nonclinical data, and is a dose hypothesised to be pharmacologically relevant based on animal challenge data (see below).

TEAE reports were similar between placebo and active treatment groups. There was an increase in treatment-related headaches recorded among participants receiving single dose m102·4 compared to those administered placebo (66·7% vs 10%); there was no increase in treatment-related headaches among those with repeat dosing. Dose-related changes in ALT of three participants were noted; however these were mild abnormalities and were not considered clinically significant. There were no deaths or severe adverse events leading to withdrawals or premature discontinuations during the study. Pharmacokinetics based on those receiving active treatment (n=30) were linear, with the mean half-life ranging from 16·5 to 27·6 days across Cohorts 1–4. There was a small to moderate accumulation of mAb in those receiving repeat dose (Cohort 5), with elimination kinetics that were comparable to those receiving single dose regimens.

A variety of active immunisation strategies have been previously explored for HeV and NiV infection involving various recombinant viral vector-based vaccines in animal challenge models.[4] These studies demonstrated the possibility of inducing strong neutralising antibody response that could afford protection in livestock or an emergency-use scenario.[4] Among the most notable studies was the identification of the m102·4, the first fully-human monoclonal antibody that cross-neutralised *in vivo* all HeV and NiV isolates, including those from the Australian 1994 HeV outbreak and the Malaysian and Bangladesh NiV outbreaks. A single dose of m102·4 administered to ferrets 10 hours after lethal virus challenge was shown to be fully protective with no evidence of toxicity.[20,22]

In subsequent studies designed to closely reflect real-world human exposure, non-human primates were challenged with lethal doses of HeV and NiV by an intratracheal route, then infused twice with m102·4 mAb (15 mg/kg), beginning at 1, 3, or 5 days ~~at various time points~~ post-infection, and again 2 days later. Untreated control animals succumbed to disease eight days post-infection, while those in the treatment groups recovered by day 16.[23,24] Also tissue samples collected from treated animals had no evidence of infectious HeV or NiV at the termination of the study, demonstrating the potential of m102·4 to treat HeV and NiV infections in human and animal populations. A potential challenge to developing antiviral therapies for RNA viruses is their heterogeneity and mutational instability. However, the m102·4 recognises epitopes that are highly conserved among virus variants, making it unlikely that the virus will escape neutralisation due to mutation activity, and in the context of therapeutic dosing, in an immunocompetent individual.

Prior to this trial, a total of 14 individuals received prophylactic high-dose m102·4 on compassionate grounds following high level exposure to HeV (13 in Australia) or NiV (one in the United States; Supplementary Table 2). Individuals ranged from 8–59 years of age. No individual demonstrated clinical or laboratory evidence of infection prior to, or following m102·4 administration. The m102·4 administration was generally well tolerated; two individuals developed infusion-related febrile reactions to early production lots of antibody.

Because of the available data and findings on m102·4, including those presented here, the recent outbreak of NiV in Kerala, India, encouraged a number of organisations, including the Indian Council of Medical Research (ICMR), the Henry M. Jackson Foundation (HJF), the WHO, and the US National Institute of Allergy and Infectious Diseases (NIAID) and US National Institutes of Health (NIH) to work towards the development of an NiV therapeutics protocol for the use of m102·4 in the event of another outbreak of NiV in India or Bangladesh. To date, treatment for NiV has been limited to supportive care and management of acute encephalitis syndrome, as well as standard infection control practices to limit person-to-person transmission.[29] One pharmacologic option that was used for post-exposure prophylaxis during the Malaysian NiV outbreak of 1999 was the anti-viral Ribavirin. While this was associated with a significant reduction in mortality due to acute encephalitis, treatment allocation was not randomised, and the lack of clinical trial safety data beyond short-term emergency administration limits its use in outbreak circumstances.[30] The safety profile of both single and repeat dosing of m102·4, and the standard elimination pharmacokinetic profile presented in this study, underpins the current standard of care to offer a single dose of 20 mg/kg of m102·4 to humans with high levels of exposure to HeV and NiV. It also supports its potential use for patients with established clinical manifestations of henipavirus infection; based upon African green monkey challenge studies,[23,24] two doses separated by 48 hours would seem appropriate.

The main limitation of our study is the small number of participants, which is not uncommon in phase 1 studies. Also pharmacokinetic parameters were based on active treatment cohorts only consisting of 30 individuals. Future studies will be needed to ascertain the efficacy of m102·4 for treatment and prophylaxis against different viral strains of NiV and HeV virus, particularly among populations living in settings where there is the potential for an outbreak. Also, while there is no evidence of escape mutants to m102·4, the potential for this cannot be ruled out with RNA viruses. It may be necessary to consider a cocktail of mAbs with additional targets to combat the likelihood of diminished efficacy of m102·4.

In summary, m102·4 single and repeated dosing of up to two doses, three days apart appears to be safe and well tolerated when administered to healthy volunteers. Given that a 57% mortality rate has been observed from the seven known cases of human infection with HeV, and that m102·4 has demonstrated protective efficacy in non-human challenge studies, m102·4 is the most promising therapeutic opportunity to date for addressing this unmet medical need. This study will inform future dosing regimens for m102·4 to achieve systemic efficacy against HeV and NiV as post-exposure prophylaxis.

Contributors

EGP was the lead investigator involved in study design, data collection and analysis, and interpretation; TM was responsible for pre-clinical and clinical monoclonal antibody production; PPG and SMM were responsible for study design; MG was responsible for antibody quality review and data analysis; MLJ, KLH, BH, EH and KH were responsible for antibody manufacture and project management; CdB was involved in analytical and stability testing of m102·4 for clinical trial, and animal toxicology studies; RK implemented virus neutralization studies; L-FW and ILS designed and analysed virus neutralization studies;

PG and SE were involved in clinical trial study design, conduct, supervision and data interpretation; MEG was the clinical trial coordinator for patient screening procedures; SBL, HC, DES and KDL were involved in the planning and implementation phase of the clinical trial. SBL also reviewed and interpreted safety data from the trial; CCB supplied preclinical materials including the master cell bank and data on monoclonal antibody, and contributed to study design and data analysis; MGS negotiated the transfer of preclinical materials and data on mAb from the USA to Australia; DSD contributed to trial design and supplied the cell line for production of antibody for trial. All authors reviewed and approved the final manuscript.

Declaration of interests

CCB is a US federal employee; and CCB and DSD are co-inventors on US patent Nos. 7,988,971 and 8,313,746 "Human monoclonal antibodies against Hendra and Nipah viruses"; assignees are The United States of America as represented by the Henry M. Jackson Foundation for the Advancement of Military Medicine Inc. (Bethesda, MD). All other authors declare no competing interests.

Data Sharing

Data generated in this study will be shared as deidentified data. Study protocol, statistical analysis plan, and informed consent forms are available with this publication. Any additional data may be made available upon request to the corresponding author following review by the protocol team.

Acknowledgements

We thank the participants who generously gave their time to participate in this study, and without whom the study would not have been possible. We acknowledge Roz Glazebrook and Wendy Morotti (Queensland Health) for initial coordination and preparation of trial related activity, and support with maintaining the study; Terry Hurst (Q-Pharm Pty Limited) for initial preparation and planning of initial grant funding of this phase 1 clinical trial; Gary Crameri (CSIRO Australian Animal Health Laboratory) for technical assistance with virus neutralization studies. We thank Clinic Manager Sharon Rankine and the Q-Pharm clinic team. We thank Drs Sonya Bennett (Executive Director) and Jeannette Young (Chief Health Officer) of Queensland Health, for ongoing support for this project, and securing a significant portion of the funding for this trial. The manufacture of m102·4 drug substance was performed at the National Biologics Facility (NBF), at the University of Queensland. The NBF is supported by Therapeutic Innovation Australia (TIA), which is supported by the Australian Government through the National Collaborative Research Infrastructure Strategy (NCRIS) program. The authors thank Dr Sharon Johnatty of SugarApple Communications for providing medical writing support, which was funded by Queensland Health, Brisbane, Australia, in accordance with Good Publication Practice (GPP3) guidelines (https://www.ismpp.org/gpp3).

References

1. Wang L-F, Mackenzie JS, Broder CC. Henipaviruses. In: Knipe DM, Howley PM, eds. Fields Virology. Philadelphia: Lippincott Williams & Wilkins; 2013: 1070-85.
2. Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol* 2000; **81**(Pt 8): 1927-32.
3. Chua KB, Koh CL, Hooi PS, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect* 2002; **4**(2): 145-51.
4. Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. *Vaccine* 2016; **34**(30): 3525-34.
5. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol* 2012; **359**: 153-77.
6. Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral research* 2013; **100**(1): 8-13.
7. Wong KT, Ong KC. Pathology of acute henipavirus infection in humans and animals. *Patholog Res Int* 2011; **2011**: 567248.
8. Playford EG, McCall B, Smith G, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis* 2010; **16**(2): 219-23.
9. Wong KT, Tan CT. Clinical and pathological manifestations of human henipavirus infection. *Curr Top Microbiol Immunol* 2012; **359**: 95-104.
10. Murray K, Rogers R, Selvey L, et al. A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis* 1995; **1**(1): 31-3.
11. International Society for Infectious Diseases. HENDRA VIRUS - AUSTRALIA: (NEW SOUTH WALES) HORSE. June 2019. Archive Number: 20190621.6531307. http://www.promedmail.org/post/6531307 (accessed June 2019).
12. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet* 1999; **354**(9186): 1257-9.
13. Chattu VK, Kumar R, Kumary S, Kajal F, David JK. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. *J Family Med Prim Care* 2018; **7**(2): 275-83.
14. Sazzad HM, Hossain MJ, Gurley ES, et al. Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. *Emerg Infect Dis* 2013; **19**(2): 210-7.
15. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Scientific reports* 2016; **6**: 30916.
16. Luby SP. The pandemic potential of Nipah virus. *Antiviral research* 2013; **100**(1): 38-43.
17. World Health Organization. WHO publishes list of top emerging diseases likely to cause major epidemics. 10 December 2015. https://www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/; (accessed June 2019).
18. Rottingen JA, Gouglas D, Feinberg M, et al. New Vaccines against Epidemic Infectious Diseases. *N Engl J Med* 2017; **376**(7): 610-3.
19. Zhu Z, Dimitrov AS, Bossart KN, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J Virol* 2006; **80**(2): 891-9.
20. Zhu Z, Bossart KN, Bishop KA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J Infect Dis* 2008; **197**(6): 846-53.
21. Xu K, Rockx B, Xie Y, et al. Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog* 2013; **9**(10): e1003684.

22. Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS Pathog* 2009; **5**(10): e1000642.

23. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Sci Transl Med* 2011; **3**(105): 105ra3.

24. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med* 2014; **6**(242): 242ra82.

25. Queensland Government. World-first Hendra treatment one step closer. Oct 31 2013. http://statements.qld.gov.au/Statement/2013/10/31/worldfirst-hendra-treatment-one-step-closer: Queensland Government; (accessed June 2019).

26. EMEA/CHMP/SWP. Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. EMEA/CHMP/SWP/28367/07 Rev.1. July 2017. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-strategies-identify-mitigate-risks-first-human-early-clinical-trials-investigational_en.pdf: European Medicines Agency; (accessed June 2019). p. 1-12.

27. US Food and Drug Administration. Guidance for Industry: Bioanalytical Method of Validation. May 2001. http://www.labcompliance.de/documents/FDA/FDA-Others/Laboratory/f-507-bioanalytical-4252fnl.pdf; (accessed June 2019).

28. Luby SP, Broder CC. Paramyxoviruses: Henipaviruses. In: Kaslow RA, Stanberry LR, Le Duc JW, eds. Viral Infections of Humans, Epidemiology and Control. New York: Springer Science+Business Media; 2014: 519-36.

29. Centers for Disease Control and Prevention. Treatment. Nipah Virus (NiV). https://www.cdc.gov/vhf/nipah/treatment/index.html; (accessed on 16 July, 2019).

30. Banerjee S, Gupta N, Kodan P, et al. Nipah virus disease: A rare and intractable disease. *Intractable Rare Dis Res* 2019; **8**(1): 1-8.

Tables

Table 1: Participant baseline characteristics

	Cohort 1 (1 mg/kg) (n=6)	Cohort 2 (3 mg/kg) (n=6)	Cohort 3 (10 mg/kg) (n=6)	Cohort 4 (20 mg/kg) (n=6)	Cohort 5 (2 x 20 mg/kg) (n=6)	Pooled placebo (n=10)	Overall (n=40)
Sex							
Male	1 (17%)	4 (67%)	5 (83%)	3 (50%)	4 (70%)	7 (70%)	24 (60%)
Female	5 (83%)	2 (33%)	1 (17%)	3 (50%)	2 (30%)	3 (30%)	16 (40%)
Age, years							
Mean (SD)	29·8 (9·1)	30·5 (5·1)	24·7 (3·9)	29·7 (10·9)	27·7 (5·5)	26·6 (5·0)	28·0 (6·7)
Range	18–42	23–36	21–32	20–44	21–35	20–37	18–44
Weight, kg							
Mean (SD)	71·5 (15·2)	84·9 (14·1)	74·7 (10·8)	67·7 (7·3)	80·5 (12·1)	79·3 (8·0)	76·7 (11·9)
Range	51·3–95·7	65·2–98·0	60·4–86·4	58·2–77·0	67·1–98·5	71·8–95·6	51·3–98·5
BMI, kg/m^2							
Mean (SD)	24·6 (5·3)	26·6 (5·3)	24·0 (4·0)	23·1 (2·5)	26·0 (4·4)	26·3 (3·4)	25·2 (3·9)
Range	19·5–33·9	21·8–31·3	19·9–29·5	19·7–26·2	20·3–32·9	22·6–32·7	19·5–33·9
Race, n (%)							
White	5 (83%)	5 (83%)	5 (83%)	6 (100%)	6 (100%)	9 (90·0%)	36 (90·0%)
Other	1 (17%)	1 (17%)	1 (17%)	0 (0%)	0 (0%)	0 (0%)	3 (7·5%)
Pacific Islander	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	1 (2·5%)

Data are n (%), mean (SD), range (minimum – maximum); BMI=body-mass index; Race='Other' were Eurasian, white/Asian/Aboriginal or South American Indigenous

Table 2: Summary of adverse events

	Cohort 1 (1 mg/kg) (n=6)	Cohort 2 (3 mg/kg) (n=6)	Cohort 3 (10 mg/kg) (n=6)	Cohort 4 (20 mg/kg) (n=6)	Cohort 5 (20 mg/kg) (n=6)	Pooled placebo (n=10)	Overall (n=40)
Participants with ≥1 TEAEs	5 (83%) [12]	5 (83%) [10]	6 (100%) [14]	6 (100%) [13]	6 (100%) [11]	9 (90%) [26]	37 (93%) [86]
Participants with serious TEAEs	0	0	0	0	0	0	0
Participants with any TEAE leading to discontinuation of study drug	0	0	0	0	0	0	0
Gastrointestinal disorders	1 (17%) [1]	0	2 (3%) [3]	1 (17%) [3]	1 (17%) [1]	1 (10%) [1]	6 (15%) [9]
General disorders and administration site conditions	1 (17%) [1]	2 (3%) [3]	3 (50%) [3]	0	1 (17%) [1]	4 (40%) [7]	11 (28%) [15]
Infections and infestations	4 (67%) [5]	2 (33%) [2]	2 (33%) [2]	3 (50%) [4]	2 (33%) [2]	5 (50%) [6]	18 (45%) [21]
Investigations	0	0	0	0	2 (33%) [2]	1 (10%) [1]	3 (8%) [3]
Metabolism and nutrition disorders	0	0	1 (17%) [1]	0	0	0	1 (3%) [1]
Musculoskeletal and connective tissue disorders	0	0	0	0	0	2 (20%) [3]	2 (5%) [3]
Nervous system disorders (headaches)	1 (17%) [1]	1 (17%) [1]	2 (33%) [2]	4 (67%) [6]	4 (67%) [4]	3 (30%) [4]	15 (38%) [18]
Psychiatric disorders	0	1 (17%) [1]	0	0	0	1 (10%) [1]	2 (5%) [2]
Renal and urinary disorders	0	0	0	0	0	1 (10%) [1]	1 (3%) [1]
Respiratory, thoracic and mediastinal disorders	2 (33%) [2]	1 (17%) [1]	0	0	0	2 (20%) [2]	5 (13%) [5]
Skin and subcutaneous tissue disorders	2 (33%) [2]	1 (17%) [2]	2 (33%) [2]	0	1 (17%) [1]	0	6 (15%) [7]
Surgical and medical procedures	0	0	1 (17%) [1]	0	0	0	1 (3%) [1]

Data are displayed as number of participants (% of participants within cohort) [number of TEAEs]

Table 3: Pharmacokinetic parameters for each cohort (8 treated participants per cohort) of healthy adults treated with m102·4

	C_{max} (µg/mL)		T_{max} (h)	$AUC_{0\text{-}last}$ (h·µg/mL)		$t_{1/2}$ (h)	CL (mL/h/kg)		V_z (mL/kg)	
	Mean (SD)	**Coefficient of Variation**	**Median (range)**	**Mean (SD)**	**Coefficient of Variation**	**Median (range)**	**Mean (SD)**	**Coefficient of Variation**	**Mean (SD)**	**Coefficient of Variation**
Cohort 1 (1 mg/kg); n=6	29·9 (5·8)	19·5%	8 (4·0–12·0)	5,528·7 (953·0)	17·2%	663·3 (474·3–735·1)	0·18 (0·03)	16·4%	167·3 (54·1)	32·3%
Cohort 2 (3 mg/kg); n=6	130·4 (38·9)	29·8%	4 (4·0–8·0)	21874·2 (4942·0)	22·6%	466·3 (382·8–522·3)	0·14 (0·03)	21·3%	95·2 (29·5)	31·0%
Cohort 3 (10 mg/kg); n=6	357·2 (72·7)	20·3%	2·5 (1·0–12·1)	79102·2 (15061·2)	19·0%	397 (333·9–491·8)	0·13 (0·03)	20·6%	75·1 (11·6)	15·4%
Cohort 4 (20 mg/kg); n=6	692 (104·7)	15·1%	4·1 (1·0–8·0)	131648·3 (18344·0)	13·9%	466·7 (351·0–889·6)	0·15 (0·02)	15·5%	112·6 (36·8)	32·7%
Cohort 5 (20 mg/kg): first dose (day 1); n=6	834·3 (150·6)	18·0%	6·0 (1·0–12·1)	33082·4 (6222·7)	18·8%					
Cohort 5 (20 mg/kg): second dose (day 4); n=6	1076·7 (187·7)	17·4%	2·5 (1·0–8·0)	337,574·6 (50058·1)	14·8%	472 (385·6–592·0)	0·06 (0·001)	14·6%	40·5 (6·6)	16·3%

Pharmacokinetic data for mAb m102·4 serum concentrations were calculated using all participants receiving m102·4 (n=30). C_{max} =maximum observed serum concentration (mean, SD and coefficients of variation). T_{max} = time to C_{max} (hours, median and range) $AUC_{0\text{-}last}$ =Area under the m102·4 concentration curve from pre-dose to last measurable concentration (mean, SD and coefficients of variation), $t_{1/2}$ =elimination half-life (hours, median and range), CL = clearance (mean, SD and coefficients of variation), V_z = volume of distribution (mean, SD and coefficients of variation).

Figure Legends

Figure 1: Study Profile

* Withdrawals were due to time constraints (n=10), personal or family illness (n=4), withdrew no reason given (n=9).

Figure 2: Mean log-scaled concentrations of total m102·4 concentrations (µg/mL) in serum from healthy adults (8 treated participants per cohort), by nominal time and treatment group, as determined by ELISA

Figure 1 Trial Profile

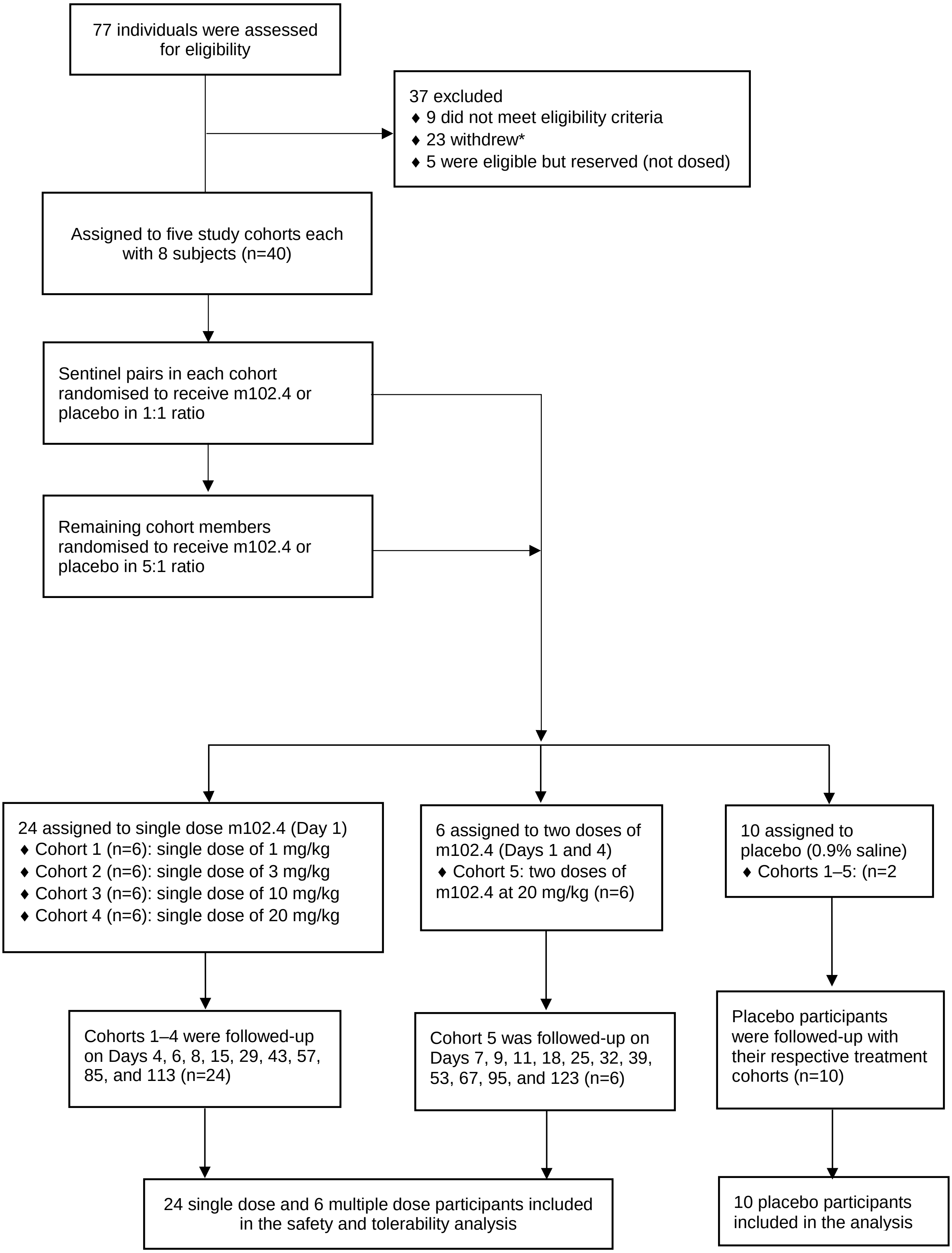
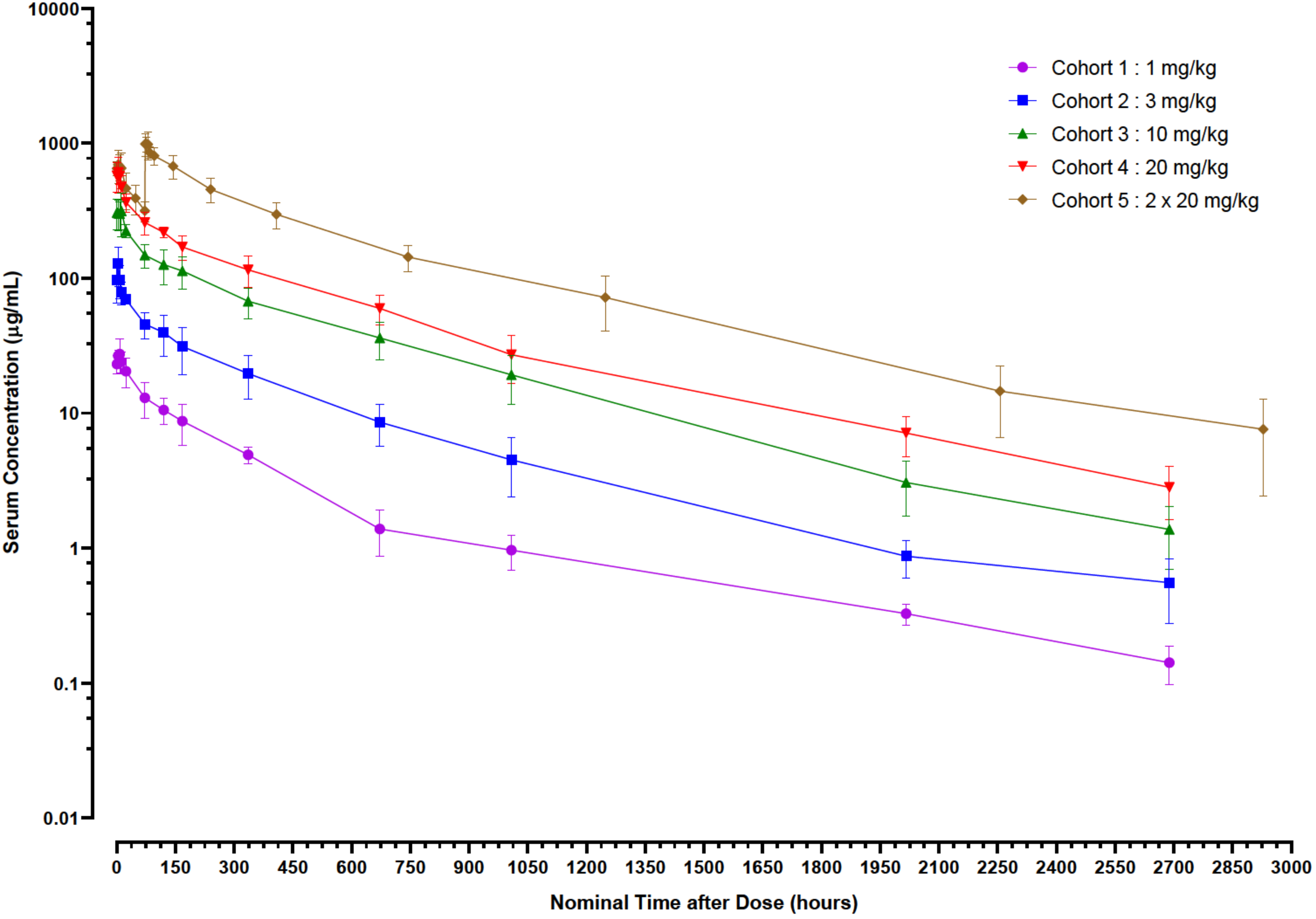


Figure 2



10000
1000
100
10
1
0.1
0.01
Serum Concentration (µg/mL)
0
150
300
450
600
750
900
1050
1200
1350
1500
1650
1800
1950
2100
2250
2400
2550
2700
2850
3000
Nominal Time after Dose (hours)
● Cohort 1 : 1 mg/kg
■ Cohort 2 : 3 mg/kg
▲ Cohort 3 : 10 mg/kg
▼ Cohort 4 : 20 mg/kg
◆ Cohort 5 : 2 x 20 mg/kg

Supplementary Tables

Supplementary Table 1: Reported changes in alanine aminotransferase levels of three participants.

	Placebo	Cohort 5 (2x20 mg/kg) Participant #1	Cohort 5 (2x20 mg/kg) Participant #2
Screening	44	18	32
Day -1	24	18	38
Day 2	22	17	41
Day 4	45	21	69
Day 5	62	31	78
Day 6			71
Day 7	101		
Day 9			67
Day 11	56	61	57
Day 18	49	36	35
Day 25	38	23	33
Day 39	68	21	32
Day 67	62	18	37
Day 95	54	21	39
Day 123	28	15	38

Day number refers to the number of days relative to the day of dose administration of m102·4 where Day 1 is the first dosing day. Participants in Cohort 5 received two doses of 20 mg/kg 72 hours apart. Alanine aminotransferase is reported as U/L; normal range is considered 5–40 U/L; blank cells indicate measurements not taken on those days.

Supplementary Table 2: Treatment data and outcomes for 13 compassionate use cases in Australia who received m102·4 as post-exposure prophylaxis.

Patient	Year	Exposure	Dose (mg/kg)	Adverse events	Follow up
1	2010	HeV-infected horse	17·9	Infusion-related febrile reaction	No seroconversion
2	2010	HeV-infected horse	18·3	Infusion-related febrile reaction *Pseudomonas aeruginosa* intravenous line-related bloodstream infection	No seroconversion
3	2012	HeV-infected horse	20·0	Nil	No seroconversion
4	2012	HeV-infected horse	20·0	Nil	No seroconversion
5	2013	NiV-Bangladesh infected NHP	2 x 20·0 at 0 and 48 hours	Nl	No seroconversion
6	2014	HeV-infected horse	19·8	Nil	No seroconversion
7	2014	HeV-infected horse	20·0	Nil	No seroconversion
8	2014	HeV-infected horse	20·0	Nil	No seroconversion
9	2014	HeV-infected horse	20·0	Nil	No seroconversion
10	2014	HeV-infected horse	20·0	Nil	No seroconversion
11	2015	HeV-infected horse	20·1	Nil	No seroconversion
12	2017	HeV-infected horse	20·0	Nil	No seroconversion
13	2017	HeV-infected horse	20·1	Nil	No seroconversion
14	2017	HeV-infected horse	20·1	Nil	No seroconversion

NHP: nonhuman primate (African green monkey)

*Protocol

CLINICAL PROTOCOL

Protocol No.: QH-M102.4-002

A Phase I, Randomized, Double-blind, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics and Immunogenicity of Various Dosing Regimens of Intravenous anti-Hendra Virus Antibody (mAb m102.4) in Healthy Subjects

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Investigational Product: mAb m102.4

Study Site: Q-Pharm Pty Ltd, Herston, QLD

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PROTOCOL SYNOPSIS

Protocol Title:	A Phase I, Randomized, Double-blind, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics and Immunogenicity of Various Dosing Regimens of Intravenous anti-Hendra Virus Antibody (mAb m102.4) in Healthy Subjects
Protocol Number:	QH-M102.4-001
Sponsor:	Queensland Department of Health, QLD, Australia
Compound no:	Human monoclonal antibody (mAb m102.4)
Phase of Development:	Phase I
Methodology:	A Phase I, randomized, double-blind, placebo-controlled, dose escalation study.
Study Duration:	Study duration is approximately 5 months per subject (including screening). For subjects in Cohorts 1-4, study duration will include a Screening period of up to 28 days prior to first dose of investigational product (IP), one dose of IP and a maximum 16-week follow-up period. For subjects in Cohort 5, there will be two dosing days, 3 days (72 hours) apart, in addition to the Screening period and maximum 16-week post second dose follow-up period.
Study Center:	Q-Pharm Pty Ltd, Herston, QLD, Australia
Objectives:	1. To determine the safety and tolerability of single and multiple intravenous (IV) administration of mAb m102.4 in healthy subjects 2. To determine the pharmacokinetics (PK), and immunogenicity of single and multiple IV administration of mAb m102.4 in healthy subjects 3. To explore the pharmacodynamics (PD) of single and multiple IV administration of mAb m102.4 in healthy subjects against Hendra (and possibly Nipah) virus.
Sample Size:	40 healthy subjects in 5 cohorts with 8 subjects in each cohort (6 randomized to receive mAb m102.4 and 2 randomized to receive placebo)
Investigational Medicinal	mAb m102.4 at doses of 1 mg/kg and 3 mg/kg in a 100 mL bag of

Product (IMP):	normal saline and 10 mg/kg and 20 mg/kg in a 500 mL bag normal saline administered IV for approximately 1 hour. In Cohort 5, 20 mg/kg will be administered twice, with 3 days (72 hours) between dosing. Doses may be adjusted following cohort reviews by the Safety Review Committee (SRC).
Reference Therapy:	Placebo (normal saline, prepared to maintain the blind)
Study Design:	All subjects will undergo Screening procedures within 28 days of the first dose of IP. Subjects in Cohorts 1-4 will be admitted to the clinical research unit (CRU) on the afternoon of Day -1 and receive a single dose of IP on Day 1 following overnight fast. Cohort 1-4 subjects will be allowed to leave the CRU on the morning of Day 3. The subjects for Cohort 5 will be admitted to the CRU on the afternoon of Day -1 to be dosed with IP on the mornings of Day 1 and Day 4 following overnight fast in each case. Cohort 5 subjects will be allowed to leave the CRU on the morning of Day 6. Safety assessments for **Cohort 1-4** will be conducted upon admission to the CRU, at baseline (Day 1) and during the clinical stay. Subjects will return to the CRU on Days 4, 6, 8, 15, 22, 29, 43, 57, 85 and 113 for PK and immunogenicity sampling and/or safety assessments. Safety assessments for **Cohort 5** will be conducted upon admission to the CRU, at baseline (Day 1) and during the clinical stay. Subjects will return to the CRU on Days 7, 9, 11, 18, 25, 32, 39, 53, 67, 95 and 123 for PK and immunogenicity sampling and/or safety assessments. The final safety assessment is scheduled for a maximum of 16 weeks after the last dose of IP. This is based on a mAb m102.4 t½ of 21-23 days. Where applicable, serious adverse events (SAEs) will be followed for a further 30 days after the final safety assessment, or until resolved, whichever is the earlier. For all cohorts two sentinel subjects will be randomized in a blinded fashion in which one subject receives mAb m102.4 and one subject receives placebo. The remaining six subjects in each cohort will receive IP (5 x mAb m102.4: 1 x placebo) a minimum of 14 days after the sentinel pair, following review of safety and tolerability data by the Principal Investigator (PI) and Medical Monitor. The decision to progress to the next higher dose will be made after

	data are reviewed for each dose group for at least six subjects per group through to a minimum of Day 29, and after the SRC determines that it is safe to proceed to the next dose level. PK data for Cohort 1 through to Day 43 will be analyzed and data reviewed by the SRC whilst Cohort 2 is ongoing. Subsequent cohort assessments, sampling times and the overall period of monitoring may be adjusted depending on the outcome of the data review. All PK data for Cohorts 1-4 will be reviewed prior to the commencement of Cohort 5 to confirm that dose and planned dosing interval for Cohort 5 is optimal.
Safety:	The following assessments will be performed during the study: physical examination, clinical laboratory tests (haematology [including coagulation], clinical chemistry, urinalysis, virus neutralization and anti-m104.2 antibodies), vital signs (blood pressure, pulse rate, temperature and respiratory rate), electrocardiogram (ECG), signs and symptoms of tolerability, infusion site reactions and adverse events (AEs). Assessments according to the schedule of events will be conducted at the following time points (vital signs will be performed at all time points): **Cohorts 1-4:** Screening, on admission (Day -1), pre-dose, Days 1, 2, 3, 4, 6, 8, 15, 22, 29, 43, 57, 85 and 113 post-dose (after the start of the infusion) **Cohort 5:** Screening, on admission (Day -1), pre-dose, Days 1, 2, 3, 4, 5, 6, 7, 9, 11, 18, 25, 32, 39, 53, 67, 95 and 123 post-dose (after the start of the first infusion). AEs will be collected from the commencement of the first dose of IP until the end of the study, and in the case of SAEs until resolution or up to 30 days after study completion whichever is the earlier. A full physical examination will be performed at Screening and at the final visit (Day 113 for Cohorts 1-4 and Day 123 for Cohort 5). A brief physical examination will be performed pre-dose on Days 1, 3, 29 and 57 for Cohorts 1-4 and pre-dose, Days 4, 6, 39 and 67 for Cohort 5. ECGs will be performed at Screening, pre-dose on Day 1, Days 2,

	8, 29, 57, 85 and 113 post-dose for Cohorts 1-4. For Cohort 5 ECGs will be performed at Screening, pre first dose on Day 1, Days 2, 4, 5, 6, 11, 39, 67, 95 and 123 post-dose.
Pharmacokinetics (PK):	PK samples will be taken at the following time points for **Cohorts 1-4:** pre-dose on Day 1 and at 1, 4, 8, 12, and 24, hours post commencement of infusion and at the following visit to the CRU on Days 4, 6, 8, 15, 29, 43, 85 and 113 post dose. PK samples will be taken at the following time points for **Cohort 5:** pre first dose on Day 1 and at 1, 4, 8, 12, 24 and 48 hours post commencement of the first infusion; pre second dose on Day 4 and at 1, 4, 8, 12, and 24 hours post commencement of the second infusion and at the following visits to the CRU on Days 7, 11, 18, 32, 53, 95 and 123 post first dose. The PK sampling for Cohort 5 may be adjusted depending on the PK data obtained from Cohorts 1-4.
Immunogenicity and Virus Neutralization:	The presence of anti mAb m102.4 antibodies will be assessed pre-dose and on Days 29 and 113 for Cohorts 1-4 and pre-dose and on Days 39 and 123 for Cohort 5. Blood samples for mAb m102.4 Hendra (and possibly Nipah) virus neutralization studies will be taken and potentially assessed at pre-dose, 12 hours, Days 2 (24 hours), 4, 8, 29, and 113 for Cohorts 1-4 and pre-dose, 12 hours, Days 2 (24 hours), 4, 6, 9, 39, and 123 for Cohort 5.

Inclusion Criteria:	Subjects who meet all of the following criteria are eligible for the study: 1. Written informed consent from the subject; 2. Healthy males and females as determined by medical history and physical examination between 18 and 50 years of age (inclusive); 3. Body mass index (BMI) of 18.0 to 35.0 kg/m^2 (inclusive) at screening (Weight $\geq$50 kg or $\leq$100 Kg); 4. Haematology, coagulation, clinical chemistry, and urinalysis test results not deviating from the normal range to a clinically relevant extent at screening; 5. Able to comply with requirements for concomitant medications and consumption of caffeine, alcohol, tobacco and nicotine; 6. Willing to attend all the study visits.
Exclusion Criteria:	Subjects are not eligible for this study if they meet any of the following criteria: 1. Close, unprotected exposure to sick horses with an unexplained illness within 4 weeks of Day 1; or known exposure to potential Hendra infected horses; 2. Clinically significant abnormalities in laboratory test results at Screening or Baseline (Day-1) (biochemistry, haematology and urinalysis parameters) (Liver function tests > 1.5 x upper limit of normal [ULN]); 3. Immunoglobulin G (IgG) and complement level outside normal limits (at Screening); 4. Positive urine screen for potential drugs of abuse at Screening and prior to randomization; 5. Presence or history (in last 2 years) of alcohol abuse confirmed by medical history, or daily alcohol intake of more than 2 standard drinks (16 g) for females or more than 3 standard drinks for males, or a positive breath alcohol test at screening or prior to randomization, and the inability to stop alcohol intake for 2 weeks post-infusion; One standard drink is (8 g) is equivalent to 280 mL of beer, one measure (25 mL) of spirits or 1 small glass (125 mL) of wine; 6. Excessive use of caffeine-containing beverages including coffee, tea, cola, energy drinks or chocolates, exceeding 500 mg caffeine/day (approximately 5 cups of coffee or 10 cups of tea) and the inability to refrain from the use of caffeine-containing beverages during confinement in the CRU;

	7. Smoke >5 cigarettes per day; 8. Positive tests for human immunodeficiency virus (HIV) or viral hepatitis (hepatitis B surface antigen [HepBsAg], or anti-hepatitis C [HCV]); 9. Treatment with an investigational drug or biologic within 60 days preceding treatment or plans to take another investigational drug or biologic in the 3 months following IP administration; 10. Blood donation or significant blood loss 60 days prior to Day 1; 11. Plasma donation within 60 days prior to Day 1 or planned donation in the 6 months following IP administration; 12. Males who are sexually active and have a partner who is capable of becoming pregnant, neither of which have had surgery to become sterilized, who are not using an effective method of birth control (e.g., surgically implanted hormonal therapy, intrauterine devices, or oral birth control with barrier method). In addition, male subjects must agree not to donate sperm during the study and in the 6 months following IP administration; 13. Females who are capable of becoming pregnant and who have not had surgery to become sterilized and who are not using an effective method of birth control (e.g., surgically implanted hormonal therapy, intrauterine devices, or oral birth control with barrier method [female condom or male partners to use condom]). For exclusively homosexually active females, not planning a pregnancy during or in the 6 month period following completion of the study, the restrictions for adequate contraception will not apply (if lifestyle choices change, contraceptive requirements will be applicable); 14. Poor peripheral venous access; 15. Subjects with clinically significant findings from medical history that, in the opinion of the investigator, could interfere with the objectives of the study or put the subject at risk; 16. Subject is unwilling or unable to abide by the requirements of the protocol or inability to be available for follow-up assessments or protocol related procedures; 17. Subject is on staff at the investigator site; or a relative of personnel of the investigator site or of the Sponsor (or Sponsor's affiliates); 18. Use of prescription or non-prescription drugs within 14 days or 5 half-lives (whichever is longer) prior to IP administration and for the duration of the study.

	Contraceptives for females are allowed – must have been using the same contraceptive for a minimum period of 3 months and must not change the contraceptive during the study; 19. Presence or history of drug hypersensitivity, or allergic disease diagnosed and treated by a physician or history of a severe allergic reaction, anaphylaxis or convulsions following any vaccination or infusion; 20. BP systolic >160 mmHg or diastolic >90 mmHg. Allow one repeat measure; 21. Personal or family history of congenital QT syndrome or sudden unexplained death; 22. ECG clinically significant abnormalities including (QTc>450 msec for males and >460 msec for females), AF or atrial flutter, notably bradycardia (<40 bpm) or tachycardia (>100 bpm), complete left or right bundle branch block, Wolf-Parkinson White Syndrome or presence of cardiac pacemaker; 23. Any vaccination in the last month. 24. Pregnant or breastfeeding females.
Statistical Methods:	The sample size for this study was not based on statistical power calculation, but is consistent with the typical sample size used for similar studies to assess preliminary safety, PK and immunogenicity data.

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IN-TEXT TABLES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	**Definition**
AE	Adverse Event
AIBN	Australian Institute for Bioengineering and Nanotechnology
ALP	Alkaline Phosphatase
ALT/SGPT	Alanine Aminotransferase
aPTT	Activated Partial Thromboplastin Time
AST/SGOT	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CRF	Case Report Form
CRU	Clinical Research Unit
CTCAE	Common Terminology Criteria for Adverse Events
eGFR	Estimated Glomerular Filtration Rate
GCP	Good Clinical Practice
HeV	Hendra Virus
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IgG	Immunoglobulin G
INR	International Normalized Ratio
IP	Investigational Product
IV	Intravenous
mAb	Monoclonal Antibody
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	No Observed Adverse Effect Level
PD	Pharmacodynamics
PI	Principal Investigator

PK	Pharmacokinetics
PT	Prothrombin Time
RBC	Red Blood Cell
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
SRC	Safety Review Committee
ULN	Upper Limit of Normal
WBC	White Blood Cell

1 INTRODUCTION

1.1 Background

Hendra virus (HeV) infection in humans is a rare serious zoonotic disease with a high mortality rate. In Australia the natural hosts of HeV are bats (flying foxes), which can then pass the virus onto horses. There are seven known cases of human HeV infection where humans have become unwell with an influenza-like illness or encephalitis following close contact with an infected horse. One case had an initial mild clinical illness, which re-emerged 13 months later and resulted in encephalitis and death. Human HeV infection has resulted in fatal severe encephalitis and multi-system involvement in 4 of the 7 (57%) infected individuals. The sole survivor of acute encephalitis has residual high-level cognitive impairment.

There is no known effective treatment for HeV infection and clinical management is based on treating symptoms as they arise.

A neutralizing human monoclonal antibody (mAb), m102.4, was developed by researchers in the United States. These antibodies have been successful in protecting ferrets and African green monkeys challenged with HeV. The mAb m102.4 are currently the only pharmaceutical agent available to prevent infection in people exposed to the HeV.

In 2010, the Queensland Department of Health, Chief Health Officer obtained a license from the US Department of Defence, Uniformed Services University of the Health Sciences to produce the experimental mAb m102.4 for the purpose of compassionate use in human cases of HeV exposure.

The mAb m102.4 has not been assessed in any safety or efficacy trials in humans. A predecessor of the product has previously been administered to eleven humans. The first person was late in disease progression with encephalitis in the Intensive Care Unit (ICU) and subsequently died. The second and third had moderately high-risk exposures, but no evidence of infection and did not develop illness or seroconversion. They experienced transfusion-like reactions to administration of mAb m102.4 which was attributed to endotoxins in the predecessor product. The fourth person received mAb m102.4 in 2012 after high-level exposure to HeV and did not experience any adverse reactions to the antibodies. The mAb m102.4 were given to a male (the 5th person) in the United States who was accidently bitten by a Nipah virus infected monkey. The male was administered treatment in 2013 under the same regime used by Dr Geoffrey Playford (the Principal Investigator [PI] for this trial) for the Queensland patients. This included a test dose and two IV administrations of m102.4 three days apart. No untoward affects associated with the administration of the antibodies were noted. The sixth and seventh individuals received the mAb on 20 March 2014 and remained well. Four more people received mAb 102.4 in June 2014, including one from Queensland and three from New South Wales.

These four people were assessed as having had high exposure to Hendra virus infected horses. With the exception of the male in the United States who received two doses, all other individuals received a single dose of mAb m102.4.

Currently mAb m102.4 are offered to people with high exposure to infected horse blood/secretions following ethics approval for each individual and appropriate consenting. Monoclonal antibodies are not offered to people with lower exposures because of the potential for unknown adverse effects of mAb m102.4 administration and a low risk of infection.

The mAbs prepared for use in this study were refined and purified at the Brisbane Based Australian Institute for Bioengineering and Nanotechnology (AIBN), which is part of the University of Queensland.

Phase 1 trials are normally conducted in healthy subjects. These trials are designed to test an experimental drug or treatment in a small group of people (20-80) for the first time to evaluate its safety, determine a safe dosage range, and identify side effects.

All medicines in use today must be proven safe and effective in clinical trials before they can be made available for use within the community. Early phase clinical trials are a vital step in this development process.

HeV is not used at any stage for the production of the mAbs and this safety trial is the final stage before the experimental drug is considered to be safe for use in humans.

Monoclonal antibodies have shown great promise in treating human diseases including arthritis and tumors associated with breast and colon cancer. They are also being trialed as treatments for viral infections such as human immunodeficiency virus (HIV) and hepatitis.

It is important to understand that mAb therapy is not a vaccine for the general population, but a treatment, which maximizes a person's chance of survival once they have been exposed to the disease.

This trial is necessary in humans to ensure there are no significant adverse side effects. Success of this safety trial will provide clinicians with the confidence to offer treatment to patients who have been exposed to medium to high levels of HeV without the fears of potentially serious side effects.

1.1 Justification for Dose Chosen

The maximum therapeutic human dose given for compassionate reasons was based on doses administered in animal studies, and was around 15-20 mg/kg. It is know from the animal studies that they were challenged with lethal doses of Hendra and Nipah virus and mAb m102.4 doses were effective. Typically humans are not exposed to such high levels of the virus through natural infection. Animals are challenged via the intra-tracheal route, whereas humans are exposed via the mucosal route and with much lower levels of virus. A dose that is effective in ferrets and monkeys for such large challenge doses of virus should be more than adequate to cope with much lower levels of the HeV that humans are experiencing.

Fourteen monkeys were challenged intra-tracheally with HeV and 12 animals were infused twice with a 100 mg dose (20 mg/kg) of m102.4 beginning at either 10, 24 or 72 hours after introduction to the virus and received a second infusion of m102.4 48 hours later. All 12 animals that received m102.4 survived the infection; whereas the untreated control monkeys succumbed to severe systemic disease by Day 8[1]. There was no evidence of HeV mediated pathology in any of the m102.4-treated animals and no infectious HeV could be recovered from any tissues from any m102.4-treated animals.

The starting dose for this study has been set at 1 mg/kg. This is based on a repeat dose toxicology study where four groups of male and female rats, 30 per group, were administered a once weekly dose of m102.4 or vehicle control via IV injection for four weeks. Necropsies were performed at the end of the dosing period (20 rats per group) and after a 2-week recovery (10 rats per group). The m102.4 treatment was well tolerated and there were no unscheduled deaths prior to completion of the main study or recovery period. Test article treatment was not associated with morbidity or clinical signs of toxicity. There was no adverse treatment related effect on body weight, or food and water consumption. There was a trend towards elevated WBC count in male and female rats at the end of the main study (significant in male rats treated with high-dose m102.4, and in female rats treated with mid- and high-dose m102.4). There were significant findings in differential white cell counts mostly in the high-dose group. While the trend in elevated total WBC count persisted at all doses at the end of the recovery phase, the findings were not significant. Significant findings in differential white cell count at the end of the recovery phase were observed at all doses in male rats, and at the low and high dose groups in female rats. There were no adverse treatment dependent differences in serum biochemical profile or urinalysis findings. A treatment-related increase in incidence of lung granuloma was seen at the end of the treatment phase, particularly in female rats all receiving doses of m102.4. Both male and female animals treated with m102.4 showed evidence of decreased incidence of lung granuloma following the recovery phase. There was no treatment-related induction of anti-drug antibodies following once weekly dosing with m102.4, for 28 days at all doses administered.

The no observed adverse effect level (NOAEL) for m102.4 was 100 mg/kg. This amount is well in excess of the dose range (1 mg/kg – 20 mg/kg) planned for this trial.

2 STUDY OBJECTIVES AND PURPOSE

2.1 Purpose of the Study

The purpose of the study is to assess the safety, tolerability, Pharmacokinetics (PK), immunogenicity and exploratory Pharmacodynamics (PD) of mAb m102.4 administered via Intravenous (IV) infusion to healthy subjects in four single escalating doses and one multiple dose at the highest concentration.

2.2 Objectives

The objectives of this study are:

1 To determine the safety and tolerability of single and multiple IV administration of mAb m102.4 in healthy subjects
2 To determine the PK and immunogenicity of single and multiple IV administration of mAb m102.4 in healthy subjects
3 To explore the PD of single and multiple IV administration of mAb m102.4 in healthy subjects against Hendra (and possibly Nipah) virus

3 STUDY DESIGN

3.1 Overall Study Design

This is a Phase I, randomized, double-blind, placebo-controlled, dose escalation study. Subjects will be dosed with four concentrations of mAb m102.4 across five cohorts. The first four cohorts (Cohorts 1 to 4) will receive a single, IV infusion of mAb m102.4 at a concentration of 1 mg/kg, 3 mg/kg, 10 mg/kg or 20 mg/kg over a period of 1 hour. The fifth cohort (Cohort 5) will receive two IV infusions of mAb m102.4 at a concentration of 20 mg/kg given over the period of 1 hour, with 72 hours between each infusion.

All subjects will undergo Screening procedures within 28 days of the first dose of investigational product (IP). Subjects in cohorts 1-4 will be admitted to the clinical research unit (CRU) on the afternoon of Day -1 and receive a single dose of IP on Day 1 following overnight fast. Each subject dosed will be confined for a total period of 3 days (Day -1 to Day 3) and allowed to leave the CRU on the morning of Day 3. The subjects in Cohort 5 will be admitted to the CRU on the afternoon of Day -1 to be dosed with IP on the mornings of Day 1 and Day 4 following overnight fast in each case. Each Cohort 5 subject dosed will be confined for a total period of 6 days (Day -1 to Day 6) and allowed to leave the CRU on the morning of Day 6.

Safety assessments for **Cohort 1-4** will be conducted upon admission to the CRU, at baseline (Day 1) and during the clinical stay. Subjects will return to the CRU on Days 4, 6, 8, 15, 22, 29, 43, 57, 85 and 113 for PK and immunogenicity sampling and safety assessments.

Safety assessments for **Cohort 5** will be conducted upon admission to the CRU, at baseline (Day 1) and during the clinical stay. Subjects will return to the CRU on Days 7, 9, 11, 18, 25, 32, 39, 53, 67, 95 and 123 for PK and immunogenicity sampling and safety assessments.

The final safety assessment is scheduled for a maximum of 16 weeks after the last dose of IP. This is based on a mAb m102.4 t½ of 21-23 days.

Where applicable, Serious Adverse Events (SAEs) will be followed for a further 30 days after the final safety assessment, or until resolved, whichever is the earlier.

For all cohorts, two sentinel subjects will be randomized in a blinded fashion in which 1 subject receives mAb m102.4 and 1 subject receives placebo. The remaining 6 subjects in each cohort will receive IP (5 x mAb m102.4: 1 x placebo) a minimum of 14 days after the sentinel pair, following review of by the PI and the Medical Monitor.

The decision to progress to each successive higher dose will be made after completion of safety data review through Day 29 for at least six subjects per group, and after the Safety Review Committee (SRC) determines that it is safe to proceed to the next dose level.

The assessments, sampling times and follow-up period for subsequent cohorts may be adjusted following SRC review of PK data through to Day 43 for Cohort 1. PK analysis and review will occur whilst Cohort 2 is ongoing.

All PK data for Cohorts 1-4 will be reviewed by the SRC prior to the commencement of Cohort 5 to ensure that the dose and planned dosing interval are optimal. Adjustments to the dose and dosing interval for Cohort 5 may be made depending on the outcome of the review.

3.1.1 Study Centre

This study will be conducted at QPharm Pty Ltd, who have extensive experience in phase I studies involving mAbs and vaccines as well as an excellent record of recruitment for studies of this nature and compliance with study protocols and regulations.

3.1.2 Study Duration

The duration of this study will be approximately 5 months per subject, based on a Screening period of up to 28 days prior to dosing, one (Cohorts 1-4) or two doses (72 hours apart, Cohort 5) of IP and a maximum 16-week follow-up period.

The schedules of assessment for Cohorts 1-4 and Cohort 5 are presented in Table 1 to Table 5.

Table 1 Schedule of Events (Cohorts 1-4)

	Screening		Dose												
Procedures	**Day -28 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 6**	**Day 8**	**Day 15**	**Day 22**	**Day 29**	**Day 43**	**Day 57**	**Day 85**	**Day 113 (EOS)**
Informed Consent	X														
Inclusion/Exclusion Criteria	X	X	X												
Demographic Data	X														
Weight and Height	X	X[1]													
Medical History	X														
Prior and Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X		X[2]		X[2]						X[2]		X[2]		X
Vital Signs[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Tests[4]	X[5, 6]	X[5]		X[5]				X[5]	X		X		X	X	X[5]
Serology[7]	X														
Urinalysis	X	X													X
Pregnancy Test (females only)[8]	X	X													X
Alcohol Breath Test	X	X													
Urine Drug Screen	X	X													
Randomization			X												

	Screening		Dose												
Procedures	**Day -28 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 6**	**Day 8**	**Day 15**	**Day 22**	**Day 29**	**Day 43**	**Day 57**	**Day 85**	**Day 113 (EOS)**
Investigational Product; one hour IV			X												
Adverse Events[9]			X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X		X	X				X			X		X	X	X
mAb m102.4 Antibodies			X								X				X
Virus Neutralization[10]			X	X		X		X			X				X
PK Sampling		X	X	X		X	X	X	X		X	X		X	X
SRC Review											DE				

1 Weight only
2 Brief physical examination
3 Blood pressure, pulse rate, respiratory rate, temperature
4 Haematology (Red blood count, haemoglobin, haematocrit, mean cell volume, platelets, white blood cell count with differential including neutrophils, lymphocytes, eosinophils, basophils) and biochemistry (albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate, total and direct bilirubin, blood urea nitrogen, calcium, chloride, creatinine, glucose, potassium, uric acid, total protein)
5 Coagulation (PT, INR, aPTT) only at Screening, Day -1, Days 2, 8 and 113 (EOS)
6 IgG and complement at Screening only
7 Test for Hepatitis B and C, HIV, Retain sample for Hendra and Nipah virus
8 Pregnancy test: Serum pregnancy test at Screening and urine pregnancy test at other time points. Positive or equivocal urine pregnancy results will be confirmed by serum test. IP should be held during the interval between positive urine test and receipt of serum test results.
9 AEs will be collected from commencement of the infusion
10 Samples to be stored
DE: Minimum data required per Cohort for dose escalation review
EOS: End of Study
SRC: Safety Review Committee

Table 2 Schedule of Events (Cohort 5)

	Screening		**Dose 1**			**Dose 2**													
Days since 1st dose	**Day -28 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 9**	**Day 11**	**Day 18**	**Day 25**	**Day 32**	**Day 39**	**Day 53**	**Day 67**	**Day 95**	**Day 123 (EOS)**
Days since 2nd dose						**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 6**	**Day 8**	**Day 15**	**Day 22**	**Day 29**	**Day 36**	**Day 50**	**Day 64**	**Day 92**	**Day 120**
Informed Consent	X																		
Inclusion/Exclusion Criteria	X	X	X			X													
Demographic Data	X																		
Weight and Height	X	X[1]			X[1]														
Medical History	X																		
Prior and Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X		X[2]			X[2]		X[2]							X[2]		X[2]		X
Vital Signs[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Tests[4]	X[5, 6]	X[5]		X[5]		X	X[5]				X[5]	X	X		X		X	X	X[5]
Serology[7]	X																		
Urinalysis	X	X				X													X
Pregnancy Test (females only)[8]	X	X																	X
Alcohol Breath Test	X	X																	
Urine Drug Screen	X	X																	
Randomization			X																
Investigational Product: one hour IV			X			X													
Adverse Events[9]			X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X

	Screening		**Dose 1**			**Dose 2**													
Days since 1st dose	**Day -28 to -2**	**Day -1**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 9**	**Day 11**	**Day 18**	**Day 25**	**Day 32**	**Day 39**	**Day 53**	**Day 67**	**Day 95**	**Day 123 (EOS)**
Days since 2nd dose						**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 6**	**Day 8**	**Day 15**	**Day 22**	**Day 29**	**Day 36**	**Day 50**	**Day 64**	**Day 92**	**Day 120**
12-lead ECG	X		X	X		X	X	X			X				X		X	X	X
mAb m102.4 Antibodies			X												X				X
Virus Neutralization[10]			X	X		X		X		X					X				X
PK Sampling		X	X	X	X	X	X		X		X	X		X		X		X	X

1 Weight only
2 Brief physical examination
3 Blood pressure, pulse rate, respiratory rate, temperature
4 Haematology (Red blood count, haemoglobin, haematocrit, mean cell volume, platelets, white blood cell with differential including neutrophils, lymphocytes, eosinophils, basophils) and biochemistry (albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate, total and direct bilirubin, blood urea nitrogen, calcium, chloride, creatinine, glucose, potassium, uric acid, total protein,
5 Coagulation (PT, INR, aPTT) only at Screening, Day -1, Days 2, 5, 11 and 123 (EOS)
6 IgG and complement at Screening only.
7 Test for Hepatitis B and C, HIV Retain sample for Hendra and Nipah virus.
8 Pregnancy test: Serum pregnancy test at Screening and urine pregnancy test at other time points. Positive or equivocal urine pregnancy results will be confirmed by serum test. IP should be held during the interval between positive urine test and receipt of serum test results.
9 AEs will be collected from commencement of the infusion
10 Samples to be stored
EOS: End of Study

Table 3 **Detailed Schedule of Events for Days -1, 1, 2 and 3 (Cohorts 1-4)**

	Day -1	**Day 1**								**Day 2**		**Day 3**
Procedures	**Admission**	**Pre-dose**	**0 hour**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**8 hours**	**12 hours**	**24 hours**	**36 hours**	**48 hours**
Inclusion/Exclusion Criteria	X	X										
Demographic Data	X											
Weight	X											
Prior and Concomitant Medications	X	X		X	X	X	X	X	X	X	X	X
Brief Physical Examination		X										X
Vital Signs[1]	X	X		X	X	X		X	X	X		X
Blood Tests[2]	X[3]									X[3]		
Urinalysis	X											
Pregnancy Test	X											
Alcohol Breath Test	X											
Urine Drug Screen	X											
Randomization		X										
Investigational Product: one hour IV			X									
Adverse Events[4]			X	X	X	X	X	X	X	X	X	X
12-lead ECG	X	X			X	X		X		X		
mAb m102.4		X										

	Day -1	**Day 1**								**Day 2**		**Day 3**
Procedures	**Admission**	**Pre-dose**	**0 hour**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**8 hours**	**12 hours**	**24 hours**	**36 hours**	**48 hours**
Antibodies												
Virus Neutralization[5]		X							X	X		
PK Sampling		X		X		X		X	X	X		
CRU	IN											OUT

1 Blood pressure, pulse rate, respiratory rate, temperature
2 Haematology (Red blood count, haemoglobin, haematocrit, mean cell volume, platelets, white blood cell with differential including neutrophils, lymphocytes eosinophils, basophils) and biochemistry (albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate, total and direct bilirubin, blood urea nitrogen, calcium, chloride, creatinine, glucose, potassium, uric acid, total protein).
3 Coagulation (PT, INR, aPTT) only at Day -1 and 24 hours.
4 AEs will be collected from commencement of the infusion
5 Samples to be stored
CRU: Clinical Research Unit

Table 4 Detailed Schedule of Events for Days -1, 1, 2 and 3 (First Dose) (Cohort 5)

	Day -1	**Day 1**								**Day 2**		**Day 3**
Procedures	**Admission**	**Pre-dose**	**0 hour**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**8 hours**	**12 hours**	**24 hours**	**36 hours**	**48 hours**
Inclusion/Exclusion Criteria	X	X										
Demographic Data	X											
Weight	X											X[1]
Prior and Concomitant Medications	X	X		X	X	X	X	X	X	X	X	X
Brief Physical Examination		X										
Vital Signs[2]	X	X		X	X	X		X	X	X		X
Blood Sample[3]	X[4]									X[4]		
Urinalysis	X											
Pregnancy Test	X											
Alcohol Breath Test	X											
Urine Drug Screen	X											
Randomization		X										
Investigational Product: one hour IV			X									
Adverse Events[5]			X	X	X	X	X	X	X	X	X	X
12-lead ECG	X	X			X	X		X		X		

	Day -1	**Day 1**								**Day 2**		**Day 3**
Procedures	**Admission**	**Pre-dose**	**0 hour**	**1 hour**	**2 hours**	**4 hours**	**6 hours**	**8 hours**	**12 hours**	**24 hours**	**36 hours**	**48 hours**
mAb m102.4 Antibodies		X										
Virus Neutralization[6]		X							X	X		
PK Sampling		X		X		X		X	X	X		X
CRU	IN											

1 For confirmation of weight for dosing next day (Day 4)
2 Blood pressure, pulse rate, respiratory rate, temperature
3 Haematology (Red blood count, haemoglobin, haematocrit, mean cell volume, platelets, white blood cell count with differential including neutrophils, lymphocytes, eosinophils, basophils) and biochemistry (albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate, total and direct bilirubin, blood urea nitrogen, calcium, chloride, creatinine, glucose, potassium, uric acid, total protein)
4 Coagulation (PT, INR, aPTT) only at Day -1 and 24 hours.
5 AEs will be collected from commencement of the infusion
6 Samples to be stored

Table 5 Detailed Schedule of Events for Days 3, 4, 5 and 6 (Second Dose) (Cohort 5)

	Day 4								Day 5		Day 6
Hours since 2nd dose	**Pre-2nd dose**	**0 hours**	**1 hour post-dose**	**2 hours post-dose**	**4 hours post-dose**	**6 hours post-dose**	**8 hours post-dose**	**12 hours post-dose**	**24 hours post-dose**	**36 hours post-dose**	**48 hours post-dose**
Hours since 1st dose		**72 hours**	**73 hours**	**74 hours**	**76 hours**	**78 hours**	**80 hours**	**84 hours**	**96 hours**	**108 hours**	**120 hours**
Inclusion/Exclusion Criteria	X										
Prior and Concomitant Medications	X		X	X	X	X	X	X	X	X	X
Brief Physical Examination	X										X
Vital Signs[1]	X		X	X	X		X	X	X		X
Blood Tests[2]	X								X[3]		
Urinalysis	X										
Investigational Product: one hour IV		X									
Adverse Events	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG	X			X	X		X		X		X
Virus Neutralization[4]	X										X
PK Sampling	X		X		X		X	X	X		
CRU											OUT

1 Blood pressure, pulse rate, respiratory rate, temperature

2 Haematology (Red blood count, haemoglobin, haematocrit, mean cell volume, platelets, white blood cell with differential including neutrophils, lymphocytes, eosinophils, basophils) leukocytes, and biochemistry (albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bicarbonate, total and direct bilirubin, blood urea nitrogen, calcium, chloride, creatinine, glucose, potassium, uric acid, total protein)

3 Coagulation (PT, INR, aPTT) at Day 5;

4 Samples to be stored

3.2 Summary of Study Procedures

All subjects must give their informed consent before any study-specific procedures are performed.

The following deviations of the timing of assessments will be allowed:

All Cohorts during confinement period:

Pre-dose samples up to 45 minutes prior to dosing
Samples between 0-4 hours inclusive post infusion +2 minutes
Samples between 4-14 hours inclusive post infusion +5 minutes
Sampling between 14-16 hours inclusive post infusion +15 minutes
Sampling at more than 16 hours post infusion ± 30 minutes

Cohorts 1-4:

± 4 hours for Day 5-11, ±1 day for Days 12-15, ±2 days for Days 16-22, ±3 days for Day 23-32, ±4 days for Day 33-43, ±5 days for Day 44-57, ±6 days for Day 58-85 and ± 7 days for Day 86-113.

Cohort 5:

± 4 hours for Day 8-11, ±1 day for Days 12-15, ±2 days for Days 16-22, ±3 days for Day 23-32, ±4 days for Day 33-43, ±5 days for Day 44-57, ±6 days for Day 58-85 and ± 7 days for Day 86-123.

The following procedures will be performed at each study visit.

3.2.1 Screening Visit

- Demographic data (Date of birth, gender, race, ethnicity)
- Obtain medical history
- Collect prior and concomitant medication (for the last 90 days for contraception and last 60 days for other medications)
- Inclusion and exclusion criteria check
- Physical examination
- Weight and height (with calculation of BMI)
- Perform alcohol breathalyzer
- Collect urine for urinalysis and urine drug screen
- Obtain vital signs (systolic and diastolic blood pressure, pulse rate, respiration rate and body temperature)

- Perform 12-lead ECG
- Collect blood for pregnancy test (females of child-bearing potential only)
- Blood samples for haematology, biochemistry, coagulation, serology (test for HIV, HepBsAg and anti-HCV, retain samples for Hendra and Nipah virus), IgG and complement

3.2.2 Day -1

Subjects will be admitted to the CRU in the late afternoon for dosing the next morning. The following procedures will be performed following admission to the CRU:

- Update prior and concomitant medication
- Weight
- Perform alcohol breathalyzer
- Collect urine for urinalysis, urine drug screen and urine pregnancy test
- Vital signs
- Inclusion and exclusion criteria check
- Blood samples for haematology, biochemistry and coagulation

3.2.3 Day 1 (pre-dose)

- Update prior and concomitant medications
- Brief physical examination
- Vital signs
- Perform 12-lead ECG
- Inclusion and exclusion criteria check
- Randomization
- Baseline blood samples for mAb m102.4 antibodies
- Baseline blood samples for virus neutralization
- PK sampling

3.2.4 Day 1/Dosing

All of the time points provided for Day 1 to End of Study are provided relative to the commencement of the first infusion of IP for all subjects.

- Time 0: commencement of IV IP administration for 1 hour
- Blood samples for virus neutralization (12 hours)
- PK sampling (1, 4, 8 and 12 hours)
- Vital signs (1, 2, 4, 8 and 12 hours)

- Perform 12-lead ECG (2, 4 and 8 hours)
- Record all concomitant medication use
- Record all AEs

3.2.5 Day 2/24 hours

- Blood samples for haematology, biochemistry and coagulation
- Vital signs
- Perform 12-lead ECG
- Blood samples for virus neutralization
- PK sampling
- Record all concomitant medication use
- Record all AEs

3.2.6 Day 3/48 hours

Cohorts 1-4

- Brief physical examination, vital signs
- Record all concomitant medication use
- Record all AEs
- Leave the CRU

Cohort 5

- Record all concomitant medication use
- Weight
- Vital signs
- PK sampling
- Record all AEs

3.2.7 Day 4

Cohorts 1-4

- Vital signs
- Blood samples for virus neutralization
- PK sampling
- Record all concomitant medication use
- Record all AEs

Cohort 5

Pre-second dose:

- Record all AEs
- Record all concomitant medication use
- Brief physical examination
- Urine sample for urinalysis
- Vital signs
- Perform 12-lead ECG
- Blood samples for haematology and biochemistry
- Blood samples for virus neutralization
- PK sampling
- Inclusion and exclusion criteria check
- Time 0: commencement of IV IP administration for 1 hour (2nd dose)

Post-dose: (Dosing is at 72 hours post first dose; Time = 0 h post second dose)

- Vital signs (73, 74, 76, 80 and 84 hours post 1st dose)
  (1, 2, 4, 8 and 12 hours post 2nd dose)
- PK sampling (73, 76, 80 and 84 hours post 1st dose)
  (1, 4, 8 and 12 hours post 2nd dose)
- 12-lead ECG (74, 76 and 80 hours post 1st dose)
  (2, 4 and 8 hours post 2nd dose)
- Record all concomitant medication use
- Record all AEs

3.2.8 Day 5 (96 hours, Cohort 5 only)

(24 hours post 2nd dose)

- Vital signs
- Blood samples for haematology, biochemistry and coagulation
- PK sampling
- 12-lead ECG
- Record all concomitant medication use
- Record all AEs

3.2.9 Day 6

Cohorts 1-4

- Vital signs
- PK sampling
- Record all concomitant medication use
- Record all AEs

Cohort 5 (120 hours)

(48 hours post 2nd dose; before leaving the CRU)

- Brief physical examination
- Vital signs
- 12-lead ECG
- Blood samples for virus neutralization
- Record all concomitant medication use
- Record all AEs

3.2.10 Day 7 (Cohort 5 only)

- Vital signs
- PK sampling
- Record all concomitant medication use
- Record all AEs

3.2.11 Day 8 (Cohorts 1-4 only)

- Vital signs
- Blood samples for virus neutralization
- PK sampling
- Blood samples for haematology, biochemistry and coagulation
- 12-lead ECG
- Record all concomitant medication use
- Record all AEs

3.2.12 Day 9 (Cohort 5 only)

- Vital signs
- Blood samples for virus neutralization
- Record all concomitant medication use
- Record all AEs

3.2.13 Day 11 (Cohort 5 only)

- Vital signs
- PK sampling
- Blood samples for haematology, biochemistry and coagulation
- 12-lead ECG
- Record all concomitant medication use
- Record all AEs

3.2.14 Day 15 (Cohorts 1-4 only)

- Vital signs
- PK sampling
- Blood samples for haematology and biochemistry
- Record all concomitant medication use
- Record all AEs

3.2.15 Day 18 (Cohort 5 only)

- Vital signs
- PK sampling
- Blood samples for haematology and biochemistry
- Record all concomitant medication use
- Record all AEs

3.2.16 Day 22 (Cohorts 1-4 only)

- Vital signs
- Record all concomitant medication use
- Record all AEs

3.2.17 Day 25 (Cohort 5 only)

- Vital signs
- Blood samples for haematology and biochemistry
- Record all concomitant medication use
- Record all AEs

3.2.18 Day 29 (Cohorts 1-4 only)

- Brief physical examination
- Vital signs
- PK sampling

- 12-lead ECG
- Blood samples for haematology and biochemistry
- Blood samples for mAb m102.4 antibodies
- Blood samples for virus neutralization
- Record all concomitant medication use
- Record all AEs

3.2.19 Day 32 (Cohort 5 only)

- Vital signs
- PK sampling
- Record all concomitant medication use
- Record all AEs

3.2.20 Day 39 (Cohort 5 only)

- Brief physical examination
- Vital signs
- 12-lead ECG
- Blood samples for haematology and biochemistry
- Blood samples for mAb m102.4 antibodies
- Blood samples for virus neutralization
- Record all concomitant medication use
- Record all AEs

3.2.21 Day 43 (Cohorts 1-4 only)

- Vital signs
- PK sampling
- Record all concomitant medication use
- Record all AEs

3.2.22 Day 53 (Cohort 5 only)

- Vital signs
- PK sampling
- Record all concomitant medication use
- Record all AEs

3.2.23 Day 57 (Cohorts 1-4 only)

- Brief physical examination
- Vital signs

- 12-lead ECG
- Blood samples for haematology and biochemistry
- Blood samples for virus neutralization
- Record all concomitant medication use
- Record all AEs

3.2.24 Day 67 (Cohort 5 only)

- Brief physical examination
- Vital signs
- 12-lead ECG
- Blood samples for haematology and biochemistry
- Record all concomitant medication use
- Record all AEs

3.2.25 Day 85 (Cohorts 1-4 only)

- Vital signs
- 12-lead ECG
- PK sampling
- Blood samples for haematology and biochemistry
- Record all concomitant medication use
- Record all AEs

3.2.26 Day 95 (Cohort 5 only)

- Vital signs
- 12-lead ECG
- PK sampling
- Blood samples for haematology and biochemistry
- Record all concomitant medication use
- Record all AEs

3.2.27 Days 113 (Cohorts 1-4) and Day 123 (Cohort 5)

End of study visit for all subjects.

- Physical examination
- Vital signs
- Urine sample for urinalysis and pregnancy test (females of child-bearing potential only)
- PK sampling
- 12-lead ECG

- Blood samples for haematology, biochemistry and coagulation
- Blood samples for mAb m102.4 antibodies
- Blood samples for virus neutralization
- Record all concomitant medication use
- Record all AEs

3.2.28 Early Termination/Discontinuation Visit

Subjects that withdraw from the study, or who are discontinued by the Investigator will be asked to attend an early termination visit at the CRU. Assessments to be conducted at the early termination visit are as described for the end of study visit above.

3.2.29 Unscheduled Visits

Subjects will be asked to attend the CRU for unscheduled visits as the need arises. Instances where this may be required are for follow-up on any AEs/SAEs or for repeat blood tests or safety assessments.

3.3 Allocation to Treatment/Blinding

Subjects will be assigned a sequential screening number at their Screening visit. Eligible subjects be randomized on the morning of Day 1 and will be allocated a randomization number. Each subject will have a corresponding IP or placebo allocated on the randomization list. The two sentinel subjects in each cohort will be dosed first. In accordance with the randomization list one subject from the sentinel pair will receive IP and the other will receive placebo. The remaining six subjects per cohort will be randomized to receive either IP or placebo in a 5:1 ratio. In order to maintain the study blind the randomization list will only be available to the un-blinded pharmacist and will be held in a secure location with restricted access.

3.4 Breaking the Blind

Only in the case of an emergency, when knowledge of the IP is essential for the welfare of the subject, the Investigator may un-blind a subject's treatment assignment. If the blind is broken for any reason, the Investigator must notify the Medical Monitor immediately of the un-blinding incident. Emergency un-blinding may be performed using the code-break envelopes supplied to the PI at the site. The code-break envelope must be signed and dated when the code is broken and the reason for un-blinding provided on the envelope and documented in the source notes. All code-break envelopes, whether sealed or opened, must be returned to the Sponsor at the end of the study.

If an unexpected and related SAE is reported, the treatment assignment for that individual subject may be un-blinded for regulatory reporting.

If a severe AE or SAE in a patient leads to a pause or discontinuation of enrolment per protocol, treatment assignment for that individual subject may be un-blinded. During subsequent investigation, if the Medical Monitor and Safety Committee discover other subjects with relevant AEs, they may additionally request un-blinding of these subjects, if necessary for their determination of whether or not to resume enrollment.

3.5 Dose Escalation and Stopping rules

Based on emerging safety and PK data, the SRC may recommend repeating a dose cohort at a dose shown to be well-tolerated, or initiating an intermediate or lower dose cohort. Initiation of an intermediate dose cohort will only proceed when the previous lower dose(s) have been found to be well tolerated following a blinded review of available safety data through study Day 29 of the current cohort and all available safety data of the previous cohort(s), a positive recommendation by the SRC. The SRC will consist of the PI, medical monitor and Sponsor representative.

There will be a pause in dose escalation to the next planned dose and dose adjustments may be made if any of the following "pausing criteria" are met:

- An SAE reported in more than ≥ 1 subject.
- ≥ 2 subjects experience a common terminology criteria for adverse events (CTCAE) Grade 3 (or higher) event in the same organ or body system.
- ≥ 50% of the subjects in one dose cohort show clinically relevant AEs of moderate to severe intensity

Termination of the study may be considered if these events are considered related to IP to the agreement of the SRC.

3.5.1 Dose Escalation Review

The will review the blinded safety (abnormal pathology, vital signs, ECG) and tolerability (AEs and concomitant medications) of the subjects at various time points. The data for the sentinel subjects from each cohort will be reviewed by the PI and the Medical Monitor only, after a minimum of 14 days to determine whether additional dosing of the remaining six subjects in the cohort can proceed.

Data for at least six of the eight subjects in each cohort will be reviewed through to Day 29 to determine whether dose escalation to the next cohort (i.e., subsequent, higher dose) can proceed.

For both additional dosing and dose escalation the SRC members will formally document their decision to proceed or otherwise.

The SRC members will also review PK data generated for Cohort 1 through to Day 43. Review of the data will be to confirm the mAb m102.4 half-life. Assessment and sampling times and the overall monitoring period for subsequent cohorts may be adjusted depending on the outcome of the review.

In addition, PK data for Cohorts 1-4 will be reviewed prior to the commencement of Cohort 5. Cohort 5 timelines for planned dose and dosing intervals may be adjusted depending on the outcome of the review.

3.6 Concomitant Treatment(s)

3.6.1 Permitted Treatment(s)

Paracetamol is a permitted concomitant medication where clinically indicated. In addition, oral contraceptives will be allowed for females, provided that the subject has been using the oral contraceptive for at least 3 months prior to the Screening visit and no change to this contraception is planned or anticipated for the duration of the study.

The use of non-prescription medications will be discouraged during the course of the study. Prescription medications should be limited and avoided if possible, but will be allowed where clinically indicated. The use of all concomitant medication (including non-prescription medications) will be recorded for each subject for the duration of the study.

3.6.2 Lifestyle Restrictions

Subjects in Cohorts 1-4 will be admitted to the CRU on Day -1 and will be allowed to leave the unit on the morning of Day 3. Subjects in Cohort 5 will be admitted on Day -1 and will be allowed to leave the unit on the morning of Day 6.

Water is permitted without restriction during the conduct of the study, but subjects must fast from at least 8 hours prior to study dosing through 2 hours post-dosing. Meals will be provided following the restriction period and during confinement.

Alcohol will be restricted from 24 hours prior to Day -1 check-in and will not be allowed for 2 weeks following the last infusion of IP. Thereafter, no more than 2 standard drinks per female and 3 standard drinks per male per day will be allowed. One standard drink is (8 g) is equivalent to 280 mL of beer, one measure (25 mL) of spirits or 1 small glass (125 mL) of wine. Alcohol Breath test must be negative for eligibility.

No caffeine-containing beverages will be allowed during confinement in the unit. Caffeine-free beverages will be provided after 4 hours post-dose. Post confinement, no excessive use of caffeine-containing beverages including coffee, tea, cola, energy drinks or chocolates, exceeding 500 mg caffeine/day (approximately 5 cups of coffee or 10 cups of tea) will be allowed.

Subjects are not allowed to smoke > 5 cigarettes per day with only occasional/non-habitual use of nicotine considered acceptable. Subjects should refrain from smoking 48 hours prior to Day -1 check-in. Subjects will not be permitted to smoke whilst inside the study site. Cotinine test must be negative for eligibility at screening and Day -1.

Subjects should refrain from any strenuous physical activity 24 hours prior to Day-1 check-in or follow up blood tests.

3.7 Treatment Compliance

The IP will be given per IV infusion over an hour in the CRU by trained personnel and will be documented in the case report form (CRF). Any deviations will be noted.

3.8 Measures to Minimize Bias

Subjects will be allocated their treatment according to a computer generated randomization schedule.

All site personnel will be blinded, with the exception for the un-blinded pharmacist who prepares the IP for dispensing to clinical staff. The randomization list will be kept in a secure locked facility. The blind will be maintained for the subjects, Investigators and clinic staff, Sponsor personnel, the Project manager and main monitor for the study. An un-blinded pharmacy monitor will be employed to monitor pharmacy compliance and drug accountability.

IP and placebo will be identical in appearance and volume for each subject in each cohort.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Number of Subjects

A total of up to 40 subjects will be enrolled to dose in this study (eight subjects in five cohorts).

4.2 Inclusion Criteria

A subject must meet the following inclusion criteria to participate in this study:

1. Written informed consent from the subject;
2. Healthy males and females as determined by medical history and physical examination between 18 and 50 years of age (inclusive);
3. Body mass index (BMI) of 18.0 to 35.0 kg/m^2 (inclusive) at screening (Weight ≥ 50 kg or ≤ 100 kg);
4. Haematology, coagulation, clinical chemistry, and urinalysis test results not deviating from the normal range to a clinically relevant extent at screening
5. Able to comply with requirements for concomitant medications and consumption of caffeine, alcohol, tobacco and nicotine;
6. Willing to attend all the study visits.

4.3 Exclusion Criteria

A subject will be excluded from participating in this study if any of they meet any of the following criteria:

1. Close, unprotected exposure to sick horses with an unexplained illness within 4 weeks of Day 1; or known exposure to potential Hendra infected horses;
2. Clinically significant abnormalities in laboratory test results at Screening or Baseline (Day -1) (biochemistry, haematology and urinalysis parameters) (Liver function tests >1.5 x upper limit of normal [ULN]);
3. Immunoglobulin G (IgG) and complement level outside normal limits (at Screening);
4. Positive urine screen for potential drugs of abuse at Screening and prior to randomization;
5. Presence or history (in last 2 years) of alcohol abuse confirmed by medical history, or daily alcohol intake of more than 2 standard drinks (16 g) for females or more than 3 standard drinks for males, or a positive breath alcohol test at screening or prior to randomization, and the inability to stop alcohol intake for 2 weeks post-infusion
   One standard drink is (8 g) is equivalent to 280 mL of beer, one measure (25 mL) of spirits or 1 small glass (125 mL) of wine;
6. Excessive use of caffeine-containing beverages including coffee, tea, cola, energy drinks or chocolates, exceeding 500 mg caffeine/day (approximately 5 cups of coffee or 10 cups of tea) and the inability to refrain from the use of caffeine-containing beverages during confinement in the CRU;
7. Smoke >5 cigarettes per day;

8. Positive tests for HIV or viral hepatitis (hepatitis B surface antigen [HepBsAg], or anti-hepatitis C [HCV]);
9. Treatment with an investigational drug or biologic within 60 days preceding treatment or plans to take another investigational drug or biologic in the 3 months following IP administration;
10. Blood donation or significant blood loss 60 days prior to Day 1;
11. Plasma donation within 60 days prior to Day 1 or planned donation in the 6 months following IP administration;
12. Males who are sexually active and have a partner who is capable of becoming pregnant, neither of which have had surgery to become sterilized, who are not using an effective method of birth control (e.g., surgically implanted hormonal therapy, intrauterine devices, or oral birth control with barrier method). In addition, male subjects must agree not to donate sperm during the study and in the 6 months following IP administration;
13. Females who are capable of becoming pregnant and who have not had surgery to become sterilized and who are not using an effective method of birth control (e.g., surgically implanted hormonal therapy, intrauterine devices, or oral birth control with barrier method [female condom or male partners to use condom]). For exclusively homosexually active females the restrictions for adequate contraception does not apply (if lifestyle choice change, contraceptive requirements will be applicable);
14. Poor peripheral venous access;
15. Subjects with clinically significant findings from medical history that, in the opinion of the investigator, could interfere with the objectives of the study or put the subject at risk;
16. Subject is unwilling or unable to abide by the requirements of the protocol or inability to be available for follow-up assessments or protocol related procedures;
17. Subject is on staff at the investigator site; or a relative of personnel of the investigator site or of the Sponsor (or Sponsor's affiliates);
18. Use of prescription or non-prescription drugs within 14 days or 5 half-lives (whichever is longer) prior to IP administration and for the duration of the study. Contraceptives for females are allowed – must have been using the same contraceptive for a minimum period of 3 months and must not change the contraceptive during the study;
19. History of allergies, allergic reactions or hypersensitivity to medications;
20. BP systolic >160 mmHg or diastolic >90 mmHg. Allow one repeat measure;
21. Personal or family history of congenital QT syndrome or sudden unexplained death;
22. ECG clinically significant abnormalities including (QTc>450 msec for males and >460 msec for females), AF or atrial flutter, notably bradycardia (<40 bpm) or tachycardia (>100 bpm), complete left or right bundle branch block, Wolf-Parkinson White Syndrome or presence of cardiac pacemaker;
23. Any vaccination in the last month;
24. Pregnant or breastfeeding females.

4.4 Withdrawal Criteria

Subjects should be encouraged to complete all study assessments. However, subjects may withdraw consent to participate in this study at any time and for any reason without penalty or loss of benefits to which they were otherwise entitled. The Sponsor or the Investigator may withdraw subjects at any time for safety or administrative reasons.

A subject may be withdrawn, if any of the following occur during the study:

1. Subject requests withdrawal from the study;
2. Subject withdraws consent;
3. AE makes the continuation of the subject impossible or inadvisable;
4. Pregnancy;
5. Subject is lost to follow-up;
6. Subject discovered after enrollment not to have met the protocol inclusion criteria;
7. Subject refuses to comply with required study procedures;
8. Use of any additional experimental drug or device or participation in another clinical study;
9. The study sponsor terminates the Investigator Site or the study.

If a subject withdraws, the Investigator is to make a reasonable effort to determine the reason for the subject's withdrawal from the study. The reason(s) for subject withdrawal will be recorded on the CRF for all subjects.

Subjects who withdraw from the study will not be replaced. All subjects that withdraw or are discontinued from the study by the Investigator will be asked to complete and early termination visit at the CRU.

5 INVESTIGATIONAL PRODUCT (IP)

5.1 Treatment Groups

There will be five cohorts dosed with mAb m102.4 in this study. The first four cohorts will receive a single dose of 1 mg/kg, 3 mg/kg, 10 mg/kg or 20 mg/kg. Cohort 5 will receive 20 mg/kg in two separate doses, 72 hours apart. There will be eight subjects in each cohort with six randomized to receive mAb m102.4 and two randomized to receive placebo.

5.2 Description of Investigational Product

The IP in this study is the human mAb m102.4, and placebo (normal saline control). The mAb m102.4 is a fully human, IgG_1 κ directed against the HeV G protein.

5.3 Investigational Product Packaging and Labeling

Active mAb m102.4 IP will be packaged with an open-label in single use, 10 mL glass vials containing 10 mL of 10 mg/mL mAb m102.4 formulated with 1x DPBS pH 7.4. Vials are closed with a rubber stopper and flip-off aluminum seal.

5.4 Investigational Product Storage and Disposal

All active IP will be shipped to the CRU frozen and should be stored at -15°C or lower until ready to use. The IP must be thawed before compounding, see the Pharmacy manual for instructions.

IP will be stored in a secure location with limited access under appropriate environmental conditions. Only appropriate personnel will have access to the IP.

Any unused IP will be returned to AIBN/UQ for storage or to organize storage at an appropriate facility.

5.5 Administration of Investigational Product

The IP will be delivered intravenously in normal saline in a volume of 100 mL (Cohorts 1 and 2) and 500 mL (Cohorts 3-5) over a 1-hour period using a peristaltic infusion pump. An experienced and qualified staff member will perform placement of the IV access. Before and after each IV infusion, the IV access will be flushed with normal saline.

The preparation of the IP is described in detail in the Pharmacy manual.

Administration of each individual subject will not occur until 30 minutes from the previous subject infusion. Medical therapy and supportive care should be deployed as necessary for any symptoms of drug hypersensitivity. Physicians and nurses involved with the administration of IP are trained in intermediate life support techniques and appropriate medical therapies will be immediately available.

5.6 Investigational Product Accountability

The Investigator and study pharmacist are responsible for maintaining a current record of the IP. IP received from the Sponsor, dispensed to the subjects and returned to the Sponsor will be recorded with corresponding dates.

For more information regarding IP handling and preparation please see the Pharmacy manual, provided as a separate document.

An un-blinded pharmacy monitor will be employed to review product accountability and verify appropriate pharmacy procedures for the study are adhered to.

6 ASSESMENT OF PHARMACODYNAMICS (PD) AND PHARMACOKINETICS (PK)

6.1 Immunogenicity and Virus Neutralization

Immunogenicity and Hendra (possibly also Nipah) virus neutralization will be collected per the study schedule and potentially assessed at the end of the study.

6.2 Pharmacokinetics

Blood samples for the measurement of mAb m102.4 levels will be collected by a qualified staff member during the visits as indicated in the schedule of assessments. Blood samples will be collected via direct venipuncture or cannula.

Detailed instructions for the collection, labeling, handling, processing, storage, and shipment of the PK samples will be documented in the site laboratory manual. All study personnel will be trained in the appropriate study procedures prior to the initiation of subject enrollment.

PK samples for Cohort 1 will have an interim analysis after collection of the Day 43 sample. Data will be reviewed by the SRC and adjustments to assessments, sample timings and follow-up period for subsequent cohorts may be implemented. Analysis and review of the Cohort 1 PK samples and data will be conducted whilst Cohort 2 is ongoing.

PK samples for the remainder of Cohort 1 and Cohorts 2-4 will be analyzed after completion of Cohort 4. All PK samples for Cohorts 1-4 will be reviewed by the SRC prior to the commencement of Cohort 5 to ensure that the dose and planned dosing interval is optimal. Adjustments may be made to the dose and dosing interval for Cohort 5 as deemed appropriate following review.

7 ASESSMENT OF SAFETY

7.1 Safety Endpoint(s)

Safety will be assessed during the study through physical examination, urinalysis, vital signs, ECG and laboratory tests as outlined in the Schedules of assessment (Table 1 to Table 5).

7.2 Physical Examination

A complete physical examination will include assessments of general appearance; skin and lymphatics; eyes; ears; nose; throat; cardiovascular system; respiratory system; abdomen/gastrointestinal system; musculoskeletal and neurological systems. Other body systems may also be examined.

A brief physical examination will include assessments of general appearance; cardiovascular system; respiratory system and abdomen/gastrointestinal system. Other body systems may also be examined if indicated through subject complaints.

Clinically significant changes from baseline (Day 1) will be recorded as AEs.

7.3 Height, Weight and BMI

Height will be recorded in centimeters (cm) and only at Screening. Weight will be recorded in kilograms (kg) and will be taken at Screening, Day -1 (all subjects) and Day 3 (Cohort 5 only). Weight is measured with subjects wearing light clothing without shoes. BMI will be calculated using the formula kg/m^2.

7.4 Vital signs

Vital signs include systolic and diastolic blood pressure, pulse rate, respiration rate and body temperature. Blood pressure and heart rate will be measured in a sitting position after resting for 5 minutes. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) should be measured in millimeters of mercury (mmHg), heart rate in beats per minute, respiration rate in breaths per minute and temperature (oral) in degrees Celsius (°C). Vital signs will be measured at every visit.

7.5 12-lead ECGs

ECGs will be taken as indicated in the schedules of assessments.

The subject must be resting in a semi-supine or supine position for at least 5 minutes prior to performing the ECG. The same ECG machine should be used for all recordings from an individual subject, when possible. ECGs will be read by the Investigator or designated physician at the unit. Machine-read automated ECG intervals (PR, QRS, QT and HR), and calculated RR intervals will be captured on the CRF and if applicable any other abnormalities noted. QTcF values will be derived from the data available.

Single ECG recordings will be taken at each time point. One repeat is allowed for screening purposes if abnormal findings are observed.

For safety monitoring purposes, the investigator or designee must review, sign and date all ECG tracings. Paper copies will be kept at the study center with the subject's clinical file as part of the permanent record. The ECG intervals and interpretation will be recorded on the CRF. Paper ECG recordings should be photocopied and maintained as a permanent source document. The Sponsor reserves the right to request copies of paper ECG recordings for independent cardiology review.

7.6 Clinical Laboratory Tests

Laboratory data obtained on Day -1 (pre-dose) will be used as baseline in the safety analyses. Safety Laboratory Data include:

- Complete blood count (CBC): haemoglobin, haematocrit, RBC count, MCV, WBC with differential (neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils), and platelets.
- Coagulation: Prothrombin time (PT), international normalized ration (INR), activated partial PT (aPTT).
- Chemistry panel: Albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT, SGPT), aspartate aminotransferase (AST, SGOT), bicarbonate, total and direct bilirubin, blood urea nitrogen (BUN), calcium, chloride, creatinine, glucose, potassium, uric acid and total protein). Estimated glomerular filtration rate (eGFR) will be calculated with each serum creatinine measurement using the Cockroft Gault equation.
- Urinalysis: specific gravity, pH, protein, glucose, ketones, bilirubin, blood, nitrite, and leukocyte esterase.
    - Specimens with abnormal Protein, WBC or RBC will be submitted for microscopy testing and reporting on WBCs, RBCs, epithelial cells, casts, crystals, yeast, and bacteria. Specimens with elevated WBC may be submitted for microbiological testing for infection.

Screening blood tests include:

- Serology: hepatitis (HepB surface antigen, anti-HCV), HIV
- Immunology: IgG and complement (Screening only)

Drug Screening includes:

- Urine Drug Screen:
  - Cocaine, narcotics, cannabis, barbiturates, benzodiazepines, amphetamines
  - Cotinine
- Breath Alcohol test

Immunogenicity and Virus Neutralization

- Formation of anti-mAb m102.4 antibodies will be tested. These samples will be stored as frozen samples for future testing upon completion of the study.
- mAb m102.4 virus neutralization for Hendra (and possibly Nipah) will possibly be assessed (samples to be stored)

7.7 Pregnancy Tests

All females of child-bearing potential will have pregnancy tests performed at the designated time points. A serum pregnancy test will be performed at Screening and urine pregnancy tests at the other time points. In the event of a positive or equivocal urine pregnancy test, a confirmatory serum pregnancy test will be performed.

7.8 Adverse Events (AEs)

7.8.1 Definitions

AEs are any untoward medical occurrence in a patient or clinical investigational subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

Treatment-emergent AEs (TEAEs) are events that are not present at baseline, or if present at baseline, have worsened in severity.

A pre-existing condition is one that is present at the start of the study and is reported as part of the subject's medical history. A pre-existing condition should be reported as an AE only if its frequency, intensity or character worsens during study treatment.

Study subjects should be instructed to report all AEs to the Investigator and all AEs that occur from the time of commencement of IP through the follow-Up Visit or premature termination visit will be recorded. All AEs must be appropriately documented in the subject's original source documents and on the CRFs. Investigators should report syndromes rather than list symptoms.

7.8.2 Grading of Adverse Events

The severity of each AE will be categorized using the following criteria:

Grade 1 (Mild): usually transient; requires no special treatment and does not interfere with the subject's daily activities. No limitation in daily activity.

Grade 2 (Moderate): produces a low level of inconvenience to the subject and may interfere with daily activities. These events are usually ameliorated by simple therapeutic measures. Some limitation in daily activity.

Grade 3 (Severe): interrupts daily activity and requires systemic drug therapy or other medical treatment. Unable to perform daily activity.

7.8.3 Relationship to Investigational Product

The Investigator must make an assessment of the relationship of the event to the IP using the following scale:

Unrelated: The event is definitely not or unlikely to be associated with IP administration and is judged due to causes other than the IP (alternative cause to be specified on the CRF).

Related: The event is possibly or probably related with IP administration. If related, the event should be further classified as possibly or probably related:

- Events considered to be possibly related are those that follow a reasonable temporal sequence from administration of the IP, but that may be due to another cause and could also be reasonably explained by the subject's clinical state or other modes of therapy administered to the subject.
- Events considered to be probably related are those that follow a reasonable temporal sequence from administration of the IP, that is not easily explained by another cause such as known characteristics of the subject's clinical state or other treatment, and is confirmed by improvement on stopping or slowing administration of the study agent (de-challenge), if applicable.

7.8.4 Laboratory Test and other Test Abnormalities as Adverse Events

Only laboratory abnormalities or other tests (ECG, vital sign) that have been judged by the Investigator as clinically significant, have clinical manifestations, or requires therapeutic intervention should be reported by the Investigator as AEs. When possible, syndromes/diagnosis, not laboratory values, should be reported as AEs. For example, decreased haemoglobin and haematocrit requiring iron supplementation should be recorded as "anaemia."

7.9 Serious Adverse Events

The Investigator is required to determine if each AE is an SAE. According to the ICH guidelines for Good clinical practice (GCP) (E6), an SAE is any untoward medical occurrence during the course of the clinical investigation that is characterized by one or more of the following:

- Results in death, or
- Is life-threatening, or
- Requires inpatient hospitalization or prolongation of existing hospitalization, or
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

In the event of death, the cause of death should be recorded as the SAE (death is an outcome, not the AE itself).

Any planned procedures that require admission to hospital for compliance with this protocol as well as any planned elective procedures are not considered SAEs (unless the underlying condition has worsened or the procedure results in a worsening of the subject's condition). In addition pregnancy that occurs during the course of the study or absence of a treatment effect will also not be considered to be an SAE.

7.9.1 Reporting for Serious Adverse Events (24 Hours)

An SAE must be followed clinically until resolution or stabilization.

All SAEs after the time of consent must be reported to the Sponsor or the Sponsor's representative within 24 hours of the CRU's knowledge of the occurrence. The CRU will either telephone the report to the Sponsor's Medical Monitor or transmit by email or facsimile a SAE Report to the Sponsor's Medical Monitor of the Sponsor's representative. If the initial report is made via telephone, a completed SAE report (SAER) must be emailed or faxed within 24 hours of the CRU's knowledge of the event. Investigational sites will be provided with the necessary forms.

The SAE information phoned/emailed/faxed to the Sponsor or the Sponsor's representative will include the following (as available):

- Subject identification, Investigator name

- SAE information: event term, onset date, severity, and casual relationship
- Basic demographic information (e.g., age, gender, weight)
- The outcomes attributable to the event (death, a life-threatening adverse drug experience, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, other important medical event[s])
- The first and last dates of IP administration, if applicable
- A statement whether IP was discontinued or IP administration schedule modified
- A statement whether event recurred after reintroduction of IP if administration had been discontinued or withheld
- Supplemental information may include the following hospital records: laboratory results, progress notes, admission and emergency room notes, observation notes, discharge summaries, autopsy reports, and death certificates, etc.

The SAER should be emailed/faxed within 24 hours with as much of the above information as available at the time. Supplemental information may be transmitted using a follow-up report and should not delay the initial report.

For regulatory purposes, initial reports of SAEs should be transmitted within the prescribed time frame as long as the following minimum information is available: subject identification, suspect IP, reporting source, and an event or outcome that can be identified as being both serious and unexpected for which the Investigator can make a relationship assessment.

The SAE report will be sent Dr Stephen Lambert, Medical Epidemiologist, Queensland Department of Health.

8 STATISTICS

8.1 Statistical Methods

This is a randomized, parallel arm, double-blind, placebo-controlled study designed to determine the safety, tolerability, and PK of mAb m102.4 in healthy subjects. No formal hypothesis tests will be conducted in this study. The statistical analysis will comprise summary descriptive statistics of the 5 dose groups and placebo group over the time points of observation. For continuous variables, the summary statistics will include: the number of observations, number of missing observations, mean, median, standard deviation, interquartile range, minimum and maximum. The interquartile range, minimum and maximum values will be presented to the same number of decimal places as recorded in the CRF. Mean, median and standard deviation will be presented to 1 more decimal place than the raw data. For binary or categorical variables the summary statistics will include frequency counts and percentages. Percentages will be rounded to 1 decimal place, with the denominator being the number of subjects in the relevant population, unless otherwise stated. All confidence intervals reported will be two-sided 95% confidence levels.

Subjects who withdraw from the study, for any reason, prior to study completion will have all data applied to planned analyses, regardless of treatment duration, up to the point of discontinuation.

Planned change from baseline analyses will utilize the last available assessment prior to drug administration as the baseline value. If the subject has a missing baseline assessment then that subject will be excluded from any change from baseline analysis.

All planned analyses will be completed using SAS Software® (version 9.1 or greater), unless otherwise specified.

A comprehensive statistical analysis plan will be completed that will describe the full details of prospective analyses for the study.

8.2 Sample Size

The sample size for this study was not based on statistical power calculation, but is consistent with the typical sample size used for similar studies to assess preliminary safety, PK and immunogenicity data. If a safety signal occurred in 25% of randomly selected subjects, then with 6 subjects in each dose cohort, the probability of observing the safety signal in at least one patient equals 0.82, which is judged to be adequate power.

8.3 Analysis Populations and/or Subsets

Safety Population: The Safety population shall consist of all randomized subjects who receive at least one dose of IP whether a complete dose or not. The Safety Population will be analyzed as treated regardless of the randomized treatment.

Pharmacokinetic Population: The PK population will comprise all randomized and dosed subjects who have sufficient observed plasma concentrations to allow calculation of the PK parameters. PK parameters will be calculated for mAb m102.4 based on the actual treatment received. A review of the plasma samples obtained will be completed by a pharmacokineticist to determine if sufficient samples exist for each subject to allow for computation of PK parameters. A listing will be provided of excluded subjects, with the reason for exclusion for the PK population.

8.4 Safety Analyses

Safety analyses will be conducted on the safety population and summarized by the 5 dose cohorts and placebo cohort.

AEs will be grouped by the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. The summary tables will present the number and percentage of subjects and number of events, by system organ class and preferred term. AE summaries will be by 5 dose cohorts and placebo cohort. For each subject, the maximum severity of each AE that is mapped to the same preferred term will be considered in the summaries. In addition, separate summaries will be provided for AEs by severity and relationship to IP. SAEs and AEs that lead to premature discontinuation or death will be listed separately.

Safety laboratory data (hematology, biochemistry, coagulation and urinalysis), vital signs, weight and ECG data will be summarized by time points and the 5 dose cohorts and placebo cohort. Shifts from normal to abnormal will be summarized for ECG, hematology, biochemistry, coagulation, selected urinalysis parameters and selected vital signs parameters.

Physical examination, and pregnancy test data may be listed only. All safety parameters will be presented for individual subjects in data listings.

8.5 PK Analyses

PK parameters will be calculated for each subject using actual sampling times and standard non-compartmental methods. In all calculations, concentrations below the quantification limit will be set to zero.

Individual and mean plasma mAb m102.4 concentrations at each sampling time point will be summarized in a graphical and tabular manner by dose cohort, and time. Individual and mean concentrations versus time plots will be depicted on linear and semi-logarithmic scales. Summary statistics (including means, geometric mean, ranges, standard deviation, and coefficients of variation) of mAb m102.4 plasma concentration by time point and PK parameters will be presented.

8.6 Missing Data

There will be no imputation for missing data.

8.7 Interim Analyses

PK data through Day 43 for Cohort 1 will be analyzed in at least 6 of the 8 subjects. Additionally, PK data for Cohorts 1-4 will be analyzed at the end of Cohort 4, before Cohort 5 will be dosed.

9 STUDY ADMINISTRATION

9.1 Ethical Considerations

9.1.1 Ethical Principles

This study will be conducted under a protocol reviewed by an Human Research Ethics Committee (HREC), the study is to be conducted by scientifically and medically qualified persons, the benefits of the study are in proportion to the risks, the rights and welfare of the subjects will be respected, the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits and the results to be reported will be accurate. The study will conform to ICH GCP guidelines, the ethical principles that have their origin in the Declaration of Helsinki, and all applicable regulatory requirements.

9.1.2 Informed Consent

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The Investigator or his/her designee will fully explain in layman's terms the nature of the study, along with the aims, methods, anticipated benefits, potential risks, and any discomfort participation may entail.

This consent form will be submitted with the protocol for review and approval by the HREC. The Investigator is responsible for obtaining the formal consent of a subject (competent to do so and not under duress), using the approved Informed Consent Form (ICF). Consent will be obtained before the subject undergoes any study procedure(s). This ICF must be signed by the subject or legally acceptable representative, and the Investigator-designated study personnel obtaining the consent. A copy of the signed ICF will be given to the subject and the original retained by the Investigator with the site's copy of the CRFs.

9.1.3 Human Research Ethics Committee Approval

The PI at the site is responsible for obtaining written HREC approval for the final protocol, Sponsor-approved ICF, subject information sheet and any advertisements to recruit subjects or tools used by subjects during the study. Written approval for these documents must be obtained from the committee before any subject is enrolled. The PI is also responsible for:

- Obtaining HREC approval for any protocol amendments and ICF revisions before implementing the changes.
- Providing the HREC with any required information before or during the study.

- Submitting progress reports to the HREC, as required, during the duration of the study.
- Notifying the HREC of all serious and unexpected AEs reported by the Sponsor.

9.2 Data Handling and Record Keeping

9.2.1 Data Collection and Reporting

The Investigator must maintain required documents for all study subjects. Data for this study will be recorded in the subject's source documents and on the CRFs. The source documents are to be separate and distinct from the CRFs, except for cases in which the Sponsor has predetermined that direct data entry into specified pages of the subject's CRF is appropriate.

All data on these CRFs should be recorded completely and promptly. A copy of the completed CRFs for each subject will be retained by the CRU.

9.2.2 Monitoring

All aspects of the study will be monitored carefully by the Sponsor's designees with respect to current GCP and standard operating procedures (SOPs) for compliance with applicable government regulations, the study protocol and Investigator's obligations. It is the responsibility of the Investigator to provide all study records, including CRFs, source documents, among other records, for review and inspection by the monitor.

All CRFs will be 100% source verified against corresponding source documentation for each subject. Monitors will periodically evaluate the progress of the study, including the verification of the accuracy and completeness of the CRFs. The completed CRFs must be promptly reviewed, signed and dated by a qualified physician who is an investigator on the study.

9.2.3 Study Records Retention

A file for each subject is maintained, and it includes the signed ICF and the copies of all source documents for that subject. Investigators are required to maintain all documentation, including documents in electronic format, for a minimum of 15 years following the end of the study. The Investigator must inform the Sponsor immediately if any documents are to be destroyed, transferred to a different facility or transferred to a different owner. The Sponsor will notify the site if the trial records are no longer needed.

The study may be selected for an audit to inspect the facilities and review the study-related records in order to evaluate the trial conduct and compliance with the protocol, ICH, GCP and applicable regulatory requirements.

9.3 Financing and Insurance

The study is fully financed by Queensland Department of Health.

The Sponsor has provided a Medicines Australia Form of Indemnity for Clinical Trials in the standard form. The Sponsor has agreed to indemnify and hold harmless Q-Pharm Pty Ltd and its employees, agents in respect of and against all claims and proceedings made or brought by or on behalf of Participants for personal injury to Participants arising out of or relating to the administration and/or use of the products under investigation or any clinical intervention or procedure provided for or required by the Protocol to which the Participants would not have been exposed but for the participation of the Participants in the Study. The indemnity provided by the Sponsor does not extend to any claim or proceeding to the extent that:

a) such personal injury is caused by the negligent or wrongful acts or omissions or breach of statutory duty by those indemnified; and
b) such personal injury is caused by the failure of Q-Pharm Pty Ltd, its employees or agents to conduct the Study in accordance with the Protocol.

If injury is suffered by a Participant while participating in the Study, the Sponsor agrees to adhere to the 'Guidelines for Compensation for Injury Resulting From Participation in a Company-sponsored Clinical Trial' published by Medicines Australia ('Guidelines').

The Sponsor will ensure that it maintains sufficient insurance to enable it to provide the indemnity referred to above and comply with the Guidelines.

9.4 Use of Information

All information not previously published concerning the test product and Queensland Department of Health is considered confidential and is the sole property of Queensland Department of Health. Site personnel agree to use this information only in connection with this study and will not use it for other purposes without written permission from Queensland Department of Health.

9.5 Data Disclosure and Subject Confidentiality

In order to maintain subject confidentiality, all CRFs, study reports and communications that relate to the study, will identify subjects by their initials and assigned subject numbers. Subjects will not be identified by name. The laboratory samples, forms, report or any other records that leave the site will only be identified by a code in order to maintain subject confidentiality. Records are to be kept in a locked facility.

The Investigator will allow the Sponsor or their designee's access to all pertinent records in order to verify the data gather on the CRFs. This access is in accordance to local, national and federal regulations. Regulatory authorities might also request access to the study records, including the source documents. Clinical information will not be released without the written permission of the subject as outlined in the subject consent form.

9.6 Publication Policy

It is agreed that before publication of any study-related data, Queensland Department of Health will be given the opportunity to review and comment upon any manuscript that contains data derived from this study.

10 REFERENCES

1 Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng Y-R, Brining D, Scott D, Wang Y, Dimitrov AS, Callison J, Chan Y-P, Hickey AC, Dimitrov DS, Broder CC and Rockx B. A Neutralizing Human Monoclonal Antibody Protects African Green Monkeys from Hendra Virus Challenge. *www.ScienceTranslationalMedicine.org. 2011; 3:105 (105ra103.doi:10.1126/scitranslmed.3002901)*

11 INVESTIGATOR'S AGREEMENT/SIGNATURE

I have read and understand the attached Final protocol entitled **A Phase I, Randomized, Double-blind, Placebo-controlled Study of the Safety, Tolerability, Pharmacokinetics and Immunogenicity of Various Dosing Regimens of Intravenous anti-Hendra Virus Antibody (mAb m102.4) in Healthy Subjects** dated, 25 August 2014 and agree to abide by all described protocol procedures. I agree to comply with:

- The World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects,
- The ICH Tripartite Guidelines on GCP,
- NHMRC National Statement on Ethical Conduct in Human Research (2007) - Updated December 2013,
- Local Institutional Review Board (IRB) guidelines and policies.

Principal Investigator Signature: (b) (6)

Principal Investigator Name: Dr. Geoffrey Playford

Date: 1/9/2014

From:	Sharon Johnatty on behalf of Sharon Johnatty <sharon@sugarapple.net.au>
To:	"Trent Munro"; "Stephen Mahler"; "Elliott, Suzanne"; "Michael GEROMETTA"; "Kym Hoger"; "Martina Jones"; "Griffin, Paul"; "Ms Kathleen Lynch"; "Heidi Carroll"; "Debra El Saadi"; "Lis Gilmour"; "Ben and Karen Hughes"; "Edwin Huang"; chris.debakker@servatus.com.au; "Klein, Reuben \(AAHL, Geelong AAHL\)"; "Mark Scher"; "Smith, Ina \(H&B, Black Mountain\)"; "Wang Linfa"; "Stephen Lambert"; "Dimitrov, Dimiter Stanchev"; "Peter Gray"; "Broder, Christopher"
Cc:	"Geoffrey Playford"
Subject:	FW: THELANCETID-D-19-01274
Date:	Friday, October 18, 2019 6:19:56 PM
Attachments:	THELANCETID-D-19-01274R1.pdf
Importance:	High

Dear authors

We've had positive feedback from the Lancet senior editor on our resubmitted HeV manuscript that was uploaded on Wednesday of this week - - see comments below. The resubmission which includes reviewers comments and our responses, is attached for your records. It is now with the editor-in-chief who hopefully will render a final decision soon.

Will keep you posted.

Kind regards

Sharon

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E sharon@sugarapple.net.au | www.sugarapple.net.au

From: Geoffrey Playford [mailto:Geoffrey.Playford@health.qld.gov.au]
Sent: Friday, 18 October 2019 12:12 PM
To: Sharon Johnatty
Subject: FW: THELANCETID-D-19-01274
Importance: High

Hi Sharon

Good news thus far.

Please forward to the entire authorship group.

Regards

Geoffrey

From: De Ambrogi, Marco (ELS-CAM) <marco.deambrogi@lancet.com>
Sent: Friday, 18 October 2019 1:48 AM
To: Geoffrey Playford <Geoffrey.Playford@health.qld.gov.au>
Subject: THELANCETID-D-19-01274
Importance: High

Dear Professor Playford,

I hope this email finds you well. I have received your revised version of the article THELANCETID-D-19-01274 “Safety, tolerability, pharmacokinetics, and immunogenicity of a human monoclonal antibody targeting the G glycoprotein of Henipaviruses in healthy adults: a randomised, first-in-human phase 1

study”. I just wanted to let you know that I am happy with the revision and I have passed now the paper to our Editor-in-Chief who needs to make his check.
I had asked you about the delay between trial and submission because we always need to ask when we notice this (there have been instances when major issues were disclosed after probing authors for this issue): I had no doubt that there was a good reason in your case and I want to reassure that the explanation is fine and I am very happy that you sent the paper to us.
Kind regards,

Marco

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June 2019

Development of Medical Countermeasures for Human Nipah Virus Infection: A Research and Development (R&D) Roadmap

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The work for this project was completed with generous support from the Wellcome Trust. Dr. de Wit is supported by the intramural research program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA.The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

The authors would also like to acknowledge Mr. Carlos R. Cruz; Center for Infectious Disease Research and Policy, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

June 2019

Word Count: 2,081(text only, not including References)

(Note: The word count will be below 2,000 once the references are in number format.)

Introduction

As part of implementing the World Health Organization’s (WHO’s)*R&D Blueprint for Action to Prevent Epidemics*(WHO 2016), in 2017 WHO embarked on a process to generate a research and development (R&D) roadmap for medical countermeasures (diagnostics, therapeutics, and vaccines) aimed at human infections caused by Nipah virus (NiV). WHO considers NiV a priority pathogen because of its disease severity, the potential for broad global spread of the virus, and the lack of available medical countermeasures. The entire roadmap can be found in the Supplemental Appendix and this report provides a summary of key highlights. NiV, a paramyxovirus of the Henipavirus genus,was first identified after an outbreak of respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chew 2000, Chua 1999, Chua 2010, Parashar 2000). No additional outbreaks have been reported in these countries; however, since 2001 outbreaks of NiV infection have occurred nearly annually in Bangladesh (Hsu 2004, WHO 2018). Outbreaks also have recently been recognized in India (Arunkumar 2019, Sharma 2018, Chattu 2018) and a possible NiV outbreak occurred in the Philippines in 2014 (Ching 2015).NiV infection is most commonly associated with severe, rapidly progressive encephalitis,which may be of late onset or relapsing (Goh 2000, Hossain 2008, Tan 2002). Some patients also have respiratory involvement or respiratory illness only, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The case-fatality rate is estimated at 40% to 75% (Hossain 2008, WHO 2019).

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Fruit bats of the Pteropus genus, which have a wide geographic range, are considered the primary natural reservoir for NiV. Evidence of NiV infection has been found in Pteropus bat

species and other bat species in South Asia, Southeast Asia, the Western Pacific region, and Africa(Anderson 2019, Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010,Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2019, Yob 2001). The wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing. Person-to-person transmission is well-documented (Arunkumar 2019, Chadha 2006,Gurley 2007, Luby 2009, Nikolay 2019) and respiratorytransmission via droplet spread may play an important role in propagating outbreaks (Nikolay 2019**;** Spiropoulou 2019). Concern has been raised about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Cross-Cutting Issues

A major challenge for development of NiV medical countermeasures is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Further efforts are needed to ensure that policy makers in affected countries and global stakeholders understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control. To accomplish this, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species (e.g., bats, swine, and equids) and proximate

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human populations in areas of predicted risk using a One Health approach (Chattu 2018, Kulkarni 2013).

Optimization of relevant animal modelsis needed, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval of therapeutics or vaccines(Rockx 2014). Issues include: (1) determining the most appropriate animal model(s) for screening assay development; (2) standardizing the challenge strain and dosing parameters (including identifying the most appropriate lethal challenge dose); (3) determining when after challenge medical countermeasures should be administered to best mimic realistic timing of use in humans; (4) bridging data from animal models to humans;and (5) identifying the best models for studying chronic (relapsing) infection(Dhondt 2013, Johnston 2015, Mathieu 2015, Mire 2019). Currently, the African green monkey is considered the most relevant animal model for evaluating candidate therapeutics and vaccines intended for human use (Bossart 2012, Geisbert 2010, Johnston 2015); however, costs, space requirements, and ethical concernsconstrain its use.

To inform NiV medical countermeasure development, additional researchalso is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. Important topics include evaluating pathophysiologic differences between different NiV strains, determining mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to infection.Standardized and well-characterized assays, reagents, antibodies, nucleic acids, and NiV challenge strains are also needed to advance research for medical countermeasures(Satterfield 2016).

Diagnostics

The most commonly used methods for diagnosing NiV infection includeenzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies and real time-polymerase chain reaction (RT-PCR) nucleic acid testing (Chadha 2006, Guillaume 2004,Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009, Mazzola 2019). Most laboratories use in-house NiV assays; also only a few commercial PCR kits are available and there is only one commercial source for reagents for ELISA testing (Mazzola 2019). More recently,neutralization assays using non-infectious pseudotyped virus particleshave been developed, which can be used under biosafety level 2 (BSL-2) conditions in laboratories with limited diagnostic capabilities(Bae 2019,Kaku 2009, Kaku 2012, Tamin 2009). Newer technologies are under investigation, such as antigen-capture ELISAs using monoclonal antibodies (Chiang 2010) and reverse transcription-loop-mediated isothermal amplification(RT-LAMP) (Ma 2019); however, more information is needed regarding their performance characteristics, particularly under field conditions.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012);thus, etiologic diagnosis is not always pursued. Rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure and are affordable are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samplesis needed to move NiV diagnostic testing forward. Development of a repository requires addressing complex issues such as determining the governance structure, establishing protocols for sample collection and maintenance, and prioritizing use and access to samples. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients. Comparison of outcomes using 54 historical controls found a lower mortality rate among treated patients (Chong 2001); however, findings may have been biased by use of historical controls.No additional clinical studies using ribavirin have been conducted and limited studies in animal models have not demonstrated efficacy following NiV or Hendra virus challenge(Georges-Courbot 2006, Rockx 2010). Ribavirin, however, may have utility for post-exposure prophylaxis (PEP) (Banerjee 2019).

A promising therapeutic option is monoclonal antibodies (mAbs) against viral envelope proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004).The human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009) and has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Other potential options for NiV treatment include favipiravir, fusion inhibitory peptides, and remdesivir (GS-5734). Initial animal model

studies involving these agents have reported favorable results (Dawes 2018, Mathieu 2018, Lo 2019); however, additional preclinical data are needed to further assess their utility.

Additional considerations for NiV therapeutics include: (1) expanding the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents; (2) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promisingtreatments;(3) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (4) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in endemic areas.

A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that occur each year. To address this concern, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how to best allocate scarce resources.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane

proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus,Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Keshwara 2019, Satterfield 2016).

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials) are not feasible.

Commented [Shahana3]: Also rapid diagnosis of the suspected cases

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized.Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012, Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research should focus primarily on the humoral immune response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful increating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

Anderson DE, Islam A, Crameri G, et al. Isolation and full-genome characterization of Nipah viruses from bats, Bangladesh. Emerg Infect Dis 2019 Jan;25(1):166-70 [Full text]

Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect DisJune 2019;219(12): 1867–1878 [Full text]

Bae SE, Kim SS, Moon ST, et al. Construction of the safe neutralizing assay system using pseudotyped Nipah virus and G protein-specific monoclonal antibody.BiochemBiophys Res Commun 2019 Jun 11;513(4):781-786; Epub 2019 Apr 15 [Abstract]

Banerjee S, Niyas VKM, Soneja M, et al. First experience of ribavirin postexposure prophylaxis for Nipah virus, tried during the 2018 outbreak in Kerala, India.J Infect. J Infect 2019 Jun;78(6):491-503. Epub 2019 Mar 6 [PubMed]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. SciTranslMed 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics.CurrOpinVirol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India.Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA.Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirusinfection in fruit bats (Pteropusgiganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. SciTransl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly($C_{12}U$) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. EmergInfec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats.PloS One 2008 Jul 23;3(7):e2739Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of Nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

HomairaN, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar.Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Keshwara R, Shiels T, Postnikova E, et al. Rabies-based vaccine induces potent immune responses against Nipah virus. NPJ Vaccines. 2019;4:15. Published online 2019 Apr 15 [Full text]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. SciTransl Med 2019 May 29;11(494):eaau9242 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007.Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh.Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Ma L, Chen Z, Guan W, et al. Rapid and specific detection of all known Nipah virus strains' sequences with reverse transcription-loop-mediated isothermal amplification. Front Microbiol. 2019;10:418 [Full text]

Mathieu C, Horvat B.Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. BMJ Global Health 2019;4:e001118 [Full text]

Mire CE, Geisbert JB, Agans KN, et al. Use of single-injection recombinant Vesicular Stomatitis virus vaccine to protect nonhuman primates against lethal Nipah virus disease. Emerg Infect Dis 2019 Jun;25(6):1144-52 [Full text]

Nikolay B, Salje H, Hossain MJ, et al. Transmission of Nipah virus – 14 years of investigations in Bangladesh. N Engl J Med 2019 May 9;380(19):1804-14[Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia.Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment.J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropusvampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou CF. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis Jun 2019;219(12):1855-57 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onsetNipahencephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand.Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top MicrobiolImmunol 2012;359:179-96 [Abstract]

WHO. An R&D Blueprint for Action to Prevent Epidemics. Plan of Action, May 2016 [Full text]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed June 10, 2019[Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

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Subject:	RE: Revised Nipah R&D roadmap manuscript--please respond by June 25
Date:	Friday, June 28, 2019 7:30:41 AM
Attachments:	Nipah Roadmap manuscript June 2019V3_Shahana.docx image001.png

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Dear Kris,

Thank you for sharing the revised draft.

I have attached some minor comments for your consideration.

I concur with the content of the roadmap, reviewed the draft and agree to be listed as a co-author on the manuscript.

Regards,
Shahana

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Cc: Kris Moore
Subject: FW: Revised Nipah R&D roadmap manuscript--please respond by June 25
Importance: High

Dear all. Please read the email below. This is just a reminder to send me any comments on the June 2019 version of the Nipah roadmap paper and to confirm that you are willing to be a co-author on the manuscript as outlined below.

Thank you so much for your time!

Warm regards,

Kris

From: Kris Moore [mailto:kamoore@umn.edu]
Sent: Saturday, June 15, 2019 1:26 PM
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Cc: kamoore@umn.edu; jto@umn.edu; mto@umn.edu; 'Anje Mehr'
Subject: Revised Nipah R&D roadmap manuscript--please respond by June 25
Importance: High

Dear Colleagues: I hope this message finds each of you well. Per the email that you received in May, I have provided an updated version of the Nipah R&D roadmap manuscript. I have also provided the full roadmap—just in case you want to refer back to that.

Our current plan is to submit this to the NEJM as a Sounding Board article per advice from Marie-Pierre. They will also provide a Supplemental Appendix with the full roadmap. WHO will then publish the roadmap once it has been published in NEJM.

Sounding Board articles have a 2,000 word limit and are structured as commentaries rather than as reviews, so the article has been substantially shortened—please keep that in mind as you go through it.

The process for submission is as follows:

1. Please review this version of the manuscript and **send any comments or edits to me by June 25** (through replying to this email). Please send a response even if you have no comments.
2. In your email response, also please state that you are willing to be a co-author on the manuscript and that you agree you fulfill the criteria for authorship, which for NEJM are:
    - Substantial contributions to conception and design; or acquisition, analysis, or interpretation of data AND
    - Drafting of the article or critical revision for important intellectual content AND
    - Final approval of the version to be published AND
    - Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved

3. If NEJM decides to request a revised version and move to the next step of accepting the manuscript, there will be three forms each co-author will need to complete—one on conflict of interest, one on copyright transfer, and one stating that you fulfill the authorship requirements. Those will be sent to you at a later time.
4. You will also need to sign off on the final version of the manuscript.

Again, thank you for your time in this important process.

Warm regards,

Kris

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June 2019

Development of Medical Countermeasures for Human Nipah Virus Infection: A Research and Development (R&D) Roadmap

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The work for this project was completed with generous support from the Wellcome Trust. Dr. de Wit is supported by the intramural research program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

The authors would also like to acknowledge Mr. Carlos R. Cruz; Center for Infectious Disease Research and Policy, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

June 2019

Word Count: 2,081 (text only, not including References)

(Note: The word count will be below 2,000 once the references are in number format.)

Introduction

As part of implementing the World Health Organization's (WHO's) *R&D Blueprint for Action to Prevent Epidemics* (WHO 2016), in 2017 WHO embarked on a process to generate a research and development (R&D) roadmap for medical countermeasures (diagnostics, therapeutics, and vaccines) aimed at human infections caused by Nipah virus (NiV). WHO considers NiV a priority pathogen because of its disease severity, the potential for broad global spread of the virus, and the lack of available medical countermeasures. The entire roadmap can be found in the Supplemental Appendix and this report provides a summary of key highlights. NiV, a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chew 2000, Chua 1999, Chua 2010, Parashar 2000). No additional outbreaks have been reported in these countries; however, since 2001 outbreaks of NiV infection have occurred nearly annually in Bangladesh (Hsu 2004, WHO 2018). Outbreaks also have recently been recognized in India (Arunkumar 2019, Sharma 2018, Chattu 2018) and a possible NiV outbreak occurred in the Philippines in 2014 (Ching 2015). NiV infection is most commonly associated with severe, rapidly progressive encephalitis, which may be of late onset or relapsing (Goh 2000, Hossain 2008, Tan 2002). Some patients also have respiratory involvement or respiratory illness only, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The case-fatality rate is estimated at 40% to 75% (Hossain 2008, WHO 2019).

Commented [MR1]: Disease severity can be measured by hospitalization rate, ICU care rate and CFR. In my opinion WHO considered it due to high CFR therefore, I would propose to use high CRF instead of Disease severity to be more specific.

Commented [MR2]: Currently, it is average 72% and may give rise to 100%.

Fruit bats of the Pteropus genus, which have a wide geographic range, are considered the primary natural reservoir for NiV. Evidence of NiV infection has been found in Pteropus bat

species and other bat species in South Asia, Southeast Asia, the Western Pacific region, and Africa (Anderson 2019, Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2019, Yob 2001). The wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing. Person-to-person transmission is well-documented (Arunkumar 2019, Chadha 2006, Gurley 2007, Luby 2009, Nikolay 2019) and respiratory transmission via droplet spread may play an important role in propagating outbreaks (Nikolay 2019**;** Spiropoulou 2019). Concern has been raised about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Cross-Cutting Issues

A major challenge for development of NiV medical countermeasures is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Further efforts are needed to ensure that policy makers in affected countries and global stakeholders understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control. To accomplish this, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species (e.g., bats, swine, and equids) and proximate

human populations in areas of predicted risk using a One Health approach (Chattu 2018, Kulkarni 2013).

Optimization of relevant animal models is needed, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval of therapeutics or vaccines (Rockx 2014). Issues include: (1) determining the most appropriate animal model(s) for screening assay development; (2) standardizing the challenge strain and dosing parameters (including identifying the most appropriate lethal challenge dose); (3) determining when after challenge medical countermeasures should be administered to best mimic realistic timing of use in humans; (4) bridging data from animal models to humans; and (5) identifying the best models for studying chronic (relapsing) infection (Dhondt 2013, Johnston 2015, Mathieu 2015, Mire 2019). Currently, the African green monkey is considered the most relevant animal model for evaluating candidate therapeutics and vaccines intended for human use (Bossart 2012, Geisbert 2010, Johnston 2015); however, costs, space requirements, and ethical concerns constrain its use.

To inform NiV medical countermeasure development, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. Important topics include evaluating pathophysiologic differences between different NiV strains, determining mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to infection. Standardized and well-characterized assays, reagents, antibodies, nucleic acids, and NiV challenge strains are also needed to advance research for medical countermeasures (Satterfield 2016).

Diagnostics

The most commonly used methods for diagnosing NiV infection include enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies and real time-polymerase chain reaction (RT-PCR) nucleic acid testing (Chadha 2006, Guillaume 2004, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009, Mazzola 2019). Most laboratories use in-house NiV assays; also only a few commercial PCR kits are available and there is only one commercial source for reagents for ELISA testing (Mazzola 2019). More recently, neutralization assays using non-infectious pseudotyped virus particles have been developed, which can be used under biosafety level 2 (BSL-2) conditions in laboratories with limited diagnostic capabilities (Bae 2019, Kaku 2009, Kaku 2012, Tamin 2009). Newer technologies are under investigation, such as antigen-capture ELISAs using monoclonal antibodies (Chiang 2010) and reverse transcription-loop-mediated isothermal amplification (RT-LAMP) (Ma 2019); however, more information is needed regarding their performance characteristics, particularly under field conditions.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. Rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure and are affordable are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of a repository requires addressing complex issues such as determining the governance structure, establishing protocols for sample collection and maintenance, and prioritizing use and access to samples. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients. Comparison of outcomes using 54 historical controls found a lower mortality rate among treated patients (Chong 2001); however, findings may have been biased by use of historical controls. No additional clinical studies using ribavirin have been conducted and limited studies in animal models have not demonstrated efficacy following NiV or Hendra virus challenge (Georges-Courbot 2006, Rockx 2010). Ribavirin, however, may have utility for post-exposure prophylaxis (PEP) (Banerjee 2019).

A promising therapeutic option is monoclonal antibodies (mAbs) against viral envelope proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). The human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009) and has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Other potential options for NiV treatment include favipiravir, fusion inhibitory peptides, and remdesivir (GS-5734). Initial animal model

studies involving these agents have reported favorable results (Dawes 2018, Mathieu 2018, Lo 2019); however, additional preclinical data are needed to further assess their utility.

Additional considerations for NiV therapeutics include: (1) expanding the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents; (2) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising treatments; (3) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (4) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in endemic areas.

A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that occur each year. To address this concern, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how to best allocate scarce resources.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane

proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Keshwara 2019, Satterfield 2016).

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012, Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research should focus primarily on the humoral immune

response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

Anderson DE, Islam A, Crameri G, et al. Isolation and full-genome characterization of Nipah viruses from bats, Bangladesh. Emerg Infect Dis 2019 Jan;25(1):166-70 [Full text]

Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis June 2019;219(12): 1867–1878 [Full text]

Bae SE, Kim SS, Moon ST, et al. Construction of the safe neutralizing assay system using pseudotyped Nipah virus and G protein-specific monoclonal antibody. Biochem Biophys Res Commun 2019 Jun 11;513(4):781-786; Epub 2019 Apr 15 [Abstract]

Banerjee S, Niyas VKM, Soneja M, et al. First experience of ribavirin postexposure prophylaxis for Nipah virus, tried during the 2018 outbreak in Kerala, India. J Infect. J Infect 2019 Jun;78(6):491-503. Epub 2019 Mar 6 [PubMed]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C_{12}U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of Nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Keshwara R, Shiels T, Postnikova E, et al. Rabies-based vaccine induces potent immune responses against Nipah virus. NPJ Vaccines. 2019;4:15. Published online 2019 Apr 15 [Full text]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med 2019 May 29;11(494):eaau9242 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Ma L, Chen Z, Guan W, et al. Rapid and specific detection of all known Nipah virus strains' sequences with reverse transcription-loop-mediated isothermal amplification. Front Microbiol. 2019;10:418 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. BMJ Global Health 2019;4:e001118 [Full text]

Mire CE, Geisbert JB, Agans KN, et al. Use of single-injection recombinant Vesicular Stomatitis virus vaccine to protect nonhuman primates against lethal Nipah virus disease. Emerg Infect Dis 2019 Jun;25(6):1144-52 [Full text]

Nikolay B, Salje H, Hossain MJ, et al. Transmission of Nipah virus – 14 years of investigations in Bangladesh. N Engl J Med 2019 May 9;380(19):1804-14 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou CF. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis Jun 2019;219(12):1855-57 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. An R&D Blueprint for Action to Prevent Epidemics. Plan of Action, May 2016 [Full text]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed June 10, 2019 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

From:	Mahmudur Rahman on behalf of Mahmudur Rahman <mahmudur57@gmail.com>
To:	Kris Moore
Cc:	christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; Emily Gurley; kim.halpin@csiro.au; Steve Luby; gnmillig@utmb.edu; dtmourya@gmail.com; Shahana Parveen; Linfa Wang; hana.weingartl@inspection.gc.ca; pierrerollin2019@gmail.com; Josie Golding; BENASSI, Virginia; PREZIOSI, Marie-pierre; jto@umn.edu; Michael Osterholm; Anje Mehr
Subject:	Re: Revised Nipah R&D roadmap manuscript--please respond by June 25
Date:	Tuesday, June 25, 2019 12:39:48 AM
Attachments:	Nipah Roadmap manuscript.June 2019,V3 comments Mahmudur Rahman.docx

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Dear Kris,

Greetings from Bangladesh !!!

Please find attached herewith the manuscript with my comments for your consideration. However, I concur with the content agree to be a co-author.

Best regards,

Mahmud

Professor Dr. Mahmudur Rahman
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On Sun, Jun 16, 2019 at 1:26 AM Kris Moore <kamoore@umn.edu> wrote:

> Dear Colleagues: I hope this message finds each of you well. Per the email that you received in May, I have provided an updated version of the Nipah R&D roadmap manuscript. I have also provided the full roadmap—just in case you want to refer back to that.
>
> Our current plan is to submit this to the NEJM as a Sounding Board article per advice from Marie-Pierre. They will also provide a Supplemental Appendix with the full roadmap. WHO will then publish the roadmap once it has been published in NEJM.
>
> Sounding Board articles have a 2,000 word limit and are structured as commentaries rather than as reviews, so the article has been substantially shortened—please keep that in mind as you go through it.
>
> The process for submission is as follows:
>
> 1. Please review this version of the manuscript and **send any comments or edits to me by**

June 25 (through replying to this email). Please send a response even if you have no comments.

2. In your email response, also please state that you are willing to be a co-author on the manuscript and that you agree you fulfill the criteria for authorship, which for NEJM are:

- Substantial contributions to conception and design; or acquisition, analysis, or interpretation of data AND
- Drafting of the article or critical revision for important intellectual content AND
- Final approval of the version to be published AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved

3. If NEJM decides to request a revised version and move to the next step of accepting the manuscript, there will be three forms each co-author will need to complete—one on conflict of interest, one on copyright transfer, and one stating that you fulfill the authorship requirements. Those will be sent to you at a later time.

4. You will also need to sign off on the final version of the manuscript.

Again, thank you for your time in this important process.

Warm regards,

Kris

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NipahResearch and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus infection.

*(**Note:**This draft roadmap is organized into the three main categories of MCMs—diagnostics, therapeutics, and vaccines—to allow reviewers an opportunity to examine the development of the roadmap components for each countermeasure. Some of the topics and issues apply to more than one MCM; these are highlighted as cross-cutting.This document is relatively detailed to ensure that it includes the most important topics and issues. As this draft is refined, the content may be reorganized and certain items may be "rolled up" into a smaller number of broader topics and issues, strategic goals, and priority areas and activities.)*

INTRODUCTION

Nipah virus (NiV)is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported inthese countries since May 1999.NiV infection was subsequently recognized, however,in Bangladeshin 2001 and nearly annual outbreaks have occurred in that country since, with disease also ~~occurring~~ being recognized periodically in eastern India; associated case fatality rates in these countries have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropusbatspecies)and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected human-to-human NiV transmission. Additionally, several other animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In theoutbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary NiV sources being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely

related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for cases. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, dizziness,cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated ~~that two distinct~~multiple strains of NiV. The strain responsible for the outbreak in Malaysia is different from the strains seen in ~~were responsible for the outbreaks in Southeast Asia (NiV$_M$) and in~~ Bangladesh/India (NiV$_B$).

Commented [SPL1]: We have isolated multiple different strains in Bangladesh.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease.

Commented [SPL2]: I recommend spelling out this acronym, so that readers don't need to learn a new acronym to understand the article.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, respond, prevent, and control outbreaks of NiV infection(and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING TOPICS AND ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value propositionfor industry and international philanthropic public-private partnershipsare needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs.

- Regulatory approval pathways for MCMs can be prohibitively expensive for product developers. For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore, licensure of vaccines and therapeutics using alternative regulatory pathways also can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions.
- To date, NiV spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh and East India; the healthcare facilities that serve these communities ~~have very rudimentary~~ are primary in nature in terms of laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.
- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses forlivestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Conducting phase 1 clinical trials, and possibly phase 2 clinical trials,is likely feasible in endemic regions. However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting phase 3 clinical trials for demonstrating efficacy. Therefore, alternative regulatory pathways and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time) may need to be

considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Key needs

- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- Standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research.
- A determination regardingthe feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection, which is needed before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities may vary between countries; therefore, early engagement is essential to identify country-specific considerations.
- Outreach and education to clinicians in endemic and at-risk areas to improve NiV awareness and training, and increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capabilities for data reporting in resource-limited settings to promote collaborative sharing of NiV sequence and strain data, which can be used to support MCM development.
- Collaboration with ~~local~~ government authorities in endemic ~~and at-risk areas~~countries to support NiV surveillance and facilitate effective communication with communities regarding disease prevention activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturingto endemic and at-risk areas.

Commented [Mahmudur3]: It will be necessary to involve the development partners who support the countries

Knowledge gaps

- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats.
- Additional research is needed torefine, standardize, and validate relevant animal challenge models(e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in

supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for development and evaluation of MCMs. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) determine the most appropriate lethal NiV dose for MCM development; (3)determine when MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.

- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between ~~NiV$_B$ and NiV$_M$ infection~~different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Some evidence suggests that there are differences in ~~NiV$_B$ and NiV$_M$~~ pathogenesis among different strains of NiV that may have implications for the development of safe and effective NiV vaccines or therapies.More information on this issue is needed.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hostsand pathogenicity.
- Additional research is needed to develop and assess next generation sequencing (NGS) for whole gene analysis, which can be used to enhance understanding of the global distribution of henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage at-risk populations (including vulnerable populations such as pregnant women, children, and immunocompromised persons) for participation in clinical trials and to ensure acceptance of new NiVMCMs, especially if therapeutics and vaccines do not consistently prevent disease. Efforts are needed to: (1) assess potential barriers for conducting clinical trials; (2) assess MCM acceptability in at-risk populations; (3) determine culturally appropriate messaging to enhance MCM acceptance; and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.
2. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.

3. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.
4. Engage NRAs (particularly in endemic and at-risk areas) to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

~~4.~~5.

Commented [Mahmudur4]: Consider adding "Enhance disease detection capabilities through active and passive surveillance"

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform**phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** the key differences between NiV$_{B}$ and NiV$_{M}$ pathogenesisthat may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate, and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research on the uses of NGS for understanding henipavirus distribution.
- **Determine** the feasibility of conducting phase 3 clinical trials or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Commented [SPL5]: As noted above, I do not consider this dichotomous framing appropriate. It has been used in the literature, but I see this as erroneous. There is not a single strain of NiV$_{B}$, but rather multiple.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote**early communication between developers and appropriate NRAs forclarity and guidance on the regulatory aspects ofMCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** surveillance capacityto: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiVsurveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorizationfrom appropriate NRAs for NiVMCMs.This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Clarify**the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Commented [SPL6]: This is a key component. The general description of research needs and capacity development, seem to me to be fairly complete, but a thoughtful consideration of priority in sequence could help direct development.

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection arenonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in instituting effective and timely infection control measures. Additionally, latent disease can occur long after initial infection.

- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using PCR and ELISA methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species)appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge forensuring diagnostic preparednessto respond tofuture outbreaks.

Commented [SPL7]: A related problem is that in many of the contexts where these patients present, etiologic diagnosis is often not pursued. We need more than an inexpensive sensitive and specific Nipah diagnostic. For this to be used clinicians need to see value in conducting the tests. Having a specific therapeutic treatment could help motivate this. A multiplex diagnostic tool that provided insight on other pathogens (especially if they were treatable) would also encourage more frequent testing.

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobankof human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course,are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- International reference standards to calibrate diagnostic assays.

- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the Differentiating Infected from Vaccinated Animals (DIVA) test.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detectionat various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Develop and assess affordable, highly sensitive and specific, point-of-care NiVdiagnostic tests for use in humans and animalsthat are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.
3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.

4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiVinfectionat different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.
- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protectionagainst lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials using both NiV$_M$ and NiV$_B$are needed to assess the safety, tolerability, and efficacyof m102.4 (and possibly other mAbs) for PEPand potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the mostpromising novel treatments (such as fusion inhibitory peptides, antifusion peptides, and GS-4734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV$_B$ and NiV$_M$, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV$_B$ to be shorter than for NiV$_M$.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- Additional studies of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer.This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies,diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines.

Commented [SPL8]: I suspect there is differences of opinion on this. On the one hand there is observational data from the Malaysian outbreak that NiV patients who received ribavirin had lower case fatality than NIV patients who did not. The unanswered question is whether this improved survival was a result of the ribavirin or whether it was primarily a result of ribavirin being used later in the outbreak once clinicians recognized the disease and possibly implemented better supportive care. The absence of impact on animal models suggests (but does not prove) that the improvement in human survival was due to confounding rather than biological effect. Given a fixed amount of resources and the opportunity cost of identifying patients who might benefit, I personally would not be particularly enthusiastic to conduct a phase III trial of ribavirin in Nipah patients in Bangladesh.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.
2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ribavirin and m102.4) for treating and preventing NiV infection, including conduct of animal studies andclinical trialsas appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infectionthat are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and $NiV_M$and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company to advance therapeutic use of m102.4 and **secure** financing for its manufacture and distribution.
- **Develop**guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human, veterinary, and public health systems, which could impact acceptance of NiV vaccinesfor use in humans or animals.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that can protect against both NiV_M and NiV_Bin humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiVvaccines (or broader henipavirus vaccines)to include vaccination strategies, potentially in both humans and animals, for different epidemiologic scenarios and different vaccine attributes.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategiesover time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines, such as the soluble G glycoprotein subunit (sG) and recombinant vesicular stomatitis (rVSV) vaccine.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoetis).
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B, including recent strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates of protection in humans and animals and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Evaluation of vaccine safety in target populations is needed to better understand the risk of adverse incidents associated with vaccine use.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than

one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.

- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines, estimating the vaccine quantity for stockpiles, and in simulating various epidemiologic scenarios and possiblestrategies for vaccine use.
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. While one HeV vaccine is available for horses, antibodies against HeV have not been found to be cross-reactive with NiV. Ongoing research into developing NiV/HeV vaccines for livestock(or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.
- Additional research is needed to determine if development of multivalent vaccines for animals(that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, and deployNiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection for use in developing and evaluatingNiV vaccines.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains,and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Determine** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effectivemonovalent NiV vaccines for humans and animals.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines for use in humans that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a worthwhile approach.

Key capacities

- **Improve**surveillance capabilities,particularly in rural areas of resource-limited settings, to assess the impact of vaccination strategies, once vaccines become available.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Policy and commercialization

- **Provide**guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoSNegl Trop Dis2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95[Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoSNegl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al.Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295.[Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE.A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B.Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE.Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. CurrOpinVirol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

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Subject: Re: Revised Nipah R&D roadmap manuscript--please respond by June 25
Date: Monday, June 24, 2019 2:48:44 PM
Attachments: Nipah Draft Roadmap.revised for taskforce review.April 2018MR sl.docx

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Hi Kris,

This looks great. I attach a couple minor suggestions.

I am willing to be listed as a co-author on the manuscript. I have fulfilled the criteria for authorship.

Steve

Steve

Kris Moore wrote on 6/15/2019 12:25 PM:

> Dear Colleagues: I hope this message finds each of you well. Per the email that you received in May, I have provided an updated version of the Nipah R&D roadmap manuscript. I have also provided the full roadmapï¿½just in case you want to refer back to that.
>
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>
> Our current plan is to submit this to the NEJM as a Sounding Board article per advice from Marie-Pierre. They will also provide a Supplemental Appendix with the full roadmap. WHO will then publish the roadmap once it has been published in NEJM.
>
> ï¿½
>
> Sounding Board articles have a 2,000 word limit and are structured as commentaries rather than as reviews, so the article has been substantially shortenedï¿½please keep that in mind as you go through it. ï¿½
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> The process for submission is as follows:
>
> <!--[if !supportLists]-->1.ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->Please review this version of the manuscript and **send any comments or edits to me by June 25** (through replying to this email). Please send a response even if you have no comments.
>
> <!--[if !supportLists]-->2.ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->In your email response, also please state that you are willing to be a co-author on the manuscript and that you agree you fulfill the criteria for authorship, which for NEJM are:

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->Substantial contributions to conception and design; or acquisition, analysis, or interpretation of data AND

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->Drafting of the article or critical revision for important intellectual content AND

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->Final approval of the version to be published AND

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved

<!--[if !supportLists]-->3.ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->If NEJM decides to request a revised version and move to the next step of accepting the manuscript, there will be three forms each co-author will need to completeï¿½one on conflict of interest, one on copyright transfer, and one stating that you fulfill the authorship requirements. Those will be sent to you at a later time.

<!--[if !supportLists]-->4.ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->You will also need to sign off on the final version of the manuscript.

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Again, thank you for your time in this important process.

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Warm regards,

ï¿½

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June 2019

Development of Medical Countermeasures for Human Nipah Virus Infection: A Research and Development (R&D) Roadmap

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The work for this project was completed with generous support from the Wellcome Trust. Dr. de Wit is supported by the intramural research program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

The authors would also like to acknowledge Mr. Carlos R. Cruz; Center for Infectious Disease Research and Policy, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

June 2019

Word Count: 2,081 (text only, not including References)

(Note: The word count will be below 2,000 once the references are in number format.)

Introduction

As part of implementing the World Health Organization's (WHO's) *R&D Blueprint for Action to Prevent Epidemics* (WHO 2016), in 2017 WHO embarked on a process to generate a research and development (R&D) roadmap for medical countermeasures (diagnostics, therapeutics, and vaccines) aimed at human infections caused by Nipah virus (NiV). WHO considers NiV a priority pathogen because of its disease severity, the potential for broad global spread of the virus, and the lack of available medical countermeasures. The entire roadmap can be found in the Supplemental Appendix and this report provides a summary of key highlights. NiV, a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chew 2000, Chua 1999, Chua 2010, Parashar 2000). No additional outbreaks have been reported in these countries; however, since 2001 outbreaks of NiV infection have occurred nearly annually in Bangladesh (Hsu 2004, WHO 2018). Outbreaks also have recently been recognized in India (Arunkumar 2019, Sharma 2018, Chattu 2018) and a possible NiV outbreak occurred in the Philippines in 2014 (Ching 2015). NiV infection is most commonly associated with severe, rapidly progressive encephalitis, which may be of late onset or relapsing (Goh 2000, Hossain 2008, Tan 2002). Some patients also have respiratory involvement or respiratory illness only, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The case-fatality rate is estimated at 40% to 75% (Hossain 2008, WHO 2019).

Fruit bats of the Pteropus genus, which have a wide geographic range, are considered the primary natural reservoir for NiV. Evidence of NiV infection has been found in Pteropus bat

species and other bat species in South Asia, Southeast Asia, the Western Pacific region, and Africa (Anderson 2019, Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2019, Yob 2001). The wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing. Person-to-person transmission is well-documented (Arunkumar 2019, Chadha 2006, Gurley 2007, Luby 2009, Nikolay 2019) and respiratory transmission via droplet spread may play an important role in propagating outbreaks (Nikolay 2019**;** Spiropoulou 2019). Concern has been raised about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Cross-Cutting Issues

A major challenge for development of NiV medical countermeasures is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Further efforts are needed to ensure that policy makers in affected countries and global stakeholders understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control. To accomplish this, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species (e.g., bats, swine, and equids) and proximate

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human populations in areas of predicted risk using a One Health approach (Chattu 2018, Kulkarni 2013).

Optimization of relevant animal models is needed, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval of therapeutics or vaccines (Rockx 2014). Issues include: (1) determining the most appropriate animal model(s) for screening assay development; (2) standardizing the challenge strain and dosing parameters (including identifying the most appropriate lethal challenge dose); (3) determining when after challenge medical countermeasures should be administered to best mimic realistic timing of use in humans; (4) bridging data from animal models to humans; and (5) identifying the best models for studying chronic (relapsing) infection (Dhondt 2013, Johnston 2015, Mathieu 2015, Mire 2019). Currently, the African green monkey is considered the most relevant animal model for evaluating candidate therapeutics and vaccines intended for human use (Bossart 2012, Geisbert 2010, Johnston 2015); however, costs, space requirements, and ethical concerns constrain its use.

To inform NiV medical countermeasure development, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. Important topics include evaluating pathophysiologic differences between different NiV strains, determining mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to infection. Standardized and well-characterized assays, reagents, antibodies, nucleic acids, and NiV challenge strains are also needed to advance research for medical countermeasures (Satterfield 2016).

Diagnostics

The most commonly used methods for diagnosing NiV infection include enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies and real time-polymerase chain reaction (RT-PCR) nucleic acid testing (Chadha 2006, Guillaume 2004, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009, Mazzola 2019). Most laboratories use in-house NiV assays; also only a few commercial PCR kits are available and there is only one commercial source for reagents for ELISA testing (Mazzola 2019). More recently, neutralization assays using non-infectious pseudotyped virus particles have been developed, which can be used under biosafety level 2 (BSL-2) conditions in laboratories with limited diagnostic capabilities (Bae 2019, Kaku 2009, Kaku 2012, Tamin 2009). Newer technologies are under investigation, such as antigen-capture ELISAs using monoclonal antibodies (Chiang 2010) and reverse transcription-loop-mediated isothermal amplification (RT-LAMP) (Ma 2019); however, more information is needed regarding their performance characteristics, particularly under field conditions.

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Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. Rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure and are affordable are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of a repository requires addressing complex issues such as determining the governance structure, establishing protocols for sample collection and maintenance, and prioritizing use and access to samples. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

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Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients. Comparison of outcomes using 54 historical controls found a lower mortality rate among treated patients (Chong 2001); however, findings may have been biased by use of historical controls. No additional clinical studies using ribavirin have been conducted and limited studies in animal models have not demonstrated efficacy following NiV or Hendra virus challenge (Georges-Courbot 2006, Rockx 2010). Ribavirin, however, may have utility for post-exposure prophylaxis (PEP) (Banerjee 2019).

A promising therapeutic option is monoclonal antibodies (mAbs) against viral envelope proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). The human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009) and has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Other potential options for NiV treatment include favipiravir, fusion inhibitory peptides, and remdesivir (GS-5734). Initial animal model

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studies involving these agents have reported favorable results (Dawes 2018, Mathieu 2018, Lo 2019); however, additional preclinical data are needed to further assess their utility.

Additional considerations for NiV therapeutics include: (1) expanding the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents; (2) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising treatments; (3) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (4) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in endemic areas.

A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that occur each year. To address this concern, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how to best allocate scarce resources.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane

proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Keshwara 2019, Satterfield 2016).

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012, Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research should focus primarily on the humoral immune

response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

Anderson DE, Islam A, Crameri G, et al. Isolation and full-genome characterization of Nipah viruses from bats, Bangladesh. Emerg Infect Dis 2019 Jan;25(1):166-70 [Full text]

Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis June 2019;219(12): 1867–1878 [Full text]

Bae SE, Kim SS, Moon ST, et al. Construction of the safe neutralizing assay system using pseudotyped Nipah virus and G protein-specific monoclonal antibody. Biochem Biophys Res Commun 2019 Jun 11;513(4):781-786; Epub 2019 Apr 15 [Abstract]

Banerjee S, Niyas VKM, Soneja M, et al. First experience of ribavirin postexposure prophylaxis for Nipah virus, tried during the 2018 outbreak in Kerala, India. J Infect. J Infect 2019 Jun;78(6):491-503. Epub 2019 Mar 6 [PubMed]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C_{12}U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of Nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Keshwara R, Shiels T, Postnikova E, et al. Rabies-based vaccine induces potent immune responses against Nipah virus. NPJ Vaccines. 2019;4:15. Published online 2019 Apr 15 [Full text]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med 2019 May 29;11(494):eaau9242 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Ma L, Chen Z, Guan W, et al. Rapid and specific detection of all known Nipah virus strains' sequences with reverse transcription-loop-mediated isothermal amplification. Front Microbiol. 2019;10:418 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. BMJ Global Health 2019;4:e001118 [Full text]

Mire CE, Geisbert JB, Agans KN, et al. Use of single-injection recombinant Vesicular Stomatitis virus vaccine to protect nonhuman primates against lethal Nipah virus disease. Emerg Infect Dis 2019 Jun;25(6):1144-52 [Full text]

Nikolay B, Salje H, Hossain MJ, et al. Transmission of Nipah virus – 14 years of investigations in Bangladesh. N Engl J Med 2019 May 9;380(19):1804-14 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou CF. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis Jun 2019;219(12):1855-57 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. An R&D Blueprint for Action to Prevent Epidemics. Plan of Action, May 2016 [Full text]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed June 10, 2019 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

From: Emily Gurley on behalf of Emily Gurley <egurley1@jhu.edu>
To: Kris Moore; christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; kim.halpin@csiro.au; sluby@stanford.edu; mahmudur57@gmail.com; dtmourya@gmail.com; shahana@icddrb.org; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca
Subject: Re: Revised Nipah R&D roadmap manuscript--please respond by June 25
Date: Monday, June 24, 2019 1:19:03 PM
Attachments: Nipah Roadmap manuscript.June 2019,V3 eg.docx

Kris,

Thanks for the update and very happy to see this work move ahead in a manuscript forma.

I've attached a couple of minor comments, in case they are useful.

I agree to coauthor this work and attest that I have made significant contributions to the content of the roadmap, its interpretations, and have contributed to and reviewed this draft.

Best,
Emily

From: Kris Moore <kamoore@umn.edu>
Sent: Sunday, June 23, 2019 3:10:33 PM
To: christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; Emily Gurley; kim.halpin@csiro.au; sluby@stanford.edu; mahmudur57@gmail.com; dtmourya@gmail.com; shahana@icddrb.org; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca
Cc: Kris Moore
Subject: FW: Revised Nipah R&D roadmap manuscript--please respond by June 25

Dear all. Please read the email below. This is just a reminder to send me any comments on the June 2019 version of the Nipah roadmap paper and to confirm that you are willing to be a co-author on the manuscript as outlined below.

Thank you so much for your time!

Warm regards,

Kris

From: Kris Moore [mailto:kamoore@umn.edu]
Sent: Saturday, June 15, 2019 1:26 PM
To: christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; egurley1@jhu.edu; kim.halpin@csiro.au; sluby@stanford.edu; gnmillig@utmb.edu; mahmudur57@gmail.com; dtmourya@gmail.com; shahana@icddrb.org; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca; pierrerollin2019@gmail.com; J.Golding@wellcome.ac.uk; benassiv@who.int; preziosim@who.int
Cc: kamoore@umn.edu; jto@umn.edu; mto@umn.edu; 'Anje Mehr'
Subject: Revised Nipah R&D roadmap manuscript--please respond by June 25
Importance: High

Dear Colleagues: I hope this message finds each of you well. Per the email that you received in May, I have provided an updated version of the Nipah R&D roadmap manuscript. I have also provided the full roadmap—just in case you want to refer back to that.

Our current plan is to submit this to the NEJM as a Sounding Board article per advice from Marie-Pierre. They will also provide a Supplemental Appendix with the full roadmap. WHO will then publish the roadmap once it has been published in NEJM.

Sounding Board articles have a 2,000 word limit and are structured as commentaries rather than as reviews, so the article has been substantially shortened—please keep that in mind as you go through it.

The process for submission is as follows:

1. Please review this version of the manuscript and **send any comments or edits to me by June 25** (through replying to this email). Please send a response even if you have no comments.
2. In your email response, also please state that you are willing to be a co-author on the manuscript and that you agree you fulfill the criteria for authorship, which for NEJM are:
    - Substantial contributions to conception and design; or acquisition, analysis, or interpretation of data AND
    - Drafting of the article or critical revision for important intellectual content AND
    - Final approval of the version to be published AND
    - Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved
3. If NEJM decides to request a revised version and move to the next step of accepting the manuscript, there will be three forms each co-author will need to complete—one on conflict of interest, one on copyright transfer, and one stating that you fulfill the authorship requirements. Those will be sent to you at a later time.
4. You will also need to sign off on the final version of the manuscript.

Again, thank you for your time in this important process.

Warm regards,

Kris

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June 2019

Development of Medical Countermeasures for Human Nipah Virus Infection: A Research and Development (R&D) Roadmap

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The work for this project was completed with generous support from the Wellcome Trust. Dr. de Wit is supported by the intramural research program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

The authors would also like to acknowledge Mr. Carlos R. Cruz; Center for Infectious Disease Research and Policy, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

Word Count: 2,081 (text only, not including References)

(Note: The word count will be below 2,000 once the references are in number format.)

Introduction

As part of implementing the World Health Organization's (WHO's) *R&D Blueprint for Action to Prevent Epidemics* (WHO 2016), in 2017 WHO embarked on a process to generate a research and development (R&D) roadmap for medical countermeasures (diagnostics, therapeutics, and vaccines) aimed at human infections caused by Nipah virus (NiV). WHO considers NiV a priority pathogen because of its disease severity, the potential for broad global spread of the virus, and the lack of available medical countermeasures. The entire roadmap can be found in the Supplemental Appendix and this report provides a summary of key highlights. NiV, a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chew 2000, Chua 1999, Chua 2010, Parashar 2000). No additional outbreaks have been reported in these countries; however, since 2001 outbreaks of NiV infection have occurred nearly annually in Bangladesh (Hsu 2004, WHO 2018). Outbreaks also have recently been recognized in India (Arunkumar 2019, Sharma 2018, Chattu 2018) and a possible NiV outbreak occurred in the Philippines in 2014 (Ching 2015). NiV infection is most commonly associated with severe, rapidly progressive encephalitis, which may be of late onset or relapsing (Goh 2000, Hossain 2008, Tan 2002). Some patients also have respiratory involvement or respiratory illness only, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The case-fatality rate is estimated at 40% to 75% (Hossain 2008, WHO 2019).

Fruit bats of the Pteropus genus, which have a wide geographic range, are considered the primary natural reservoir for NiV. Evidence of NiV infection has been found in Pteropus bat

species and other bat species in South Asia, Southeast Asia, the Western Pacific region, and Africa (Anderson 2019, Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2019, Yob 2001). The wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing. Person-to-person transmission is well-documented (Arunkumar 2019, Chadha 2006, Gurley 2007, Luby 2009, Nikolay 2019) and respiratory transmission via droplet spread may play an important role in propagating outbreaks (Nikolay 2019**;** Spiropoulou 2019). Concern has been raised about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Cross-Cutting Issues

A major challenge for development of NiV medical countermeasures is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Further efforts are needed to ensure that policy makers in affected countries and global stakeholders understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control. To accomplish this, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species (e.g., bats, swine, and equids) and proximate

human populations in areas of predicted risk using a One Health approach (Chattu 2018, Kulkarni 2013).

Optimization of relevant animal models is needed, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval of therapeutics or vaccines (Rockx 2014). Issues include: (1) determining the most appropriate animal model(s) for screening assay development; (2) standardizing the challenge strain and dosing parameters (including identifying the most appropriate lethal challenge dose); (3) determining when after challenge medical countermeasures should be administered to best mimic realistic timing of use in humans; (4) bridging data from animal models to humans; and (5) identifying the best models for studying chronic (relapsing) infection (Dhondt 2013, Johnston 2015, Mathieu 2015, Mire 2019). Currently, the African green monkey is considered the most relevant animal model for evaluating candidate therapeutics and vaccines intended for human use (Bossart 2012, Geisbert 2010, Johnston 2015); however, costs, space requirements, and ethical concerns constrain its use.

To inform NiV medical countermeasure development, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. Important topics include evaluating pathophysiologic differences between different NiV strains, determining mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to infection. Standardized and well-characterized assays, reagents, antibodies, nucleic acids, and NiV challenge strains are also needed to advance research for medical countermeasures (Satterfield 2016).

Diagnostics

The most commonly used methods for diagnosing NiV infection include enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies and real time-polymerase chain reaction (RT-PCR) nucleic acid testing (Chadha 2006, Guillaume 2004, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009, Mazzola 2019). Most laboratories use in-house NiV assays; also only a few commercial PCR kits are available and there is only one commercial source for reagents for ELISA testing (Mazzola 2019). More recently, neutralization assays using non-infectious pseudotyped virus particles have been developed, which can be used under biosafety level 2 (BSL-2) conditions in laboratories with limited diagnostic capabilities (Bae 2019, Kaku 2009, Kaku 2012, Tamin 2009). Newer technologies are under investigation, such as antigen-capture ELISAs using monoclonal antibodies (Chiang 2010) and reverse transcription-loop-mediated isothermal amplification (RT-LAMP) (Ma 2019); however, more information is needed regarding their performance characteristics, particularly under field conditions.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. Rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure and are affordable are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of a repository requires addressing complex issues such as determining the governance structure, establishing protocols for sample collection and maintenance, and prioritizing use and access to samples. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients. Comparison of outcomes using 54 historical controls found a lower mortality rate among treated patients (Chong 2001); however, findings may have been biased by use of historical controls. No additional clinical studies using ribavirin have been conducted and limited studies in animal models have not demonstrated efficacy following NiV or Hendra virus challenge (Georges-Courbot 2006, Rockx 2010). Ribavirin, however, may have utility for post-exposure prophylaxis (PEP) (Banerjee 2019).

A promising therapeutic option is monoclonal antibodies (mAbs) against viral envelope proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). The human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009) and has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Other potential options for NiV treatment include favipiravir, fusion inhibitory peptides, and remdesivir (GS-5734). Initial animal model

studies involving these agents have reported favorable results (Dawes 2018, Mathieu 2018, Lo 2019); however, additional preclinical data are needed to further assess their utility.

Additional considerations for NiV therapeutics include: (1) expanding the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents; (2) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising treatments; (3) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (4) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in endemic areas.

A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that occur each year. To address this concern, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how to best allocate scarce resources.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane

proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Keshwara 2019, Satterfield 2016).

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012, Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research should focus primarily on the humoral immune

response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

Anderson DE, Islam A, Crameri G, et al. Isolation and full-genome characterization of Nipah viruses from bats, Bangladesh. Emerg Infect Dis 2019 Jan;25(1):166-70 [Full text]

Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis June 2019;219(12): 1867–1878 [Full text]

Bae SE, Kim SS, Moon ST, et al. Construction of the safe neutralizing assay system using pseudotyped Nipah virus and G protein-specific monoclonal antibody. Biochem Biophys Res Commun 2019 Jun 11;513(4):781-786; Epub 2019 Apr 15 [Abstract]

Banerjee S, Niyas VKM, Soneja M, et al. First experience of ribavirin postexposure prophylaxis for Nipah virus, tried during the 2018 outbreak in Kerala, India. J Infect. J Infect 2019 Jun;78(6):491-503. Epub 2019 Mar 6 [PubMed]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C_{12}U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of Nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Keshwara R, Shiels T, Postnikova E, et al. Rabies-based vaccine induces potent immune responses against Nipah virus. NPJ Vaccines. 2019;4:15. Published online 2019 Apr 15 [Full text]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Feldmann F, Gary JM, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med 2019 May 29;11(494):eaau9242 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Ma L, Chen Z, Guan W, et al. Rapid and specific detection of all known Nipah virus strains' sequences with reverse transcription-loop-mediated isothermal amplification. Front Microbiol. 2019;10:418 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Mazzola LT, Kelly-Cirino C. Diagnostics for Nipah virus: a zoonotic pathogen endemic to Southeast Asia. BMJ Global Health 2019;4:e001118 [Full text]

Mire CE, Geisbert JB, Agans KN, et al. Use of single-injection recombinant Vesicular Stomatitis virus vaccine to protect nonhuman primates against lethal Nipah virus disease. Emerg Infect Dis 2019 Jun;25(6):1144-52 [Full text]

Nikolay B, Salje H, Hossain MJ, et al. Transmission of Nipah virus – 14 years of investigations in Bangladesh. N Engl J Med 2019 May 9;380(19):1804-14 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou CF. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis Jun 2019;219(12):1855-57 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. An R&D Blueprint for Action to Prevent Epidemics. Plan of Action, May 2016 [Full text]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed June 10, 2019 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs)—diagnostics, therapeutics, and vaccines—against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (*Pteropus* species and several other bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species (including horses, cats, and dogs) were found to be infected with NiV on the farms involved in the outbreak. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen—Hendra virus (HeV)—which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat; disease also occurred in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

NiV infection in humans results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India. Some differences have been noted in the clinical features of infection, with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of MCMs to enable effective and timely emergency response to infectious disease epidemics. NiV infection is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this roadmap ranges from basic research to late-stage development of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include minimizing zoonotic NiV transmission, improving use of personal protective equipment (PPE), ensuring adequate hand hygiene and environmental hygiene, promoting effective community engagement, implementing adequate infection prevention and control practices, developing adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and promoting workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap but need to be addressed as part of a broader public health control strategy. Further research of NiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV (or related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

VISION

Ready availability and accessibility of r~~R~~**obust MCMs to detect, prevent, and control human outbreaks of NiV infection (and other closely related henipaviruses)** ~~that are readily available and accessible~~ **for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.**

CROSS-CUTTING ISSUES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research represents a substantial challenge, since economic incentives to invest in Nipah research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with small and sporadic outbreaks. The development of a sustainable value proposition for industry and international philanthropic public-private partnerships is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate.
- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (biosafety level 4 [BSL-4]) conditions, which can increase the cost of MCM development.
- To date, NiV spillovers to human communities have been identified most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the *Pteropus* genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to the lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- While ferrets, Syrian hamsters, and interferon-alpha/beta receptor knockout (IFNAR-KO) mice are well-established animal models for NiV research, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection occurs in relatively small, focal outbreaks, the low disease incidence poses a major

challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop and manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries and to global stakeholders to ensure they understand the potential health, societal, and economic benefits of devoting resources to improving NiV surveillance, detection, prevention, and control measures.
- Obtaining additional prospective serosurveillance data of henipavirus exposure from susceptible animal species and proximate human populations in areas of predicted risk should be explored as a strategy to assess the potential of human spillover and to build preparedness for detection of human cases and for limiting exposure.
- Standardized and well-characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains for R&D of MCMs for NiV infection. Assays that can be used at lower biosafety levels are an important priority. WHO international standards should be used (when available) as calibrators and reported in units/ml to harmonize assay results.
- Clear criteria for downselection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or other regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.

- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, depend on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of *Pteropus* bats and potentially other bat species.
- Additional research is needed to optimize relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) for promoting development and evaluation of MCMs, particularly if investigators are required to use an alternative pathway (such as the United States Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after-challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunologic clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurologic sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes that may affect the epidemiologic and clinical features of disease over time and thereby influence MCM development.

- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole-genome sequencing of NiV are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume date palm sap, healthcare workers, and workers at the human-animal interface) and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains to facilitate R&D of NiV MCMs.

2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and that may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.
- **Generate** research tools to promote R&D of MCMs for NiV infection (e.g., standardized and validated assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability of henipaviruses to improve understanding of their global distribution.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for downselection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.

- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an "affordable and fair" price is one that can reasonably be paid by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

DIAGNOSTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific, and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and investigation into the cause of infection is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.

- Owing to the biosafety precautions necessary when working with NiV, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods is problematic, given the rapid disease progression of NiV infection.
- Currently, no approved tests for diagnosis of NiV infection are commercially available.
- *Pteropus* species (and perhaps other bat species) appear to carry other henipaviruses in addition to NiV and HeV, some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate using serologic assays which henipaviruses are in circulation. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.
- A TPP for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples to assess and validate diagnostic tests. As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6) identify who would have access to the samples; (7) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature), and have a high sensitivity and specificity for different NiV strains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

- Improved diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, particularly given the costs of regulatory approval.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotype neutralization assays and antigen-capture enzyme-linked immunosorbent assays [ELISAs]) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries: Bangladesh and India.

2. By 2021, identify funding and initiate creation of the virtual reference repository in Bangladesh and India, with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.

2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnose NiV infection during future outbreaks.

Priority Areas/Activities

Research

- **Create** a virtual repository of clinical reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- **Develop and evaluate** point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed.

Policy and commercialization

- **Develop** guidance on optimal strategies for the deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

THERAPEUTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment of patients and PEP for exposed persons.
- In NiV-endemic areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis but do not have NiV infection. Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore, accurate and rapid diagnosis is critical.
- Studies in animals often evaluate the usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well a therapeutic agent will work in the field.
- NiV can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control– programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.
- A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that are detected annually.

Key needs

- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas, particularly during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).
- A transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials, given the limited number of cases that could potentially be enrolled in trials each year.

Knowledge gaps

- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to further assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed that the therapeutic window for m102.4 against a strain from Bangladesh/India was shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection and strengthen the therapeutic pipeline.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship of promising therapeutic candidates.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will affect the response to therapeutic candidates and results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials to inform best-practice guidelines and evidence-based policy decisions.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
3. By 2019, identify an approach for downselecting promising therapeutic candidates for further study in clinical trials, given limited annual case numbers for enrollment.
4. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
5. By 2020, generate a reliable source of m102.4 (or other promising agent[s] or agent combinations) to be ~~used~~ assessed in clinical trials.

Strategic Goal 2: Develop and evaluate therapeutic agents for the treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as m102.4 and favipiravir) for treating and preventing NiV infection, including conducting studies in animal models and clinical trials as appropriate and feasible.
- **Continue to expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Develop, evaluate, and license** safe and effective therapeutic agents for the treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in affected areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- **Generate** a reliable source of m102.4 to be used in clinical trials.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

VACCINES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development, and few companies are willing to invest in the generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could diminish the acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for the timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on the use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA’s Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen, and therefore multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed involving target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations and identify additional areas of potential NiV spillover.
- Additional sociological research is needed to explore perceptions and concerns of at-risk populations regarding NiV vaccine implementation.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the need for licensure and future use of NiV vaccines. This consultation should include representatives from in-country regulators, other regulatory agencies (such as the FDA or the European Medicines Agency), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of the humoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serologic assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.

- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Evaluate** the feasibility of generating multivalent vaccines (i.e., that protect against more than one disease) and determine whether or not such vaccines would be useful in future NiV control efforts.

Product development

- **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptance and promotion within the community.
- **Consider** developing a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for faster spread.

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

WHO. Nipah baseline situational analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017 [Full text]

Other Publications:

Arunkumar G, Chandni R, D T Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Med 2016;9(7):630-4 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 29 Nov 2016; site accessed 31 October 2018 [Web page]

Baseler L, Scott DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov 3;10(11):e0005120 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Pathog 2009 Oct 30;5(10):e1000642 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (US Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 20 Mar 2014 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2):235–240 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-283 [Full text]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of infectious diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PKG, de los Reyes V, Sucaldito M, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015 Feb;21(2):328-31 [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. Malaysian J Pathol 2010; 32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microb Infect 2013 Jun;2(6):e37 [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Curr Opin Virol 2017 Feb;22:97-104 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8:7604 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024 [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Abstract]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global virology I — identifying and investigating viral diseases. New York, NY: Springer US; 2015:125-46

Deka MA, Morshed N. Mapping disease transmission risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2).pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug;14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-7 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37. [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The role of animals in emerging viral diseases. Amsterdam: Elsevier; 2014:293-315 [Abstract]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis. 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2):e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lancet editors. Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137):2295 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55. [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013 Oct;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug; 15(8): 1229–1235 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015 Mar;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-227 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis. 2014;20:372–9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;6:30916 [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13;10:353 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9. Epub 2000 May 15 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sep 1;157(9):1635-41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Rahman SA. National guideline for management, prevention and control of Nipah virus infection including encephalitis. Dec 2011 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-4 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Schmidt R, Beltzig LC, Sawatsky B, et al. Generation of therapeutic antisera for emerging viral infections. NPJ Vaccines 2018 Oct 5;3:42 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013; 8(7): e69544. Published online 2013 Jul 22 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Epub ahead of print]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Steffen DL, Xu K, Nikolov DB, et al. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Sun B, Jia L, Liang B, et al. Phylogeography, transmission, and viral proteins of Nipah virus. Virol Sin 2018; Oct 11; Epub ahead of print [Full text]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

Yu J, Lv X, Yang Z, et al. The main risk factors of Nipah disease and its risk analysis in China. Viruses 2018 Oct 19;10(10). pii:E572 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, influenza, Nipah, and Semliki Forest chimeric viruses with vesicular stomatitis virus: actions in the brain. J Virol 2017 Feb 28;91(6):e02154-16 [Full text]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah Virus, Thailand. Emerg Infect Dis 2005 Dec; 11(12):1949–1951 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

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Cc:	Kris Moore
Subject:	FW: Revised Nipah R&D roadmap manuscript--please respond by June 25
Date:	Sunday, June 23, 2019 3:11:07 PM
Attachments:	Nipah Roadmap manuscript.June 2019,V3.docx Nipah RD Roadmap.January2019.docx
Importance:	High

Dear all. Please read the email below. This is just a reminder to send me any comments on the June 2019 version of the Nipah roadmap paper and to confirm that you are willing to be a co-author on the manuscript as outlined below.

Thank you so much for your time!

Warm regards,

Kris

From: Kris Moore [mailto:kamoore@umn.edu]
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Cc: kamoore@umn.edu; jto@umn.edu; mto@umn.edu; 'Anje Mehr'
Subject: Revised Nipah R&D roadmap manuscript--please respond by June 25
Importance: High

Dear Colleagues: I hope this message finds each of you well. Per the email that you received in May, I have provided an updated version of the Nipah R&D roadmap manuscript. I have also provided the full roadmap—just in case you want to refer back to that.

Our current plan is to submit this to the NEJM as a Sounding Board article per advice from Marie-Pierre. They will also provide a Supplemental Appendix with the full roadmap. WHO will then publish the roadmap once it has been published in NEJM.

Sounding Board articles have a 2,000 word limit and are structured as commentaries rather than as reviews, so the article has been substantially shortened—please keep that in mind as you go through it.

The process for submission is as follows:

1. Please review this version of the manuscript and **send any comments or edits to me by June 25** (through replying to this email). Please send a response even if you have no comments.

2. In your email response, also please state that you are willing to be a co-author on the manuscript and that you agree you fulfill the criteria for authorship, which for NEJM are:

    - Substantial contributions to conception and design; or acquisition, analysis, or interpretation of data AND
    - Drafting of the article or critical revision for important intellectual content AND
    - Final approval of the version to be published AND
    - Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved

3. If NEJM decides to request a revised version and move to the next step of accepting the manuscript, there will be three forms each co-author will need to complete—one on conflict of interest, one on copyright transfer, and one stating that you fulfill the authorship requirements. Those will be sent to you at a later time.
4. You will also need to sign off on the final version of the manuscript.

Again, thank you for your time in this important process.

Warm regards,

Kris

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Wolters Kluwer Health: Medical Practice

Chapter 14

--Manuscript Draft--

Manuscript Number:	
Component Type:	Chapter with features, contributor agreement WITHOUT honorarium
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Chapter 14

<TITLE>14 Henipaviruses

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<H1>History

Hendra virus (HeV), the first known member of the genus *Henipavirus* in the family *Paramyxoviridae*, came to light in September 1994 as the causative agent of a sudden outbreak of acute respiratory disease in thoroughbred horses at a stable in Brisbane, Australia. A total of 21 horses and 2 humans (a horse trainer and a stable hand) became infected. The horse trainer and 14 horses died.(1,2) A virus was isolated, initially called *Equine Morbillivirus* but later re-named *Hendra virus* after the Brisbane suburb where the outbreak occurred. A second person died from HeV infection 13 months after the Brisbane outbreak, a farmer from Mackay, nearly 1000 km north of Brisbane. Unlike the first case, however, the man succumbed to encephalitis caused by HeV infection14 months after his initial mild meningitic illness.(3) (4). After initial serologic evidence suggested that fruit bats (flying foxes) of the genus *Pteropus* in the suborder Yinpterochiroptera were the reservoir hosts,(5) HeV was isolated from two species of flying fox.(6) In total, there have been 60 recognized occurrences of HeV in Australia between 1994–2018, with at least one occurrence per year since 2006 (Table 14.1A-online).(7) Every occurrence of HeV has involved horses as the initial infected host, causing lethal respiratory disease and/or encephalitis, along with a total of seven human cases arising from exposure to infected horses, among which four have been fatal and the most recent in 2009 (Table 14.1B-online).(8)

Nipah virus (NiV), the second known member of the genus *Henipavirus,* emerged as the cause of an outbreak of disease in pigs and humans in Peninsular Malaysia in 1998 through 1999. The epidemic started in Perak State as clusters of cases of encephalitis among pig farmers. It was initially believed to be caused by Japanese encephalitis virus; however, various features of the outbreak, including a high proportion of cases in direct contact with pigs and illness and deaths in pigs, differed from those expected with Japanese encephalitis.(9) Indeed, respiratory illness and encephalitis in pigs preceded human cases in the same district.(10) The epidemic spread south to the intensive pig-farming areas of Negeri Sembilan in December 1998 and subsequently peaked between February and April 1999. More than 1 million pigs were destroyed to halt the spread of the epidemic, and by late May, 265 human cases of acute encephalitis with 105 deaths were recorded.(9,11) A cluster of 11 cases with 1 death occurred among abattoir workers in Singapore.(12) In early March 1999, a virus was isolated from the cerebrospinal fluid (CSF) of a patient with encephalitis and identified as the etiologic agent.(9,11) Named *Nipah virus* after the village from which the patient had come, it was shown to be closely related to HeV. NiV was subsequently isolated from the urine of Malaysian flying foxes.(13) A highly related NiV emerged in Bangladesh in 2001,(14) and outbreaks of NiV-related encephalitis have occurred in people from that country almost every year since, along with three reports of NiV encephalitis in India(15,16), albeit in Bangladesh adjacent regions. However, the most recent outbreak of NiV occurred in 2018 in Kerala (India), more than 2,500 km south-west of NiV-endemic Bangladesh counties. This outbreak claimed 21 lives out of 23 infected human cases.(17) In 2014, a NiV outbreak occurred in the Province of Sultan Kudarat, the Phillipines, which resulted in the death of 9 humans from 11 which showed an acute encephalitis syndrome.(18) The human case fatality rate of these NiV outbreaks averages around 60% (Table 14.1C). So far human NiV oubreaks have been documented in a total of five countries: Malaysia, Singapore, Bangladesh, India and the Philippines. Sero-epidemiological evidence for henipavirus spillover events from bats into high-risk human populations have also been reported in Cameroon.(19)

<TAB14.1C>

<H1>Infectious Agent

<H2>Classification

When HeV was first isolated in 1994, partial sequencing of the matrix gene (M) revealed that it most closely resembled members of the genus *Morbillivirus* in the subfamily

Paramyxovirinae.(2) Subsequent characterization of the full-length genome, however, revealed that many of the genetic features of HeV were unique among paramyxoviruses and that the virus did not fit within any of the existing genera at that time.(20,21) After the isolation of NiV in 1999, it was shown that sera raised against HeV were able to neutralize NiV and vice versa, and that both viruses shared a high degree of similarity in genome organization and protein size and sequence.(11,22-24) In 2002, the genus *Henipavirus* was created to accommodate these novel paramyxoviruses, and HeV was designated the type species.(25) Since 1994, there have been 38 isolations of HeV or NiV from humans, bats, horses, and pigs over a wide geographic area and spanning a period of approximately 20 years (Table 14.2-online). The susceptibility of humans, the virulence of the viruses, and absence of therapeutics and vaccines led to classification of HeV and NiV as biosafety level 4 (BSL4) pathogens. In the latest report from the paramyxovirus study group under the order Mononegavirales, the genus *Henipavirus* has been expanded to include three new species,(26,27) they are *Cedar henipavirus* (Cedar virus, CedV) isolated from bats in Australia, (28) *Ghanaian bat henipavirus* (Ghana virus, GhV) detected in bats from Ghana, (29) and *Mojiang henipavirus* (Mòjiāng, MojV) detected in rats in China.(30) These three new species are highly divergent from NiV and HeV, and only CedV has been isolated. GhV and MojV are only known from sequence information. *Henipavirus* is now classified as one of the seven ICTV-approved genera in the family *Paramyxoviridae*.

<H2>Propagation in Cell Culture and Cytopathic Effect

The ultrastructural characteristics of henipavirus-infected cells resemble those found in cells infected by other members of the *Paramyxoviridae.* Shared features include generation of large syncytia and the presence of viral nucleocapsids in cytoplasmic inclusion bodies and underlying electron-dense areas of the plasma membrane.(31,32) In Vero cells, NiV-induced syncytia are significantly larger than those generated by HeV and nuclei and nucleocapsids are frequently located at the cell periphery, compared with HeV-induced syncytia, where they tend to be more centrally located or distributed randomly throughout the cytoplasm (Fig. 14.1). Henipavirus-infected cells also contain structures that are not seen with other paramyxoviruses—specifically a network of membrane-like reticular structures in the cytoplasm and long tubules that appear to be continuous with the plasma membrane in NiV-infected cells. Tubules can also be observed in NiV virions (Fig. 14.2A). *In situ* hybridization suggests that these reticular structures contain viral RNA and may play a role in viral transcription.(31)

<FIG14.1><FIG14.2>

<H2>Virus Morphology

Henipavirus particles are pleomorphic, varying from spherical to filamentous and ranging in size from 40 to 1,900 nm. (2,31,32) Nucleocapsids have a diameter of 18 to 19 nm with an average pitch of 5 nm. When examined by electron microscopy (EM), HeV has a unique double-fringed appearance, caused by the presence of surface projections 15 ± 1 nm and 8 ± 1 nm in length (see Fig. 14.2B). Approximately 95% of virions contain the double fringe, and the remaining 5% display a uniform fringe length of 15 ± 1 nm. Unlike HeV, NiV possesses a single layer of surface projections with an average length of 17 ± 1 nm, and NiV particles released into the culture medium are difficult to image because they are routinely penetrated by negative stains. This suggests that the viruses may differ in the physical nature of their envelope.(32)

<H2>Genome Length and Organization

In the family *Paramyxoviridae,* the genome length of all characterized viruses is divisible by six, an observation caused by the requirement of each N protein in the viral ribonucleoprotein to bind 6 nucleotide (nt) residues (see Chapter 13). This is also true for HeV and NiV despite their much larger genome sizes.(24) The genomes of the Malaysian (MY) and Bangladesh (BD) strains of NiV differ by 6 nt because of a 6-nt increase in the 3′ untranslated region of the *F* gene in NiV-BD.(33) A minigenome replicon study confirms that NiV complies with the rule of six.(34) When the complete genome sequence of HeV was determined, its length (18,234 nt) was more than 2,700 nt, or 15% longer than the genomes of all other paramyxoviruses known at that time.(21) The size of NiV genomes at 18,246 nt to 18,252 nt are slightly larger than that of HeV.(22,23,33) These large genome sizes of >18 kb is a conserved feature of henipaviruses as even the recently discovered and more divergent CedV, GhV, and MojV all have genomes between 18,162 nt (CedV) to ~18,430 nt (GhV). Given the caveat that there is wide variation amongst individual species of the other paramyxovirus genera, the extra length of the henipavirus genome is primarily due to the longer 3' untranslated regions (3'UTRs) at the end of several of its six genes, and a larger *P* gene relative to the other paramyxovirus genera. A comparison of genome length and gene organisation of representative members of the *Paramyxoviridae* is shown in Fig. 14.3A. The nomenclature of each of the six major genes common to all paramyxoviruses have been updated to reflect the latest ICTV convention.(35) An analysis of the 3' UTR lengths between extant henipaviruses and representative species from each paramyxovirus genera that infects mammals is presented in Fig. 14.3B. The most significant differences are in the 3'UTRs of *N*, *P*, and *RBP* genes even given the wide variance seen within each genus. The significance of long 3' UTRs, especially in HeV and NiV, is generally unknown. However, the N gene 3'UTR of NiV appears to play a role in the downregulation of N mRNAs via specific interactions with hnRNP D.(36)

<FIG14.3A><FIG14.3B>

The genome organization of henipaviruses resembles that in the genera *Respirovirus* and *Morbillivirus.* The first 12 nt of the 3′ and 5′ genomic terminal sequences of paramyxoviruses are highly conserved and complementary, containing promoter elements for replication and transcription (see Chapter 13). The first 3 nt of the henipavirus genome termini are 5′-ACC-3′—a sequence that is absolutely conserved in members of the family *Paramyxoviridae* and different from that found in the family *Pneumoviridae.*

<H2>Virus Proteins and Their Properties

Analysis of purified viruses by polyacrylamide gel electrophoresis reveals RdRP (L), P, RBP (G), F_0, N, F_1, M, and F_2 proteins$^{(20,24)}$ where F_0 is the uncleaved and F_1 and F_2 the cleaved products of the *F* gene (Fig. 14.4A). Interestingly, F_0 is more readily detected in HeV compared with other paramyxoviruses, including NiV,$^{(24,37)}$ which may suggest that HeV-F is less efficiently cleaved. Overall, the proteins of henipaviruses are typical of those of *Paramyxoviridae*, with the exception of the P protein, which is significantly larger than cognate proteins in this virus family.$^{(26)}$ The P protein is translated from messenger RNA (mRNA) that is co-linear with genomic RNA. For most henipaviruses, the *P* gene also encodes V and W proteins, produced from mRNA in which one and two nontemplated G residues, respectively, are inserted at the RNA editing site during transcription. The P, V, and W proteins, therefore, are identical for the first 405 amino acid residues. A C protein is encoded by the 5′ end of the gene in an overlapping reading frame and is produced by an internal translational initiation mechanism, which is common to other members of *Paramyxoviridae*, except for rubulaviruses (see Chapter 13). The *P*-derived V, W, and C proteins are present in HeV-infected and/or NiV-infected cells.$^{(24,38)}$ It is worth noting that Cedar virus lacks both the RNA editing and expression of the V protein.$^{(28)}$ Similar to other paramyxoviruses, P itself and its derived proteins (V, W and C in the case of most henipaviruses), all antagonize innate immune responses via a diverse set of mechanisms.$^{(39,40)}$ The C protein also interacts with the host cell ESCRT machinery and the virus matrix (M) protein itself to facilitate virus budding (see Pathogenesis section for details on the function of these *P*-derived proteins).$^{(41)}$

<FIG14.4A><FIG14.4B>

Paramyxovirus N, P, and RdRP proteins are necessary and sufficient for replication of viral RNA both *in vitro* and *in vivo* as discussed in chapter 13. This has been confirmed for henipaviruses by reverse genetics using a minigenome replicon containing leader and trailer sequences of the NiV genome with its entire coding region replaced with a reporter gene.(34),(42) NiV N, P, and RdRP proteins were also able to rescue a HeV minigenome, demonstrating the close genetic relationship between the viruses. Full-length recombinant NiV bearing the matrix (M), fusion (F) and receptor binding protein (RBP) genes from HeV, singly or in combination, can also be functionally rescued. These chimeric but isogenic rNiVs replicate well in primary human endothelial and neuronal cells, further underscoring the genetic relatedness of NiV and HeV that allows for heterotypic complementation between their major structural proteins.(43) These henipavirus structural proteins contribute to unique aspects of the henipavirus life cycle (Fig. 14.4B), and play different roles in the spread and virulence of henipaviruses. Indeed, multiple groups have developed recombinant virus systems for NiV, HeV and CedV bearing various reporters and/or relevant mutations.(43-53) These additional tools have facilitated work towards understanding the virological and pathogenic similarities and differences amongst the henipaviruses. (see Pathogenesis section for details).

The RdRP protein of nonsegmented, negative-stranded RNA (NNR) viruses in the order *Mononegavirales* contains a highly conserved GDNQ motif, believed to be important for polymerase activity.(54) Henipaviruses were the first NNR viruses in which GDNQ was replaced by GDNE. It was speculated that this motif might be unique to paramyxoviruses with relatively large genomes(21,24); however, the GDNE motif has since been found in the RdRP protein of Mossman virus that has a genome length of 16,650 nt.(55) Conversely, other henipaviruses of both Asiatic (CedV) and African lineages (GhV) also have the GDNQ motif that is common to most other NNR viruses. High-resolution cryo-EM structures of VSV-RdRP (another NNR virus, family *Rhabdoviridae*) suggest that GDN is the truly conserved catalytic motif essential for the polymerase function of RdRP.(56)

The receptor binding proteins (RBPs) of the *Paramyxoviridae* display hemagglutination (H) and neuraminidase (N) activities in a predominantly genus-specific manner. Viruses in the genera *Respirovirus, Avulavirus*, and *Rubulavirus* possess both activities, hence their RBPs were formerly termed HN proteins(57) whereas viruses in the genus *Morbillivirus* do not behave uniformly and only some possess hemagglutination activity, but nonetheless their RBPs were formerly termed H.(58) This is an unfortunate misnomer. Paramyxoviruses with RBPs bearing HN activity genuinely use sialic acid based receptors for entry whereas "H" activity of some morbilliviruses arise from the ability of their RBPs to bind CD46 expressed on some non-human primate (NHP) red blood cells.(59) In contrast, henipavirus RBPs have neither of these activities(24,60); rather, they utilize at least ephrin-B2, and in many cases, also ephrin-B3 expressed on host cell surfaces as attachment and entry receptors (61-64),(28,65). The exception is MojV, which does not appear to use any known paramyxovirus receptors.(66) Recent solution structures of various henipavirus RBPs alone and in complex with the ephrin-B2 and/or ephrin-B3 receptors have revealed the details of the virus–host cell binding process, distinguishing it from other paramyxoviruses's receptor binding strategies.(67-69), (59,65,66) (Fig. 14.5)

<FIG14.5>

Proteolytic processing of paramyxovirus F proteins is essential for the generation of a fusogenic form of the protein. For most paramyxoviruses that generate systemic infections, furin-like proteases in the secretory pathway cleave their F proteins at a multibasic cleavage site.(70) Surprisingly, henipavirus F proteins are cleaved without the involvement of furin and, although cleavage occurs at a single basic residue—lysine for HeV and arginine for NiV.(37)

—activation of the NiV F protein does not require a basic amino acid at the cleavage site.(71) Instead, endosomal/lysosomal cysteine proteases such as cathepsins L and B are responsible for the cleavage of henipavirus F proteins.(72-75) To access cathepsins L/B, henipavirus F proteins are endocytosed upon initially reaching the cell surface in an uncleaved form, and subsequently have to traffic back to the cell surface once they are cleaved into a fusion-competent form (Fig. 14.4B). [55,57] Classical endocytic YXXØ motifs(76,77) in the cytoplasmic tails of henipavirus F proteins and specific residues in the transmembrane domain of at least HeV-F regulate this trafficking behaviour through the early/sorting (S490) and recycling endosomal (Y498) compartments.(78) Henipavirus F proteins also have the ability to bud by themselves forming F-only particles in the absence of M.(79,80)

The matrix protein (M) plays a major role in the efficient assembly and budding of infectious paramyxoviruses.(81,82) M interacts specifically with N, the cytoplasmic tail of F, as well as cognate lipid ligands along the vesicular trafficking pathway. These orchestrated interactions help to co-ordinate the envelopment of encapsidated viral genomes (RNPs) and the budding of infectious virions bearing a relatively high density of viral envelope glycoprotein spikes. General details on the role that matrix plays in paramyxovirus assembly and budding are given in the previous chapter 13.

Henipavirus M exhibits some relatively unique behaviours and functions. For example, NiV and HeV M have classical nuclear localization and export sequences (NLS, NES) that when mutated give the relevant nuclear exclusion and nuclear retention phenotypes, respectively.(83) Time-course monitoring of M trafficking during live NiV and HeV infection indicates that M first appears in the nucleus, localizes to subnuclear compartments, including the nucleolus, before exiting into the cytoplasm and trafficking to the plasma membrane to coordinate virus assembly and budding (Fig. 14.4B).(83,84) This nuclear transit is regulated by ubiquitination and is critical for M to acquire its budding functionality.(83,85) Henipavirus M is multi-mono and poly-ubiquitinated; a lysine to arginine mutation of a key residue in its bipartite NLS is sufficient to dysregulate ubiquitination of M and abrogate its budding function.(83,85) The latter is true not only in HeV and NiV, but also in divergent henipaviruses (GhV, CedV, MojV), albeit to varying degrees.(86) Matrix interactome studies have identified many nuclear interacting factors, including importins and exportins, consistent with the nuclear-cytoplasmic trafficking phenotype of M.(85,87)

<H2>Host Range

For most paramyxoviruses, host range is limited and interspecies transmission is rare. In contrast, henipaviruses display a broad species tropism. In addition to a large number of bat hosts at various geographic locations (see more detail in Epidemiology section), NiV has naturally infected pigs, humans, dogs, horses, and cats,(10,11,88-90) whereas HeV infects all four Australian flying fox species and has naturally infected humans, horses and dogs.(2,91-93) Confirmation of the wide host range of these henipaviruses and the identical cell tropism of HeV and NiV were obtained early using an *in vitro* cell fusion system that relies on vaccinia virus–mediated cell surface expression of G and F glycoproteins.(94-96) Bats, guinea pigs, hamsters, ferrets, squirrel monkeys, and African green monkeys are also susceptible to experimental NiV infection.(97-101) Laboratory studies have added cats, guinea pigs, hamsters, ferrets, dogs and African green monkeys to the list of HeV-susceptible species.(93,102-106) CedV, isolated from flying foxes (*Pteropus sp.*) in Cedar Grove, Australia, can establish transient but non-pathogenic infections in laboratory challenged guinea pigs and ferrets. GhV and MojV, known only from sequence information, were identified from bats(29) and rats,(30) respectively.

<H1>Pathogenesis and Pathology

<H2>Entry into the host

Epidemiological and experimental studies generally support an oronasal (8,107,108) or oropharygeal(109-111) route of entry. This occurs directly or indirectly via saliva, urine, or oronasal sceretions that are contaminated with relatively high levels of HeV or NiV.(93,105,107,112-123) Fig. 14.6 summarizes what is known about how NiV is transmitted to humans either directly from their pteropid reservoir hosts—fruit bats or flying foxes—or indirectly via a secondary amplifying host such as pigs, horses and humans. Direct bat-to-human transmission has been associated with consuming contaminated fruit or liquid (e.g. date palm sap)(109-111). Human-to-human transmission of NiV has been well-documented, especially in Bangladesh(117,124-126) and the most recent outbreak in Kerala (India).(17) However, all 7 known cases of HeV infections in humans have resulted from contact with sick horses already exhibiting symptoms of respiratory distress,(127) likely after being exposed to food or water contaminated with saliva and urine/droppings from HeV-infected flying foxes.(128) While patients that succumb to henipavirus infections eventually die from encephalitic complications that often involve brainstem neuronal dysfunction,(108,129) there is diversity of clinical presentations.(130) For example, NiV-MY infected patients in Malaysia present less often with respiratory involvement (~40%) than the vast majority of NiV-BD infected patients in Bangladesh and India. The handful of HeV infected humans have also presented with encephalitic illness with or without a respiratory component (see Clinical Features section). To better understand the pathogenesis and pathology associated with henipavirus disease in humans, many relevant animal models have been developed that either represent natural amplifying hosts (pigs and horses) or that reproduce well the respiratory and encephalitic disease seen in humans (hamsters, ferrets and African green monkeys (AGMs)).(131,132) Despite the broad host range mentioned in the prior section, other animal challenge models (guinea pigs, cats, dogs) either do not represent the full spectrum of disease seen in humans, or are relatively asymptomatic when challenged with NiV and/or HeV.(93,131)

<FIG14.6>

<H2>Site of primary replication, virus spread, and cell and tissue tropism

The clinical and pathological data from humans (see Clinical Features section), relevant animal models, and cell tropism studies (Table 14.3) indicate that the initial site of replication is within the respiratory system, likely involving cells lining the oro-/naso-pharyngeal epithelium as well as bronchiolar epithelial cells, type I pneumocytes, alveolar macrophages or pulmonary airway dendritic cells (DCs).(40,132-135) The distribution and time of appearance of lesions throughout the vasculature and in the brain and lung in NiV encephalitis suggest that secondary infection probably arises via hematogenous spread of the virus, with secondary replication occurring in vascular endothelium.(136) Inflammation of blood vessels (vasculitis) occurs in most organs but is particularly prominent in the brain, lung, heart, kidney and spleen.(9,136,137) Vasculitis is limited to small arteries, arterioles, and capillaries where NiV antigen is found in both endothelial cells and the smooth muscle of the tunica media. The pattern and time of appearance of vasculitis and viral antigen distribution are consistent with endothelial cell infection occurring before infection of the smooth muscle. The 5-day interval between maximal vasculitis in the brain and parenchymal infection in acute NiV encephalitis suggests that virus replication occurs first in endothelial cells, with infection of neurons occurring as a result of vascular damage and breakdown of the blood-brain barrier (BBB). In humans as well as in pigs and hamsters, viral antigen positive brain microvascular endothelial cells can be observed in areas of compromised BBB integrity.(136,138,139) The presence of inclusion bodies and viral antigen in neurons, along with widespread histologic and radiologic lesions, suggests that neurologic impairment in NiV (and HeV) encephalitis may be caused by both the effects of ischemia and infarction and viral infection of neurons.(12,107,136)

<TAB14.3>

In addition to the systemic vasculitis, NiV antigen is clearly present in the parenchyma of multiple organs mentioned above. The same pathology has been reported for a fatal case of acute HeV encephalitis.(140) Similar widespread infection and multiorgan vasculopathy has been demonstrated in relevant animal models with the highest viral antigen load consistently seen in the lung and brain (Fig. 14.7). *(131,132)* Interestingly, intranasal inoculation in pigs, hamsters, and ferrets suggests that NiV can also directly invade the central nervous system (CNS) through the olfactory epithelium (OE). NiV antigen positive neurons or olfactory nerves can be found extending through the cribiform plate or in the nasal turbinate into the olfactory bulb.(133,139,141) While primary human OE cells are susceptible to henipavirus infection(142), the olfactory route is unlikely to be the major mode of CNS invasion in humans (and the AGM model). Primates have a relatively smaller area of OE compared to hamsters, pigs, or ferrets where the majority of evidence regarding the olfactory route of entry has been gathered.(97,139,141) In addition, NiV antigen was not detected in the olfactory bulbs of at least nine infected patients from the autopsy series reported from the Malaysian outbreak.(136)

<FIG14.7>

The widespread cell and tissue tropism of NiV and HeV seen in humans and the various animal models is determined by the expression pattern of the henipavirus receptors, ephrin-B2 and ephrin-B3.(61-64) The ephrinB ligands and their cognate EphB receptors are both receptor tyrosine kinases that mediate bidirectional cell–cell signaling events upon engaging each other. They are critical modulators of cell remodeling events, especially within the nervous and vascular systems.(143,144) They are also highly conserved among vertebrates, which explains, at least in part, the unusually broad species tropism of henipaviruses. Ephrin-B2 is found in arteries, arterioles, and capillaries in multiple organs, and is more broadly distributed across multiple tissues and cell types (Fig. 14.8), including neurons, arterial smooth muscle and human bronchiolar epithelial cells,(143,145-147) but is absent from venous components of the vasculature.(148,149) In contrast, ephrin-B3 is essentially restricted to the brain(144) where it is expressed higher than ephrin-B2 (Fig. 14.8). Its relative absence from organs such as lung, kidney, and spleen, as well as from arterial endothelium suggest that the systemic vasculitis, respiratory distress, and other end-organ pathologies seen in henipavirus infections is largely a consequence of ephrin-B2-mediated entry. Conversely, ephrin-B3 mediated entry may play a larger role in the spread of HeV and NiV within the CNS.

<FIG14.8>

Human lymphocytes (mainly T- and B- cells), and monocytes, despite being non-permissive for NiV infection(150) or HeV-F/RBP induced fusion(94), can bind and transfer NiV to permissive microvascular endothelial cells *in vitro*.(150) This trans-infection phenotype, thought to be mediated by heparan sulfates,(151) may be an efficient form of hematogenous spread in addition to the free form viruses that make up the plasma viremia seen in acute NiV and HeV infections in humans. This acute but transient viremia appears about 4-7 days post-onset of illness (POI) and in survivors, is usually undetectable within 2-4 weeks POI depending on severity of the disease course.(17,152-154) A similar course of plasma viremia can also be detected in AGMs challenged intratracheally and orally with NiV.(98) The next section on the Immune Response will discuss possible mechanisms by which viremia is cleared in survivors.

<H2>Immune Response

Viral antagonism of innate immune responses

HeV and NiV have a broad host range that is uncommon in paramyxoviruses. This ability to replicate, spread, and cause acutely lethal disease amongst multiple species suggest that these viruses have evolved effective means to antagonize or evade innate antiviral responses that might otherwise keep them in check until adaptive immune responses develop.

In henipaviruses, as in other paramyxoviruses, (40) the major anti-interferon (IFN) activities are encoded by the *P* gene and *in vitro* studies indicate that *P* gene products inhibit both IFN induction and signalling (Fig. 14.9). (39) In IFN induction, various forms of viral double-stranded RNA (dsRNA) are detected by cytoplasmic RNA helicases(155-157) such as RIG-I and MDA-5, and by toll-like receptor-3 (TLR-3). (157,158) Both of these dsRNA signaling pathways, via a cascade of signaling events depicted in Fig. 14.9 (left) lead to activation and nuclear translocation of interferon regulatory factor (IRF)-3, or if present, IRF-7, and nuclear factor kappa B (NF-kB); transcription factors that induce the expression of IFN-α/β proteins. HeV and NiV inhibit dsRNA signaling via its V protein, which binds to MDA-5 and prevents downstream signaling(155,159,160) but does not abrogate dsRNA signaling through TLR-3.(161) Recent evidence suggest that NiV-V also binds RIG-I and its regulator (TRIM25), disrupting downstream signalling. (162) In an additional strategy unique to henipaviruses, the W protein, by virtue of a NLS located in the unique carboxy terminal, inhibits induction of IFN-α/β expression in the nucleus by targeting a process that is part of both helicase-dependent and TLR-3–dependent signaling pathways.(163) Unexpectedly, the NiV M protein, can also inhibit IFN-α/β induction by indirectly antagonizing the activity of IKKε, the kinase responsible for IRF-3 activation.(51)

<FIG14.9>

In the IFN signaling pathway (Fig. 14.9, right), IFN binds to the IFN-α/β receptor (IFNAR) on cells and initiates a signaling sequence that leads to activation of members of a family of proteins called *signal transducers and activators of transcription* (STAT).(164,165) Henipaviruses inhibit IFN signaling by sequestering STAT proteins in high molecular weight complexes and preventing their phospho-activation .(166,167) The anti-IFN signaling activity is a property of the V protein, as has been observed for other paramyxoviruses, but also of the W and P proteins.(161,166,167) (45)The P, V, and W proteins of henipaviruses have an N-terminal extension of 100 to 200 amino acids compared with cognate proteins in the subfamily,(20,23) and the STAT binding domain of NiV V maps to this region.(161,168,169) The V and P proteins bind STAT in the cytoplasm, whereas the W protein, which is imported into the nucleus via specific interactions of its NLS with importin α-3, (170) likely sequesters unactivated STAT1 in the nucleus.(161,163) Recently, NiV and HeV N proteins were also found to antagonize IFN-signaling by interfering with formation of activated phospho-(p)STAT1 complexes. This NiV-N mediated antagonism of pSTAT1 nuclear accumulation does not rely on direct binding to or inhibiting phosphorylation of STAT1 itself. (171) NiV C has also been demonstrated to interfere with IFN-signaling.(47,172) However, its mechanism and true function remains unclear. Antagonizing IFN-signaling is unlikely to be the main function of the C protein since recombinant C-deficient NiVs have an attenuated replication phenotype even in Vero cells, (41,45,46) which are genetically deficient in IFN production.

There is little doubt that inhibition of IFN-α/β production is one major contributor to the acute virulence of HeV and NiV *in vivo*. Daily administration of poly(I)-poly(C(12)U), a potent IFN-inducer, results in >80% survival in a lethal challenge hamster model for NiV infections.(173) Most of the studies described previously were carried out *in vitro* using single-gene transfection expression systems. Understanding the true function(s) of these proteins requires assessing their impact in the context of a live virus infection or the phenotype of viruses deficient in the cognate protein(s). For example, IFN signaling apparently remains functional during live HeV and NiV infection of human cell lines, whereas IFN production was inhibited.(174) However, henipaviruses also clearly antagonize both IFN production and signaling in bat cells derived from *Pteropus sp*. that serve as their natural hosts.(175)

The diverse phenotypes observed in the various animal models when challenged with recombinant viruses deficient in the various P-derived accessory proteins makes interpretation difficult. The clearest phenotype comes from rNiVs engineered to be V-deficient. rNiV-ΔV are extremely attenuated in both hamster and ferret challenge models.(46,50) Each model re-capitulates the respiratory and neurological disease seen in human infections when infected with wt NiV, albeit in different ways and in a dose-dependent fashion.(103,131,176) However, challenge with V-deficient rNiV under conditions where their wt counterparts effectuate 100% mortality always results in 100% survival, and almost never results in any overt respiratory or neurological signs.(46,50) On the other hand, C-deficient rNiVs give a variety of phenotypes that range from strong attenuation in the hamster model,(177) to 100% mortality in the ferret model, albeit with less severe histological lesions in the lung.(52) Similarly, W-deficient rNiVs did not show significant attenuation compared to their wild-type counterparts in both the hamster(46) and ferret models, although the disease symptomology appeared to be more neurologic in the latter.(50,52) Interestingly, a rNiV lacking both C and W still resulted in 60% mortality with reduced respiratory disease but markedly increased neurological disease.(52)

Adaptive immune responses.

In patients with encephalitis, anti-NiV antibodies were observed more frequently in the serum than the CSF. Immunoglobulin M (IgM) antibodies occurred more frequently than immunoglobulin G (IgG) antibodies in both locations.(9,12,136) The appearance of specific IgM antibodies in serum preceded their appearance in the CSF, a sequence consistent with viremia preceding central nervous system (CNS) infection. Anti-NiV antibodies were present in most patients with clinical NiV encephalitis; however, no difference was observed in clinical features, laboratory results, or mortality between seropositive and seronegative patients.(108,178) Seroconversion of IgG against HeV was seen in two human cases of HeV infection in 2008, with one progressing to fatal encephalitis.(8) From these cases, seroconversion in acute henipavirus disease may have diagnostic but no apparent prognostic value. Conversely, in the most recent NiV outbreak in Kerala involving 23 cases, careful monitoring of humoral and cellular immune responses in the 2 survivors revealed that there was a dramatic increase in activated (Ki67+) CD8+ T-cells with an acute effector phenotype (CD38+/HLA-DR+/Granzyme B+ /PD1+), suggesting that virus-specific naïve T-cells were actively proliferating in response to the virus. Interestingly, clearance of viremia coincided with the peak of proliferating Ki67+ CD8+ T-cells, and occurred before peak IgG titers. (152) Increases in activated CD8+ T-cells in lethal (NHP)(179) and non-lethal (Swine) animal models of NiV infection have also been observed; (180) in the latter, prevention of virus shedding appears correlated with such T-cell responses. However, most animal studies do not monitor adaptive immune responses with the requisite precision and sophistication in order to make definitive statements regarding the relative importance of T- or B- cell responses in protective immunity against acute henipavirus infections.(181)

The high CFR associated with the acute nature of henipavirus infections already suggests that the virus interferes with the timely generation of effective T- or B- cell responses during the *natural* history of the disease in the majority of infected individuals. Indeed, although NiV replicates poorly in DCs (Table 14.3),(150,182) *in vitro* NiV-infected DCs induces an inflammatory pro-apoptotic milieu (TNF-a, IL-1β, IL-8) that leads not only to defective T-cell priming, but apoptosis of cognate T-cells and DCs.(182) This in vitro phenomenon, if recapitulated *in vivo* by NiV-infected DCs that traffic to lymph nodes, may account for the lymphoid depletion and lymphoid necrotic lesions seen in humans*(136)* and various relevant animal models.*(50,183,184)*

<H2>Release from host and transmission

Transmission is generally accepted to occur via the oronasal route(8,107,108) or liquid/food (palm sap/fruit),(109-111) with transmissibility of HeV (horse-human or human-to-human)(185,186) being much less efficient than that of NiV transmission (pig-to-human or human-to-human).(9,15,17,108,115,117,126,187,188) Infectious NiV can be recovered from urine and the tracheal and nasopharyngeal secretions of infected patients in the early phase of their illness in the original Malaysian outbreak.(3,107,108) Nonetheless, human-to-human transmission in the Malaysian outbreak was extremely rare(189), although it has been reported in the more recent outbreak in the Philippines. In contrast, person-to-person transmission in Bangladesh has been well documented and typically occurs during the final stages of disease in the index patient when not only symptoms of respiratory involvement are most prominent, but also more frequent and closer contact with familial caretakers occurs. (125,187,188)

Replication in the respiratory epithelium and shedding in oronasal secretions is most likely responsible for virus release and transmission. Ferrets have been used to model the respiratory transmission of henipaviruses(112,184) as their respiratory symptoms (cough, serous nasal discharge, dyspnea) mimic the human disease.(97) Despite shedding relatively large amounts of virus in their oronasal secretions (4-5 log10 $TCID_{50}$ equivalents/ml at peak titers), passively cohoused naïve ferrets never got infected, regardless of whether the index ferrets were infected with HeV, NiV-MY, or NiV-BD.(112,184) However, following assisted or direct exposure to infectious oronasal fluids from the index ferrets, all the naïve ferrets developed acute infections, regardless of virus strain used.(112) Thus, the efficiency of NiV transmission appears low, and likely requires direct and repeated oro- or naso-mucosal exposure to infectious oronasal secretions. This is consistent with epidemiological investigations into the many recurrent outbreaks in Bangladesh where despite chains of person-to-person transmission, the estimated basic reproductive number R_0 of NiV-BD strains that have spilled over in Bangladesh have averaged 0.48.(187)

<H2>Virulence

Infection with HeV appears more severe than infection with NiV-MY and NiV-BD in the hamster(138,190) and AGM model.(191) The dose-dependent disease symptomology in the hamster model(138,192) makes it difficult to make generalize statements about any intrinsic pathogenic difference between these three genetically distinct henipaviruses. However, the intratracheal and intranasal AGM challenge model appears to recapitulate the clinical pathologies seen in human patients more robustly.(98,103) Interestingly, it is also in this model that NiV-BD is shown to be more pathogenic than NiV-MY,(191) which reflects the observed differences in pathogenicity between NiV-MY and NiV-BD. Previous studies comparing NiV-MY and NiV-BD in hamsters(190) and in ferrets(112) gave conflicting and confusing results. But more careful comparisons of fully-sequenced NiV-MY and NiV-BD strains in the AGM model provides support that NiV-BD is more pathogenic than NiV-MY,(191) consistent with the higher mortality rates associated with NiV-BD (see Table 14.1C). Under identical experimental conditions, NiV-BD is not only shown to be more pathogenic in terms of viral load in lungs, spleen, and blood, but also that the therapeutic window for post-exposure prophylaxis using human monoclonal antibody (m102.4) is significantly shorter in NiV-BD infected AGMs.(191) m102.4 is a potent neutralizing mAb previously shown to rescue NiV-MY and HeV infected AGMs even when administered up to 7 days post-infection.(193,194)

CedV lacks the editing site in the *P* gene and does not make the V protein,(28) a potent type I IFN antagonist required for henipavirus virulence *in vivo*.(39) Not surprisingly, CedV, which is naturally V-deficient, also does not induce any overt disease in ferrets and guinea pigs.(28) Reactive hyperplasia of oropharyngeal lymphoid tissues and detection of viral RNA in select bronchial associated lymphoid tissue (BALT) accompanied by increasing neutralizing Ab titers suggests that CedV likely replicates transiently in the upper and lower respiratory tracts of the challenged animals.(28) While recent evidence suggests that the CedV P protein itself is also a less efficient antagonizer of IFN-α signaling compared to HeV-P,(195) whether CedV is truly non-pathogenic in humans or horses remains to be seen. Given the fact that CedV does not appear to use ephrin-B3 as receptor, one might also predict CedV to be less encephalopathic even in more susceptible animals.

<H2>Persistence

A small percentage of NiV-infected patients (3-7%) experience late-onset or relapse encephalitis months to years after the initial NiV infection; the former describes patients with initially mild or asymptomatic NiV infections while the latter are survivors of acute NiV encephalitis.(108,129,196,197) One case of fatal late-onset HeV encephalitis has also been reported.(3,140) These cases suggest that NiV and HeV can persist in sanctuary sites like the brain especially since virus has never been isolated from oronasal secretions of relapsed encephalitis cases.(9,107,136,196-198) (see Clinical Features section for details). There is one report of NiV RNA being detected in the semen of a NiV survivor 4 weeks post-onset of illness, but this was gone 2 weeks later. Longitudinal studies on 2 HeV survivors shows no evidence of viral shedding by RT-qPCR, up to 6 years post-symptom onset.(154) It is unlikely that NiV and HeV in humans can persist in infectious forms. Thus, relapse henipavirus encephalitis might resemble measles virus associated subacute sclerosing panencephalitis, which can occur years after recovery from measles virus infection.(199,200)

<H1>Epidemiology

<H2>Age

The age of patients with encephalitis in the Malaysian outbreak ranged from 9 to 76 years, with almost 50% of cases occurring in those 40 to 44 years.(108,136,153,178,201) The male-to-female ratio was approximately 3:1, and more than 80% of the patients were Chinese, with statistics reflecting the increased risk to those working with infected pigs.(108,201) In Bangladesh, the age of patients ranged from 4 to 60 years, and males constituted 47% and 67% of the cases in the 2001 and 2003 outbreaks, respectively.(14,202) The outbreak in early 2011 in Bangladesh claimed at least 35 lives, including many children and infants, with ages ranging from 2 to 56 years.(127,203) Among the 18 suspected patients in the 2014 oubreak in the Phillipines, the age distribution is between 21-60 years old.(18) In the most recent NiV outbreak in Kerala, India, the age distribution ranged from a 17 year old male to a 75 year old female among a total of 23 human cases.(17)

<H2>Morbidity and Mortality

In Malaysia, between September 1998 and June 1999, 256 patients who developed acute NiV encephalitis were admitted to Malaysian hospitals and 105 died, a mortality rate of approximately 40%.(9,201) The rate of subclinical infection in households and farms where cases of NiV encephalitis occurred was calculated to be 8% and 11%, respectively.(196,201) In Singapore, where 11 patients were confirmed to have acute NiV encephalitis, a further 2 asymptomatic abattoir workers were serologically positive, representing a rate of subclinical infection of 15%.(204) Subsequently, 89 individuals were identified on the basis of positive serology as having experienced either an asymptomatic or mildly symptomatic NiV infection.(129) This increases the number of people infected with NiV to 345 and decreases the mortality rate to approximately 30%.(137) In Bangladesh, 98 of 135 patients died in eight outbreaks from 2001 to 2008, giving a combined case fatality rate of 73%.(117,188,202) In the Philippines outbreak, the case mortality is 53% out of all the suspected cases or 81% for those with encephalitic clinical signs.(18) The case mortality is 94% for the 2018 Kerala outbreak in India.(17) There have only been seven known human cases of HeV infection in Australia in the past 16 years, four of which have been fatal (three acute and one case of relapsed encephalitis).(2,3,8)

<H2>Origin and Spread of Epidemics

Fruit bats (flying foxes) in the genus *Pteropus*, family *Pteropodidae*, suborder *Megachiroptera* are main reservoir hosts of HeV and NiV.(5,6,13,89,205) In Australia, HeV has been shown to occur in all four flying fox species, with the crude seroprevalence of 47%, indicating an endemic pattern of infection throughout Australia.(91) Serologic tests show that NiV or NiV-related virus is widely dispersed in bats from Indonesia to the west border of

Africa (Fig. 14.10). NiV was first isolated from the urine of Island flying foxes and from the saliva on partially eaten fruit(13) and has since been isolated from Lyle's flying foxes (*Pteropus lylei*) in Cambodia(89) and *Pteropus medius* in Bangladesh.(206) The Indian flying fox (*Pteropus giganteus*) is the main pteropid species throughout Bangladesh and the Indian subcontinent with a high seroprevalence of henipavirus-specific antibody.(14,110,187,207) Additional serologic and limited nucleic acid evidence has suggested that related henipaviruses are circulating in other regions, including Thailand, Indonesia, Vietnam, China, Madagascar, and several countries and regions in Africa (Fig. 14.10 and Table 14.4-online). <FIG14.10>

Neither HeV nor NiV appear to cause clinical disease in naturally infected bats.(91,202,205,208) Experimental infection with doses of HeV that are lethal in horses generates sporadic vasculitis in the lung, spleen, meninges, kidney, and gastrointestinal tract, and even then, only in a proportion of infected bats.(105) Viral antigen is detected in the tunica media rather than endothelial cells, a fact that may spare these bats from the clinical effects associated with vasculitis.(209) In infected pregnant fruit bats, antigen was observed in similar locations and in the placenta.(210) The mode of transmission between fruit bats is unknown. Transplacental transmission has also been observed experimentally without apparent harm to the fetus.(210) Experimental infection of fruit bats with NiV produced a subclinical infection with a transient presence of virus within selected viscera along with periodic viral excretion in bat urine and seroconversion with neutralizing antibody present.(100)

The spillover and epidemic hosts of HeV and NiV were horses in Australia and Philippines, and pigs in Malaysia. All human infections with HeV in Australia and NiV in Malaysia have only occurred through transmission from these domestic animal hosts.(9,201) No evidence exists of direct transmission from pteropid bats to humans in Australia or Malaysia, despite many opportunities in Australia for transmission to bat carers.(196,211) In contrast, fruit bats apparently play a direct role in the transmission of NiV to humans in the many recent outbreaks of disease in Bangladesh, where epidemiologic evidence in support of a role for an intermediate host was lacking.(14,202) Three pathways of NiV transmission from bats to people have been identified based on epidemiologic investigations in Bangladesh.(117) Consumption of fresh date palm sap appears to be the predominant risk factor, and infrared camera studies have confirmed that *P. giganteus* bats frequently visit date palm sap trees and consume sap during collection.(212) In the 2005 NiV outbreak in Tangail District, Bangladesh, drinking raw date palm sap was the only activity significantly associated with illness (64% among cases vs. 18% among controls).(110) Another route of transmission for NiV from bats to people in Bangladesh could be via domestic animals. Contact with a sick cow in Meherpur, Bangladesh, in 2001 was strongly associated with NiV infection,(14) and contact with pigs and diseased goats have also been implicated in other occurrences of NiV in Bangladesh.(117) Although NiV has never been isolated in domestic livestock animals, serological evidence was reported for antibodies to NiV or NiV-related viruses in cattle, goats and pigs in Bangladesh.(213) Transmission via direct contact with NiV-infected bat secretions also appears possible from evidence in the Goalando outbreak in 2004, where individuals who climbed trees were more likely to develop NiV infection than controls.(214)

The mode of transmission from bats to spillover hosts in Australia and Malaysia remains to be determined. Three principal hypotheses exist. One is that masticated pellets of virus-contaminated, residual fruit pulp spat out by flying foxes are ingested by horses or pigs.(205) The second is that urine from infected animals contaminates pastures or pigsties. The third is that infected fetal tissues or fluids contaminate pastures or sties and are ingested. The latter is based largely on the fact that the HeV outbreaks have occurred during the birthing period of some species of flying fox and is supported by the isolation of virus from a pregnant flying fox and its fetus.(6)

HeV has been transmitted from horse to man on seven occasions from 1994 to 2009, twice during the initial outbreak in Brisbane,(1,2) twice during necropsy of horses that died in the field,(1,4) twice during either daily nasal cavity lavage and participating in a necropsy,(8) and once from performing an endoscopy on an infected horse.(8,215) HeV is rarely found in the bronchi or bronchioles of infected horses, which suggests that aerosol transmission to either man or horses is less likely(216) and horse-to-horse transmission of HeV has not been demonstrated.(105) The presence of HeV in equine saliva, however, suggests that close contact with infected horses, such as might occur during manual feeding of the animals, may facilitate horse-to-human transmission.(1) The presence of virus in a wide range of tissues and in the nasal discharge commonly found at the terminal stage of infection offers a range of sources for virus transmission during necropsy.(2,217) As shown in Fig. 14.7, high level of viral antigen can be detected in the nasal cavity of HeV-infected horses.

In the Malaysian NiV outbreak, contact with pigs or fresh pig products was required for transmission of the virus to humans, with greater likelihood of transfer to those in direct contact with sick or dying pigs on farms or in abattoirs.(9,201) The presence of NiV in the respiratory epithelium of naturally and experimentally infected pigs (Fig. 14.7) indicated that virus probably spread to humans and within the pig population by aerosol or by direct contact with oropharyngeal or nasal secretions.(10,121,139) The presence of the virus in a wide range of organs indicates that humans may also have been infected during processes such as slaughtering or farrowing. In Bangladesh, pigs were excluded as potential sources of NiV on epidemiological grounds, and human-to-human transmission was observed.(126,188) The virus may have been transmitted to human index cases directly through contact with fruit bat secretions in contaminated fruit or date palm sap before circulation in the human population (Fig. 14.6).(13,14,117,202) Nosocomial transmission has been detected in some of the Bangladesh and India outbreaks.(15,117,218) In the Philippines NiV outbreak, of the 17 case-patients, a total of 7 (41%) had participated in horse slaughtering and horse meat consumption; 3 (18%) had only consumed horse meat and but had no history of slaughtering or meat preparation; 5 (29%) case-patients had been exposed to other human casepatients but not to any horses. So it is possible that both horse-to-human and human-to-human transmission might have occurred (Fig. 14.6).(18) For the Kerala NiV outbreak, while it remains undefined how the index case was exposed to NiV, it was evident that human-to-human transmission was the main driver for the spread of the virus in both family/community and hospital settings (Fig. 14.6).(17)

<H2>Genetic Diversity

Genome sequencing revealed that HeV isolated from equine and human sources during the outbreak in Brisbane appears identical and differs little from HeV isolated from flying foxes 2 years later.(2,6) Sequencing of five additional horse isolates from five different locations from the 2006 to 2008 HeV occurrences has also demonstrated a very high genetic similarity.(219) Similar observations were made in Malaysia, where it was demonstrated that NiV isolated from pigs at the height of the outbreak and at its geographic focus were essentially identical to human isolates made at that time and isolates obtained from flying foxes several years later.(13,22,23,220)

In Bangladesh, four human isolates obtained in 2004 demonstrate significant genetic heterogeneity, which might suggest multiple spillovers of NiV from flying foxes into the human population.(33) The NiV sequences detected from human patients in India from 2001 to 2018 were more related to NiV from Bangladesh than NiV from Malaysia.(15,17,218) A recently whole genome sequencing study of ten NiV isolates obtained from bats in 2013 in Bangladesh at two different geographic locations revealed that the isolated viruses were very similar in sequences. This suggests that multiple strains were not co-circulating in the bat population at the time.(206) Furthermore, none of the bat NiV isolate sequences was identical with any previously detected human NiV isolate sequences, suggesting that NiV spillover into humans is a rare event and more intensive surveillance is required to appreciate the full genetic diversity of NiV in bats in an area with frequent spillover events into human populations.(206) NiV isolated from the flying fox *Pteropus lylei* in Cambodia(89) represents an evolutionary lineage that is separated from the Malaysia or Bangladesh/India cluster . Partial *N* gene sequences detected in *Pteropus lylei* in Thailand indicate the circulation of at least two lineages of NiV—one related to the Bangladesh NiV and the other more related to the Malaysian NiV.(119) On the other hand, NiV sequences detected in *Pteropus hypomelanus* in southern Thailand are clearly more related to the Malaysian NiV.(221) As the RNA-dependent RNA polymerase (RdRP), formerly known as the L protein, is the most highly conserved protein for paramyxoviruses, it has been used as a more reliable indicator of evolutionary relationship among different paramyxoviruses. Fig. 14.11 shows a RdRP-based phylogenetic tree of henipavirus species and strains, selected to represent major species origin, geographic location and time of isolation. Prototype species from other genera in the family *Paramyxoviridae* are also shown for context and comparison.

<FIG14.11>

The presence of henipavirus-reactive (but not neutralizing) antibodies and viral RNA in bats from other regions of the world (Fig. 14.10 and Table 14.4-online) indicate that a much greater genetic diversity of henipaviruses exists in different bat populations.(29,222,223) These divergent but yet to be characterized henipaviruses likely have different transmissibility, pathogenicity, and receptor usage patterns, as has already been shown for CedV,(28,53,195) GhV,(65,224,225) and MojV.(66) The latter is an outlier in many ways,(41,66,86) and is currently the only henipavirus identified in rodents rather than bats.(30)

<H1>Clinical Features

<H2>Incubation Period

Based on the time interval between the last exposure to pigs and onset of disease, the incubation period for NiV ranged from 2 to 45 days; however, for 90% of patients, it was 2 weeks or less.(108,153,178) An estimate of 2 to 3 weeks was made based on the time interval between importation of pigs from NiV-affected areas of Malaysia and development of human disease at a Singaporean abattoir.(204) A mean incubation period of 9.4 days was calculated for four patients who had a fixed period of exposure.(178) The NiV outbreak in Kerala, India, 2018, revealed a median incubation period was 9.5 days among 23 cases, 22 of which were nosocomial.(17) In the occurrence of HeV in Australia in 2008, a detailed examination of exposure histories from two infected patients (one fatal) suggested a likely incubation period of 9 to 16 days, with exposure occurring some 3 days before the onset of symptoms of HeV infection in the horse.(8)

<H2>Acute Clinical Features

The first two patients infected with HeV presented with fever, myalgia, headaches, lethargy, and vertigo. One patient recovered; however, the other developed pneumonitis, respiratory failure, renal failure, and arterial thrombosis, and died of cardiac arrest 7 days after admission to the hospital. Findings at autopsy were consistent with a viral infection; both lungs were congested, hemorrhagic, and filled with serous fluid, and the histology revealed focal necrotizing alveolitis with many giant cells, some syncytial formation, and viral inclusions.(1) Although the predominant clinical features of NiV encephalitis derived from CNS involvement in the initial Malaysia outbreak, a proportion (40%) of patients displayed pulmonary involvement, which presented as an atypical pneumonia with fever, cough, and headache.(12,108,136,178) In the Kerala, India NiV outbreak, 19 cases (83%) presented with acute respiratory distress syndrome (ARDS) and shortness of breath, and 20 cases (87%) had respiratory symptoms.(17) The clinical presentation of NiV infections in Bangladesh also shows severe respiratory disease involvement.(226) A chest X-ray of an acute NiV-infected human case showing diffuse bilateral opacities of the lung fields consistent with ARDS (Fig. 14.12A) and a similar presentation was reported in the first fatal human HeV case.(1) Chest X-ray images of NiV and HeV-infected AGMs have also demonstrated severe respiratory disease (Fig. 14.12B). Case definitions for suspected NiV infection in Bangladesh(226) and India(15,17) generally included individuals residing in an outbreak area who presented with fever, headache, vomiting or altered mental status or with history of cough or shortness of breath and fever.

<FIG14.12>

With NiV in Malaysia, most patients presented with acute encephalitis characterized by fever, headache, drowsiness, dizziness, myalgia, and vomiting, and more than 50% had a reduced level of consciousness.(9,12,108,178) The major clinical signs included drowsiness, areflexia, segmental myoclonus, tachycardia, hypertension, pinpoint pupils, and an abnormal doll's eye reflex. Such clinical features as these suggested involvement of the brain stem and upper cervical spinal cord, and were observed more frequently in patients with a reduced level of consciousness.(108,227) In Kerala, India, 2018, NiV-infected patients presented with altered sensorium (74%), along with myalgia, headache, vomiting, and seizures.(17) Patients in the NiV-Malaysia outbreak who retained normal levels of consciousness throughout their illness recovered fully; however, only 15% with reduced levels of consciousness survived. Fig.14.13 shows magnetic resonance imaging (MRI) images of human cases of acute NiV encephalitis. Such neurologic manifestations are consistent with vasculitis-induced thrombosis in the brain and the direct infection of neurons.(9,11,12,136) The multiple discrete lesions 1 to 5 mm in diameter in the cerebral white matter detected by MRI may be the site of such microinfarctions and are distinct from lesions caused by other viruses.(9,12,140,196,228) A third case of HeV infection presented first with meningitis and a 12-day history of sore throat, headache, drowsiness, vomiting, and neck stiffness. After an apparent full recovery, this patient developed fatal encephalitis 13 months later and was admitted to the hospital with a generalized tonic-clonic seizure after 2 weeks of irritable mood and low back pain. Recurrent focal motor seizures occurred over the next 7 days, as did secondarily generalized seizures and low-grade fever, followed by dense right hemiplegia, signs of brain stem involvement, and depressed consciousness requiring intubation. The patient remained comatose and died 25 days after admission.(3) Two patients in 2008(8) presented with initial influenza-like illness, although soon after apparent clinical improvement and an absence of fever, encephalitis developed in both. MRI revealed widespread cortical, subcortical, and deep white matter involvement, similar to the previous late-onset case of HeV encephalitis(3) and to NiV encephalitis cases (Fig. 14.13B).(108,196,229)

<FIG14.13>

<H2>Outcome of Infection

Most patients who survived acute NiV encephalitis in Malaysia made a full recovery; however, approximately 20% had residual neurologic deficits.(108,178,230) Neurologic sequelae included cognitive difficulties, tetraparesis, cerebellar signs, nerve palsies, and clinical depression. A few patients remained in a vegetative state. In patients with encephalitis who recovered, most brain lesions revealed by MRI disappeared or became smaller over a period of 12 to 18 months, although some remained unchanged.(230) Approximately 7.5% of patients who recovered from acute encephalitis and 3.4% of those who experienced nonencephalitic or asymptomatic infection developed late neurologic disease (Fig. 14.13).(108,129,196,197) Relapse encephalitis and late-onset encephalitis presented several months to 4 years after the initial infection, with the longest reported case 11 years after an initial acute infection.(231) Relapsed cases had elevated IgG, but not IgM, and no vasculitis, and unlike the situation in acute encephalitis, virus was not isolated from throat and nasal secretions.(9,107,136,196-198)

The clinical features associated with relapse and late-onset encephalitis resembled those found with acute NiV encephalitis, although decreased incidence was seen of fever, coma, segmental myoclonus, and meningism and an increased occurrence of seizures and focal cortical signs compared with the acute manifestation of the disease.(129) The clinical, radiologic, and pathologic features of relapse NiV encephalitis resembled those of the patient who became infected with HeV, suffered mild, transient aseptic meningitis, and recovered but died of a fatal meningoencephalitis 13 months later.(3,140) Most patients with relapse and late-onset encephalitis had only one neurologic episode, although some patients experienced two episodes separated by a mean of 7.6 months (6 weeks to 1 year).(129) The mortality rate associated with relapse and late-onset encephalitis at 18% was lower than that associated with acute encephalitis, at 30% to 40%. However, 61% of patients with relapse and late onset had further neurologic sequelae compared with 22% after acute encephalitis. Among NiV survivors in Bangladesh, some 30% have moderate to severe persistent neurologic dysfunction for years following acute infection.(196)

The demographics, clinical features, serology, and MRI of patients with relapsed and late-onset encephalitis were similar, suggesting that the two diseases have identical pathogenesis(129) and that the initial infection in late-onset encephalitis patients may not have been sufficiently severe to cause neurologic symptoms. MRI abnormalities similar to those observed in patients with acute encephalitis were also seen in 16% of asymptomatic patients, although the lesions were fewer in number. (227) The involvement of the cortex in relapse and late-onset encephalitis suggests a different pathologic mechanism compared with acute encephalitis. Relapse and late-onset encephalitis are considered to be caused by the recrudescence and rapid replication of virus that had persisted following acute or asymptomatic NiV infection.(129) NiV, however, was not isolated from CSF and brain tissue of patients with relapse and late-onset encephalitis.(129)

<FIG14.14>

The generalized understanding of the infection process along with the clinical features of human NiV and HeV infection is diagrammed in Fig. 14.14. Transmission is likely via the oronasal route as described above (Pathogenesis section). The initial site of replication in humans is likely within the respiratory system and disease onset is characterized by fever, myalgia, shortness of breath, cough, which may progress to ARDS. Possible invasion into the CNS via the olfactory bulb has been demonstrated in non-primate animal models. [133,139,141] Virus replication and hematogenous systemic spread occurs (cell-free or cell-associated viremia) and established infection is characterized by a widespread vasculitis with endothelial and smooth muscle cell tropism resulting in multinucleated syncytial cells.(136) CNS infection and encephalitis results in altered sensorium, headache, vomiting, and seizures along with fever and other systemic symptoms. Infection of other organs in addition to lung and brain in human cases occurs and has been reported in kidney, heart, spleen and lymphoid tissues,(136) and similar widespread infection has been demonstrated in multiple animal models.(131)

<H1>Diagnosis

Virus isolation, EM, immuno-EM, immunohistochemistry (IHC), serology, and polymerase chain reaction (PCR) played key roles in the initial discovery of HeV(2) and NiV.(11) They remain essential elements in the repertoire of procedures for the rapid and specific diagnosis of henipavirus infections in humans and animals.

During investigation of a suspected disease outbreak, attempts to grow henipaviruses may be initiated in a BSL3 laboratory. However, if a cytopathic effect (CPE) is observed and the growth of henipavirus is confirmed by PCR or immune staining, infected cultures needs to be handled under BSL4 conditions and subsequent work with live virus restricted to BSL4. Both HeV and NiV replicate in various cell lines—a feature that contributed to the efficiency with which they were isolated during the initial disease outbreak investigations.(2,11) Vero cells are commonly used, generating titers of virus as high as 10^8 infectious virions per milliliter.(153,232) In fatal cases, attempts should be made to isolate virus from brain, lung, kidney, and spleen.(232) For tissue specimens containing a high virus load, direct examination by immuno-EM and IHC can be very useful in providing early diagnosis. Various antibody reagents have been developed for this purpose, including polyclonal antisera, monospecific antibodies raised against recombinant antigens,(20) and monoclonal antibodies (mAb) raised against whole virions or vaccinia virus–expressed viral proteins.(233-236) Using HeV- or NiV-specific mAb, it is possible to differentiate between the two viruses.(235,236) Quantitative real-time PCR (TaqMan assay) has been the method of choice to detect viral materials in infected tissues because of its speed, specificity, and sensitivity. The first-generation henipavirus TaqMan assays are either HeV specific(237) or NiV specific.(238) Since then, several consensus henipavirus real-time PCR assays have been developed that target different conserved regions of the viral genome.(239) It should be cautioned that the current PCR tests may not work with new henipaviruses yet to be discovered, especially those from African bats, owing to expected greater genetic divergence than those detected in Australia and Asia.

For henipaviruses, serologic tests are important both during outbreak investigation and for disease surveillance. The virus neutralization test (VNT) is accepted as the reference standard.(232) Few laboratories, however, can conduct neutralization tests because of the requirement to handle live virus at BSL4. For surveillance and diagnostic purposes, three types of tests that do not require BSL4 containment have been developed:

1. *Enzyme-linked immunosorbent assay (ELISA):* Several ELISA-based tests have been reported for the detection of henipavirus antibodies.(232,240,241) For diagnosis of human infections, two different ELISA tests have been applied: an IgM capture ELISA for early diagnosis of infection and an indirect ELISA for detection of IgG antibodies.(232)

2. *Liquid protein array multiplex test:* A Luminex-based test based on recombinant soluble G proteins of HeV and NiV was developed that is capable of mimicking VNT with great sensitivity and differentiating between antibody responses of HeV versus NiV infection.(242)
3. *Pseudotype virus:* Different pseudotype systems carrying the henipavirus F and G proteins have been developed as a surrogate VNT for detecting henipavirus-specific antibodies.(18,243-245) Incorporation of reporter genes in these systems resulted in greater sensitivity and reproducibility.

<H1>Prevention, and Control

<H2>Drugs and Small molecules

Ribavirin, which inhibits replication of HeV *in vitro*,(246) was used during the NiV outbreak in Malaysia in an open-label study in which 140 patients with encephalitis were given the drug, and 54 patients who presented before ribavirin became available or who refused treatment acted as controls.(247) Mortality in the treated group was 32% compared with 54% in the control group, representing a 35% reduction ($P = 0.011$). Duration of ventilation and total hospital stay were both significantly shorter in the ribavirin group ($P = 0.0002$ and <0.0001, respectively). In the absence of other therapies, ribavirin may be an option for treatment of henipavirus infections. However, two HeV-infected patients in 2008(8) were given a high-dose intravenous regimen of ribavirin, although basal concentrations appeared inadequate given the results of *in vitro* susceptibility testing of HeV, and the efficacy of ribavirin as therapy or prophylaxis in people remains at best uncertain. Chloroquine, an antimalarial drug, was first demonstrated to block the critical proteolytic processing needed for HeV F maturation and function.(248) Not surprisingly, the drug was later shown to inhibit NiV and HeV infection in cell culture experiments.(249) Chloroquine was administered along with ribavirin to one HeV-infected individual in 2009(250) with no apparent clinical benefit. Remdesivir (GS-5734) is a nucleotide analog prodrug that showed therapeutic efficacy in a nonhuman primate model of Ebola virus infection.(251) GS-5734 has been administered under compassionate use to Ebola virus infected patients and is currently in Phase 2 clinical development for treatment of Ebola virus disease. The antiviral activities of GS-5734 has been demonstrated to be broad showing *in vitro* activity against viruses in the *Coronaviridae*, *Filoviridae* and the *Paramyxoviridae* including NiV and HeV.(252)

In vivo, ribavirin only delayed but did not prevent deaths caused by NiV and had no effect on HeV infection in a hamster model.(173,253) Ribavirin treatment also only delayed disease onset by 1 to 2 days in African green monkeys challenged with HeV with no significant benefit for disease progression or outcome.(103) Chloroquine administration, either alone or in combination with ribavirin, had no therapeutic benefit in ferrets challenged with NiV or hamsters challenged with either NiV or HeV.(253,254) Poly(I)-poly(C12U), which induces IFN-α and -β production and can completely block NiV replication *in vitro*, displayed ~80% protective efficacy in the hamster model of lethal NiV infection.(173)

<H2>Peptide Fusion Inhibitors

The first potential henipavirus-specific therapeutic was shown to be a heptad peptide-based fusion inhibitor,(95) analogous to HIV-1-specific peptide, enfuvirtide (Fuzeon) approved by the Food and Drug Administration (FDA) in March 2003. The henipavirus F_1 glycoprotein resembles other fusion glycoproteins in having α-helical heptad repeat (HR) domains proximal to both the fusion peptide at the amino (N) terminus and the transmembrane domain near the carboxy (C) terminus of the protein. The HR domains are involved in the formation of a trimer-of-hairpins structure during the membrane fusion and virus infection process. Addition of exogenous peptide from either HR domain blocks formation of the trimer-of-hairpins and abrogates membrane fusion and virus infection.(94,95,255) These observations were followed up with testing cholesterol tagged HR-derived peptides in the hamster model of NiV infection providing the first evidence of *in vivo* effectiveness of a fusion inhibiting peptide against NiV.(256)

<H2>Vaccines

A wide variety of immunization strategies have been developed for NiV and HeV prevention and include live-recombinant virus platforms, protein subunit, virus-like particles and DNA vaccines, however many of these approaches have only been examined for HeV- or NiV-specific neutralizing antibody induction.(257) A more limited number of vaccine candidates have been examined for both immune response and efficacy using a variety of animal challenge models (Table 14.5). Attenuated vaccinia virus strain NYVAC recombinants encoding either the NiV F or G glycoproteins used to immunize hamsters provided complete protection from NiV-mediated disease following challenge and demonstrated that an immune response to the viral envelope glycoproteins were an important immunogen in protection.(258) A second poxvirus-based approach was developed as a potential livestock vaccine using recombinant canarypox virus with the NiV F and G genes inserted into ALVAC vectors (Table 14.5). ALVAC vectors expressing either NiV F or NiV G were tested individually and in combination by vaccination of piglets, and animals were challenged intranasally with NiV. Piglets were protected from NiV-mediated illness by either ALVAC vector alone or in combination and immunized animals shed only low levels of nucleic acid detectable virus and no recoverable virus was evident.(259) Three other viral vector-based NiV vaccines have been examined in animal challenge studies (Table 14.5). An adeno-associated virus (AAV) platform using the NiV G glycoprotein was tested in the hamster model using NiV challenge, showing complete protection against NiV, but only low level cross-protection (3 of 6 animals) against a HeV challenge.(260) A recombinant measles virus vector encoding the NiV G glycoprotein was examined using two different vectors; the HL (rMV-HL-G) and Edmonston (rMV-Ed-G) measles virus strains(261) (Table 14.5). Here, complete protection from NiV disease was achievable in the hamster challenge model following vaccination with either rMV-HL-G or rMV-Ed-G, and the rMV-Ed-G was tested in the nonhuman primate model demonstrating 2 of 2 AGMs were protected against NiV challenge. Several groups have tested the vesicular stomatitis virus (VSV) based platform using the NiV F or G glycoproteins (Table 14.5). In the ferret model, a single immunization with VSV encoding either NiV F or NiV G could afford complete protection from NiV challenge.(262) Similarly, NiV G or F glycoproteins in VSV vectors were successful in protection from NiV challenge in the hamster model,(263,264) and a single immunization of NiV G encoding VSV was protective in the AGM model.(265,266)

<TAB14.5>

The most extensively tested vaccine against NiV and HeV has been a recombinant protein subunit strategy because of the inherent safety of such an approach. Soluble, secreted, oligomeric forms of the G glycoprotein (sG) from both NiV and HeV were developed and tested as possible vaccine candidates and the HeV-sG was show capable of eliciting a more potent cross-reactive polyclonal antibody response and also a better cross-protective response(267,268) (Table 14.5). HeV-sG is a soluble and secreted version of the molecule with a genetically deleted transmembrane and cytoplasmic tail that is produced in mammalian cell culture systems and is properly N-linked glycosylated(269) (Fig. 14.15). HeV-sG has been shown to retain many native characteristics including oligomerization and ability to bind ephrin receptors,(62,267) and is capable of eliciting potent cross-reactive (HeV and NiV) neutralizing antibody responses in a variety of animals including mice, rabbits, cats, ferrets, monkeys and horses.(257) The HeV-sG vaccine in the cat model provided complete protection against a lethal NiV challenge(268) and suggested a single subunit vaccine (HeV-sG) could be effective against both HeV and NiV. Further studies in the cat model revealed that antibody titers as low as 1:32 could protect against NiV-Malaysia challenge(270) (Table 14.5). Studies in the ferret model with low vaccine doses of HeV-sG formulated in CpG and Allhydrogel^{TM} could completely protect against high dose HeV challenge.(106) Immunization and challenge studies in ferrets with the NiV-Bangladesh strain also demonstrated complete protection.(271) In addition, good durable immunity was shown in other ferrets challenged 434 days post-vaccination.(271) However, preliminary studies with HeV-sG as a vaccine in the pig model was less effective against HeV and un-protective against NiV, and both humoral and cellular immune responses were required for protection of swine against henipaviruses in that challenge model.(180)

<FIG14.15>

The HeV-sG vaccine has also been extensively evaluated in nonhuman primates (AGMs). Monkeys immunized with HeV-sG formulated in Allhydrogel^{TM} and CpG were completely protected against intratracheal challenge with NiV-Malaysia, some with pre-challenge neutralizing titers as low as 1:28, and no evidence of clinical disease, virus replication, or pathology was observed in any vaccinated monkeys.(272) Similarly, HeV-sG vaccination and protection from a HeV challenge in AGMs has also been shown, including HeV-sG formulated in Allhydrogel^{TM} alone.(273) The inherent safety and effectiveness of the HeV-sG vaccine led to its development as an equine vaccine to prevent not only HeV infection of horses but also as the means to reduce the risk of HeV transmission to people (Figure 14.16A). HeV-sG was licensed by Zoetis™ Inc., (formerly Pfizer Animal Health) and developed as an equine vaccine for use in Australia. Horse HeV-sG vaccination and HeV challenge studies were conducted at the BSL-4 facilities of the Animal Health Laboratories (AAHL) in Geelong(274) (Table 14.5). HeV-sG was formulated in an approved equine adjuvant (Zoetis, Inc.) and two initial efficacy studies in horses tested 50 μg and 100 μg doses of the same HeV-sG used in published animal studies was used to immunize horses. Additional studies used a 100 μg dose of HeV-sG produced in CHO cells (Zoetis, Inc.).

Immunizations were two doses given intramuscularly 3-weeks apart. All horses in these efficacy studies were challenged oronasally with 2×10^6 $TCID_{50}$ of HeV. Seven horses were challenged at 28 days, and 3 horses were challenged 194 days, after the second immunization. All vaccinated horses remained clinically healthy following challenge, demonstrating protection with pre-challenge HeV neutralizing titers as low as 1:16.(274) There was no gross or histologic evidence of HeV infection in any of the vaccinated horses at study completion, and all tissues examined were negative for HeV antigen by immunohistochemistry, with no viral genome detected in any tissue. In 9 of 10 vaccinated horses, HeV nucleic acid was not detected in daily nasal, oral, or rectal swab specimens or from blood, urine, or feces samples collected before euthanasia, and no recoverable virus was present.(274)

<FIG14.16>

Equivac® HeV was launched in November 2012 on a Minor Use Permit by the regulatory authority, the Australian Pesticides and Veterinary Medicines Authority (APVMA), and is the first commercially developed and deployed vaccine against a BSL-4 agent and is currently the only licensed antiviral approach for henipavirus infection. All vaccinated horses receive a microchip and database is maintained, and in August, 2015 Equivac® HeV received full registration by the APVMA. To date, >640,000 doses of Equivac® HeV vaccine has been administered to >153,000 unique horses (Fig. 14.16B). Since vaccine release, laboratory confirmed HeV infections in horses (n=21) have only occurred in unvaccinated horses. The HeV-sG recombinant subunit vaccine for NiV and HeV is now in clinical development with support from the Coalition for Epidemic Preparedness Innovations (CEPI)(275) and Phase I clinical trials set to commence in 2019. Recent vaccination and challenge studies in nonhuman primates have shown that a single immunizing dose of HeV-sG in Allhydrogel™ can protect against HeV and NiV-Bangladesh challenge (Geisbert and Broder, unpublished) and together with the results of recombinant

VSV-based NiV vaccines, these findings highlight their potential usefulness in an emergency-use or outbreak scenario.

<H2>Passive immunization

Initial passive immunization studies using either NiV G and F-specific polyclonal antiserums, or mouse monoclonal antibodies (mAbs) specific for the NiV or HeV G or F glycoproteins were shown to be protective in the hamster model.(102,258,276) HeV- and NiV-neutralizing human mAbs reactive to the G glycoproteins of both HeV and NiV have been developed using recombinant antibody technology.(277) One mAb, m102.4, possessed strong cross-reactive neutralizing activity against HeV and NiV and was later produced in an IgG1 format in a CHO-K1 cell line.(278) The m102.4 mAb epitope maps to the receptor binding site of G and engages G in a similar fashion as the ephrin receptors(279) (Figure 14.5). The m102.4 mAb can neutralize all HeV and NiV isolates tested, including HeV-1994, HeV-Redlands, NiV-Malaysia and NiV-Bangladesh.(97,280) NiV challenge experiments in the ferret demonstrated complete protection was possible by a single dose of m102.4 mAb administered by intravenous infusion 10 hrs following a lethal virus challenge.(97) The therapeutic efficacy of mAb m102.4 has also been examined in nonhuman primates against both NiV and HeV challenge as a post-exposure therapeutic reflective of a potential real life virus exposure scenario(193,194) (Table 14.5). In one study, AGMs were challenged intratracheally with HeV and following virus administration they were infused twice with m102.4 (~15 mg/kg) beginning at 10 hrs, 24 hrs or 72 hrs post-infection followed by a the second infusion ~48 hrs after the first. In this study, all subjects became infected following challenge, and all animals that received m102.4 survived whereas all controls succumbed to severe systemic disease 8 days following virus challenge. Animals in a 72 hrs treatment group did exhibit neurological signs but all recovered by day 16, but there was no evidence of HeV-specific pathology in any of the m102.4-treated animals, and no infectious HeV could be recovered from any tissues from any m102.4-treated subjects. A later study evaluated the efficacy of m102.4 against NiV-Malaysia challenge in AGMs, at several time points after virus exposure by intratracheal challenge including as late as the onset of clinical illness.(194) Here, animals were infused twice with m102.4 (15 mg/kg) beginning at either 1, 3, or 5 days following NiV-Malaysia challenge and again 48 hrs after the first dose. All animals became infected following virus exposure, and again all subjects that received m102.4 therapy survived the infection, whereas the untreated control subjects succumbed to disease between days 8 and 10 following virus challenge. The m102.4 mAb was also shown to be effective the nonhuman primate model against NiV-Bangladesh infection, although its therapeutic window was much shorter as compared to NiV-Malaysia infected subjects.(191) Taken together, these studies have shown that m102.4 mAb therapy can prevent HeV or NiV lethal disease in exposed subjects is the only effective post-exposure therapeutic tested *in vivo* in nonhuman primates. The mAb m102.4 has since been used by emergency protocol in 13 people in Australia and one in the U.S. because of significant risk of HeV or NiV infection.(257) The m102.4 antibody has completed a Phase I clinical trial in Australia, and has recently been supplied to Government of India and included in an international Nipah treatment protocol sponsored by the Indian Council of Medical Research (ICMR) with support from the NIAID, NIH, USA.

<H1>Perspective

Nipah and henipaviral diseases remain on the latest WHO list of Blueprint priority diseases (Feb 2018).(281) This acknowledges that NiV (or related virulent henipaviruses) is a major threat to global public health, and calls for increased research and development into henipaviruses and their countermeasures, including surveillance and diagnostics. In addition, both NiV and HeV emergence and reemergence remain continuous infectious disease transboundary threats to economically important livestock throughout South Asia and

Australia.(282) NiV is also one of the top three viral agents prioritized for vaccine development by the Coalition of Epidemic Preparedness Innovations (CEPI). (275,283) The high virulence of the henipaviruses and the requirement for BSL-4 facilities have hampered investigations into the biology and pathogenesis of these novel paramyxoviruses. Nonetheless, the advent of reverse genetics for henipaviruses, coupled with the development of animal models that recapitulate the clinical features and pathology seen in virus-infected humans, have provided insights into the functions of many henipavirus proteins and the role they play in the pathogenesis of henipavirus disease. While the virulence of henipaviruses necessitates the initial functional characterization of henipavirus proteins from cloned genes, it is imperative that these functional characteristics be examined in the context of a live virus infection. Even then, it appears increasingly important to investigate why henipaviruses appear so much more virulent in many of their terrestrial hosts versus their chiropteran counterparts.(284)

Despite our expanded understanding of the henipaviruses, many questions relating to the ecology and biology of henipaviruses remain unanswered. Pteropus species of fruit bats are clearly the major reservoir host of these viruses,(202,207,208) but new studies have suggested that an even wider group of chiropteran species host not only the known henipaviruses but also multiple divergent clades of new henipavirus species.(29,222,285) Molecular or serological evidence of henipaviruses can now be found in frugivorous and insectivorous bats that range from Australia and Austronesia, to China, South-East Asia, countries of the Indian subcontinent, through to Madagascar, sub-Saharan Africa and even into the New World (Costa Rica and Brazil).(19,29,207,286-297) The emergence of these and related viruses is probably associated with the destruction of the flying fox native habitats, driving the animals to seek food from orchards and ornamental trees in urban and periurban areas. Continued deforestation and amplification of factors that drive zoonotic spillover from reservoir hosts, will undoubtedly lead to further outbreaks of HeV, NiV, and novel related members of the genus.(298-301) Continued surveillance coupled with identification and characterization of these putative new species of henipavirus is required provide a proper assessment as to the countermeasures, such as the CEPI initiatives, that would be necessary to control or prevent future outbreaks and spillovers.

<H1>References

1. Selvey LA, Wells RM, McCormack JG, et al. Infection of humans and horses by a newly described morbillivirus. *Med J Aust.* 1995;162(12):642-645.
2. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science.* 1995;268(5207):94-97.
3. O'Sullivan JD, Allworth AM, Paterson DL, et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet.* 1997;349(9045):93-95.
4. Rogers RJ, Douglas IC, Baldock FC, et al. Investigation of a second focus of equine morbillivirus infection in coastal Queensland. *Aust Vet J.* 1996;74(3):243-244.
5. Young PL, Halpin K, Selleck PW, et al. Serologic evidence for the presence in Pteropus bats of a paramyxovirus related to equine morbillivirus. *Emerg Infect Dis.* 1996;2(3):239-240.
6. Halpin K, Young PL, Field HE, et al. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol.* 2000;81(Pt 8):1927-1932.
7. Summary of Hendra virus incidents in horses. Queensland Government. Business Queensland Web site. https://www.business.qld.gov.au/industries/service-industries-professionals/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary. Published 2018. Accessed 20 Sep 2018.
8. Playford EG, McCall B, Smith G, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis.* 2010;16(2):219-223.
9. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet.* 1999;354(9186):1257-1259.
10. Mohd Nor MN, Gan CH, Ong BL. Nipah virus infection of pigs in peninsular Malaysia. *Rev Sci Tech.* 2000;19(1):160-165.
11. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science.* 2000;288(5470):1432-1435.
12. Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet.* 1999;354(9186):1253-1256.
13. Chua KB, Koh CL, Hooi PS, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect.* 2002;4(2):145-151.
14. Hsu VP, Hossain MJ, Parashar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis.* 2004;10(12):2082-2087.
15. Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis.* 2006;12(2):235-240.
16. Harit AK, Ichhpujani RL, Gupta S, et al. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. *Indian J Med Res.* 2006;123(4):553-560.
17. Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis.* 2018.
18. Ching PK, de los Reyes VC, Sucaldito MN, et al. Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis.* 2015;21(2):328-331.

19. Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. *Nat Commun.* 2014;5:5342.
20. Wang LF, Michalski WP, Yu M, et al. A novel P/V/C gene in a new member of the Paramyxoviridae family, which causes lethal infection in humans, horses, and other animals. *J Virol.* 1998;72(2):1482-1490.
21. Wang LF, Yu M, Hansson E, et al. The exceptionally large genome of Hendra virus: support for creation of a new genus within the family Paramyxoviridae. *J Virol.* 2000;74(21):9972-9979.
22. Harcourt BH, Tamin A, Halpin K, et al. Molecular characterization of the polymerase gene and genomic termini of Nipah virus. *Virology.* 2001;287(1):192-201.
23. Harcourt BH, Tamin A, Ksiazek TG, et al. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. *Virology.* 2000;271(2):334-349.
24. Wang L, Harcourt BH, Yu M, et al. Molecular biology of Hendra and Nipah viruses. *Microbes Infect.* 2001;3(4):279-287.
25. Lamb RA, Collins PL, Kolakofsky D, et al. Family Paramyxoviridae. In: Fauquet CM, Mayo J, Maniloff J, Desselberger U, Ball LA, eds. *Virus Taxonomy: 8th Report of the International Committee on Taxonomy of Viruses.* San Diego: Elsevier Academic Press; 2005:655-668.
26. Amarasinghe GK, Arechiga Ceballos NG, Banyard AC, et al. Taxonomy of the order Mononegavirales: update 2018. *Arch Virol.* 2018;163(8):2283-2294.
27. Maes P, Amarasinghe GK, Ayllon MA, et al. Taxonomy of the order Mononegavirales: second update 2018. *Arch Virol.* 2019.
28. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog.* 2012;8(8):e1002836.
29. Drexler JF, Corman VM, Muller MA, et al. Bats host major mammalian paramyxoviruses. *Nat Commun.* 2012;3:796.
30. Wu Z, Yang L, Yang F, et al. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis.* 2014;20(6):1064-1066.
31. Goldsmith CS, Whistler T, Rollin PE, et al. Elucidation of Nipah virus morphogenesis and replication using ultrastructural and molecular approaches. *Virus Res.* 2003;92(1):89-98.
32. Hyatt AD, Zaki SR, Goldsmith CS, et al. Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. *Microbes Infect.* 2001;3(4):297-306.
33. Harcourt BH, Lowe L, Tamin A, et al. Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis.* 2005;11(10):1594-1597.
34. Halpin K, Bankamp B, Harcourt BH, et al. Nipah virus conforms to the rule of six in a minigenome replication assay. *J Gen Virol.* 2004;85(Pt 3):701-707.
35. Rima BK, Balkema-Buschmann A, Dundon WG, et al. Family Paramyxoviridae Classification and features. *J Gen Virol.* 2019;in press.
36. Hino K, Sato H, Sugai A, et al. Downregulation of Nipah virus N mRNA occurs through interaction between its 3' untranslated region and hnRNP D. *J Virol.* 2013;87(12):6582-6588.
37. Michalski WP, Crameri G, Wang L, et al. The cleavage activation and sites of glycosylation in the fusion protein of Hendra virus. *Virus Res.* 2000;69(2):83-93.
38. Lo MK, Harcourt BH, Mungall BA, et al. Determination of the henipavirus phosphoprotein gene mRNA editing frequencies and detection of the C, V and W proteins of Nipah virus in virus-infected cells. *J Gen Virol.* 2009;90(Pt 2):398-404.
39. Basler CF. Nipah and hendra virus interactions with the innate immune system. *Curr Top Microbiol Immunol.* 2012;359:123-152.
40. Parks GD, Alexander-Miller MA. Paramyxovirus activation and inhibition of innate immune responses. *J Mol Biol.* 2013;425(24):4872-4892.
41. Park A, Yun T, Vigant F, et al. Nipah Virus C Protein Recruits Tsg101 to Promote the Efficient Release of Virus in an ESCRT-Dependent Pathway. *PLoS Pathog.* 2016;12(5):e1005659.

42. Freiberg A, Dolores LK, Enterlein S, et al. Establishment and characterization of plasmid-driven minigenome rescue systems for Nipah virus: RNA polymerase I- and T7-catalyzed generation of functional paramyxoviral RNA. *Virology.* 2008;370(1):33-44.
43. Yun T, Park A, Hill TE, et al. Efficient reverse genetics reveals genetic determinants of budding and fusogenic differences between Nipah and Hendra viruses and enables real-time monitoring of viral spread in small animal models of henipavirus infection. *J Virol.* 2015;89(2):1242-1253.
44. Yoneda M, Guillaume V, Ikeda F, et al. Establishment of a Nipah virus rescue system. *Proc Natl Acad Sci U S A.* 2006;103(44):16508-16513.
45. Ciancanelli MJ, Volchkova VA, Shaw ML, et al. Nipah virus sequesters inactive STAT1 in the nucleus via a P gene-encoded mechanism. *J Virol.* 2009;83(16):7828-7841.
46. Yoneda M, Guillaume V, Sato H, et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One.* 2010;5(9):e12709.
47. Lo MK, Peeples ME, Bellini WJ, et al. Distinct and overlapping roles of Nipah virus P gene products in modulating the human endothelial cell antiviral response. *PLoS One.* 2012;7(10):e47790.
48. Marsh GA, Virtue ER, Smith I, et al. Recombinant Hendra viruses expressing a reporter gene retain pathogenicity in ferrets. *Virol J.* 2013;10:95.
49. Dietzel E, Kolesnikova L, Sawatsky B, et al. Nipah Virus Matrix Protein Influences Fusogenicity and Is Essential for Particle Infectivity and Stability. *J Virol.* 2015;90(5):2514-2522.
50. Satterfield BA, Cross RW, Fenton KA, et al. The immunomodulating V and W proteins of Nipah virus determine disease course. *Nat Commun.* 2015;6:7483.
51. Bharaj P, Wang YE, Dawes BE, et al. The Matrix Protein of Nipah Virus Targets the E3-Ubiquitin Ligase TRIM6 to Inhibit the IKKepsilon Kinase-Mediated Type-I IFN Antiviral Response. *PLoS Pathog.* 2016;12(9):e1005880.
52. Satterfield BA, Cross RW, Fenton KA, et al. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J Virol.* 2016;90(14):6326-6343.
53. Laing ED, Amaya M, Navaratnarajah CK, et al. Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J.* 2018;15(1):56.
54. Poch O, Blumberg BM, Bougueleret L, et al. Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. *J Gen Virol.* 1990;71 (Pt 5):1153-1162.
55. Miller PJ, Boyle DB, Eaton BT, et al. Full-length genome sequence of Mossman virus, a novel paramyxovirus isolated from rodents in Australia. *Virology.* 2003;317(2):330-344.
56. Liang B, Li Z, Jenni S, et al. Structure of the L Protein of Vesicular Stomatitis Virus from Electron Cryomicroscopy. *Cell.* 2015;162(2):314-327.
57. Lamb RA, Parks GD. *Paramyxoviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, eds. *Fields Virology.* Vol 6th Edition. Philadelphia: Lippincott Williams & Wilkins; 2013:957-995.
58. Langedijk JP, Daus FJ, van Oirschot JT. Sequence and structure alignment of Paramyxoviridae attachment proteins and discovery of enzymatic activity for a morbillivirus hemagglutinin. *J Virol.* 1997;71(8):6155-6167.
59. Lee B, Ataman ZA. Modes of paramyxovirus fusion: a Henipavirus perspective. *Trends Microbiol.* 2011;19(8):389-399.
60. Yu M, Hansson E, Langedijk JP, et al. The attachment protein of Hendra virus has high structural similarity but limited primary sequence homology compared with viruses in the genus Paramyxovirus. *Virology.* 1998;251(2):227-233.
61. Bishop KA, Stantchev TS, Hickey AC, et al. Identification of Hendra virus G glycoprotein residues that are critical for receptor binding. *J Virol.* 2007;81(11):5893-5901.
62. Bonaparte MI, Dimitrov AS, Bossart KN, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A.* 2005;102(30):10652-10657.

63. Negrete OA, Levroney EL, Aguilar HC, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature.* 2005;436(7049):401-405.
64. Negrete OA, Wolf MC, Aguilar HC, et al. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog.* 2006;2(2):e7.
65. Lee B, Pernet O, Ahmed AA, et al. Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. *Proc Natl Acad Sci U S A.* 2015;112(17):E2156-2165.
66. Rissanen I, Ahmed AA, Azarm K, et al. Idiosyncratic Mojiang virus attachment glycoprotein directs a host-cell entry pathway distinct from genetically related henipaviruses. *Nat Commun.* 2017;8:16060.
67. Bowden TA, Aricescu AR, Gilbert RJ, et al. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. *Nat Struct Mol Biol.* 2008;15(6):567-572.
68. Bowden TA, Crispin M, Harvey DJ, et al. Dimeric architecture of the Hendra virus attachment glycoprotein: evidence for a conserved mode of assembly. *J Virol.* 2010;84(12):6208-6217.
69. Xu K, Rajashankar KR, Chan YP, et al. Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. *Proc Natl Acad Sci U S A.* 2008;105(29):9953-9958.
70. Bose S, Jardetzky TS, Lamb RA. Timing is everything: Fine-tuned molecular machines orchestrate paramyxovirus entry. *Virology.* 2015;479-480:518-531.
71. Moll M, Diederich S, Klenk HD, et al. Ubiquitous activation of the Nipah virus fusion protein does not require a basic amino acid at the cleavage site. *J Virol.* 2004;78(18):9705-9712.
72. Pager CT, Dutch RE. Cathepsin L is involved in proteolytic processing of the Hendra virus fusion protein. *J Virol.* 2005;79(20):12714-12720.
73. Diederich S, Sauerhering L, Weis M, et al. Activation of the Nipah virus fusion protein in MDCK cells is mediated by cathepsin B within the endosome-recycling compartment. *J Virol.* 2012;86(7):3736-3745.
74. Kruger N, Hoffmann M, Weis M, et al. Surface glycoproteins of an African henipavirus induce syncytium formation in a cell line derived from an African fruit bat, Hypsignathus monstrosus. *J Virol.* 2013;87(24):13889-13891.
75. Pager CT, Craft WW, Jr., Patch J, et al. A mature and fusogenic form of the Nipah virus fusion protein requires proteolytic processing by cathepsin L. *Virology.* 2006;346(2):251-257.
76. Meulendyke KA, Wurth MA, McCann RO, et al. Endocytosis plays a critical role in proteolytic processing of the Hendra virus fusion protein. *J Virol.* 2005;79(20):12643-12649.
77. Vogt C, Eickmann M, Diederich S, et al. Endocytosis of the Nipah virus glycoproteins. *J Virol.* 2005;79(6):3865-3872.
78. Popa A, Carter JR, Smith SE, et al. Residues in the hendra virus fusion protein transmembrane domain are critical for endocytic recycling. *J Virol.* 2012;86(6):3014-3026.
79. Cifuentes-Munoz N, Sun W, Ray G, et al. Mutations in the Transmembrane Domain and Cytoplasmic Tail of Hendra Virus Fusion Protein Disrupt Virus-Like-Particle Assembly. *J Virol.* 2017;91(14).
80. Johnston GP, Contreras EM, Dabundo J, et al. Cytoplasmic Motifs in the Nipah Virus Fusion Protein Modulate Virus Particle Assembly and Egress. *J Virol.* 2017;91(10).
81. El Najjar F, Schmitt AP, Dutch RE. Paramyxovirus glycoprotein incorporation, assembly and budding: a three way dance for infectious particle production. *Viruses.* 2014;6(8):3019-3054.
82. Harrison MS, Sakaguchi T, Schmitt AP. Paramyxovirus assembly and budding: building particles that transmit infections. *Int J Biochem Cell Biol.* 2010;42(9):1416-1429.
83. Wang YE, Park A, Lake M, et al. Ubiquitin-regulated nuclear-cytoplasmic trafficking of the Nipah virus matrix protein is important for viral budding. *PLoS Pathog.* 2010;6(11):e1001186.
84. Monaghan P, Green D, Pallister J, et al. Detailed morphological characterisation of Hendra virus infection of different cell types using super-resolution and conventional imaging. *Virol J.* 2014;11:200.

85. Pentecost M, Vashisht AA, Lester T, et al. Evidence for ubiquitin-regulated nuclear and subnuclear trafficking among Paramyxovirinae matrix proteins. *PLoS Pathog.* 2015;11(3):e1004739.
86. McLinton EC, Wagstaff KM, Lee A, et al. Nuclear localization and secretion competence are conserved among henipavirus matrix proteins. *J Gen Virol.* 2017;98(4):563-576.
87. Bauer A, Neumann S, Karger A, et al. ANP32B is a nuclear target of henipavirus M proteins. *PLoS One.* 2014;9(5):e97233.
88. Hooper P, Zaki S, Daniels P, et al. Comparative pathology of the diseases caused by Hendra and Nipah viruses. *Microbes Infect.* 2001;3(4):315-322.
89. Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. *Emerg Infect Dis.* 2005;11(7):1042-1047.
90. Mills JN, Alim AN, Bunning ML, et al. Nipah virus infection in dogs, Malaysia, 1999. *Emerg Infect Dis.* 2009;15(6):950-952.
91. Field H, Young P, Yob JM, et al. The natural history of Hendra and Nipah viruses. *Microbes Infect.* 2001;3(4):307-314.
92. Kirkland PD, Gabor M, Poe I, et al. Hendra Virus Infection in Dog, Australia, 2013. *Emerg Infect Dis.* 2015;21(12):2182-2185.
93. Middleton DJ, Riddell S, Klein R, et al. Experimental Hendra virus infection of dogs: virus replication, shedding and potential for transmission. *Aust Vet J.* 2017;95(1-2):10-18.
94. Bossart KN, Wang LF, Eaton BT, et al. Functional expression and membrane fusion tropism of the envelope glycoproteins of Hendra virus. *Virology.* 2001;290(1):121-135.
95. Bossart KN, Wang LF, Flora MN, et al. Membrane fusion tropism and heterotypic functional activities of the Nipah virus and Hendra virus envelope glycoproteins. *J Virol.* 2002;76(22):11186-11198.
96. Tamin A, Harcourt BH, Ksiazek TG, et al. Functional properties of the fusion and attachment glycoproteins of Nipah virus. *Virology.* 2002;296(1):190-200.
97. Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute nipah virus infection. *PLoS Pathog.* 2009;5(10):e1000642.
98. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One.* 2010;5(5):e10690.
99. Marianneau P, Guillaume V, Wong T, et al. Experimental infection of squirrel monkeys with nipah virus. *Emerg Infect Dis.* 2010;16(3):507-510.
100. Middleton DJ, Morrissy CJ, van der Heide BM, et al. Experimental Nipah virus infection in pteropid bats (Pteropus poliocephalus). *J Comp Pathol.* 2007;136(4):266-272.
101. Wong KT, Grosjean I, Brisson C, et al. A golden hamster model for human acute Nipah virus infection. *Am J Pathol.* 2003;163(5):2127-2137.
102. Guillaume V, Wong KT, Looi RY, et al. Acute Hendra virus infection: Analysis of the pathogenesis and passive antibody protection in the hamster model. *Virology.* 2009;387(2):459-465.
103. Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. *J Virol.* 2010;84(19):9831-9839.
104. Williamson MM, Hooper PT, Selleck PW, et al. A guinea-pig model of Hendra virus encephalitis. *J Comp Pathol.* 2001;124(4):273-279.
105. Williamson MM, Hooper PT, Selleck PW, et al. Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. *Aust Vet J.* 1998;76(12):813-818.
106. Pallister J, Middleton D, Wang LF, et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine.* 2011;29(34):5623-5630.

107. Chua KB, Lam SK, Goh KJ, et al. The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. *J Infect.* 2001;42(1):40-43.
108. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med.* 2000;342(17):1229-1235.
109. Islam MS, Sazzad HM, Satter SM, et al. Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011-2014. *Emerg Infect Dis.* 2016;22(4):664-670.
110. Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis.* 2006;12(12):1888-1894.
111. Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector Borne Zoonotic Dis.* 2012;12(1):65-72.
112. Clayton BA, Middleton D, Arkinstall R, et al. The Nature of Exposure Drives Transmission of Nipah Viruses from Malaysia and Bangladesh in Ferrets. *PLoS Negl Trop Dis.* 2016;10(6):e0004775.
113. Edson D, Field H, McMichael L, et al. Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PLoS One.* 2015;10(10):e0140670.
114. Middleton DJ, Weingartl HM. Henipaviruses in their natural animal hosts. *Curr Top Microbiol Immunol.* 2012;359:105-121.
115. Homaira N, Rahman M, Hossain MJ, et al. Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One.* 2010;5(10):e13570.
116. Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. *Malays J Pathol.* 2010;32(2):69-73.
117. Luby SP, Gurley ES, Hossain MJ. Transmission of human infection with Nipah virus. *Clin Infect Dis.* 2009;49(11):1743-1748.
118. Fogarty R, Halpin K, Hyatt AD, et al. Henipavirus susceptibility to environmental variables. *Virus Res.* 2008;132(1-2):140-144.
119. Wacharapluesadee S, Hemachudha T. Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats. *J Virol Methods.* 2007;141(1):97-101.
120. Looi LM, Chua KB. Lessons from the Nipah virus outbreak in Malaysia. *Malays J Pathol.* 2007;29(2):63-67.
121. Middleton DJ, Westbury HA, Morrissy CJ, et al. Experimental Nipah virus infection in pigs and cats. *J Comp Pathol.* 2002;126(2-3):124-136.
122. Chakraborty A, Sazzad HM, Hossain MJ, et al. Evolving epidemiology of Nipah virus infection in Bangladesh: evidence from outbreaks during 2010-2011. *Epidemiol Infect.* 2016;144(2):371-380.
123. Hegde ST, Sazzad HM, Hossain MJ, et al. Investigating Rare Risk Factors for Nipah Virus in Bangladesh: 2001-2012. *Ecohealth.* 2016;13(4):720-728.
124. Blum LS, Khan R, Nahar N, et al. In-depth assessment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies. *Am J Trop Med Hyg.* 2009;80(1):96-102.
125. Sazzad HM, Hossain MJ, Gurley ES, et al. Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. *Emerg Infect Dis.* 2013;19(2):210-217.
126. Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerg Infect Dis.* 2007;13(7):1031-1037.
127. Luby SP, Gurley ES. Epidemiology of henipavirus disease in humans. *Curr Top Microbiol Immunol.* 2012;359:25-40.
128. Field HE. Hendra virus ecology and transmission. *Curr Opin Virol.* 2016;16:120-125.
129. Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. *Ann Neurol.* 2002;51(6):703-708.

130. Wong KT, Tan CT. Clinical and pathological manifestations of human henipavirus infection. *Curr Top Microbiol Immunol.* 2012;359:95-104.
131. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol.* 2012;359:153-177.
132. de Wit E, Munster VJ. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *J Pathol.* 2015;235(2):196-205.
133. Baseler L, Scott DP, Saturday G, et al. Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. *PLoS Negl Trop Dis.* 2016;10(11):e0005120.
134. Hammoud DA, Lentz MR, Lara A, et al. Aerosol exposure to intermediate size Nipah virus particles induces neurological disease in African green monkeys. *PLoS Negl Trop Dis.* 2018;12(11):e0006978.
135. Lambrecht BN, Hammad H. Lung dendritic cells in respiratory viral infection and asthma: from protection to immunopathology. *Annu Rev Immunol.* 2012;30:243-270.
136. Wong KT, Shieh WJ, Kumar S, et al. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol.* 2002;161(6):2153-2167.
137. Wong KT, Shieh WJ, Zaki SR, et al. Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer Semin Immunopathol.* 2002;24(2):215-228.
138. Rockx B, Brining D, Kramer J, et al. Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *J Virol.* 2011;85(15):7658-7671.
139. Weingartl H, Czub S, Copps J, et al. Invasion of the central nervous system in a porcine host by nipah virus. *J Virol.* 2005;79(12):7528-7534.
140. Wong KT, Robertson T, Ong BB, et al. Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathol Appl Neurobiol.* 2009;35(3):296-305.
141. Munster VJ, Prescott JB, Bushmaker T, et al. Rapid Nipah virus entry into the central nervous system of hamsters via the olfactory route. *Sci Rep.* 2012;2:736.
142. Borisevich V, Ozdener MH, Malik B, et al. Hendra and Nipah Virus Infection in Cultured Human Olfactory Epithelial Cells. *mSphere.* 2017;2(3).
143. Miao H, Wang B. Eph/ephrin signaling in epithelial development and homeostasis. *Int J Biochem Cell Biol.* 2009;41(4):762-770.
144. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell.* 2008;133(1):38-52.
145. Barquilla A, Pasquale EB. Eph receptors and ephrins: therapeutic opportunities. *Annu Rev Pharmacol Toxicol.* 2015;55:465-487.
146. Bennett KM, Afanador MD, Lal CV, et al. Ephrin-B2 reverse signaling increases alpha5beta1 integrin-mediated fibronectin deposition and reduces distal lung compliance. *Am J Respir Cell Mol Biol.* 2013;49(4):680-687.
147. Genotype-Tissue Expression (GTEx) Portal: dbGaP Accession phs000424.v7.p2. The Broad Institute of MIT and Harvard; 2019. https://gtexportal.org/home/. Accessed 01/31/2019.
148. Gale NW, Baluk P, Pan L, et al. Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. *Dev Biol.* 2001;230(2):151-160.
149. Shin D, Garcia-Cardena G, Hayashi S, et al. Expression of ephrinB2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. *Dev Biol.* 2001;230(2):139-150.
150. Mathieu C, Pohl C, Szecsi J, et al. Nipah virus uses leukocytes for efficient dissemination within a host. *J Virol.* 2011;85(15):7863-7871.
151. Mathieu C, Dhondt KP, Chalons M, et al. Heparan sulfate-dependent enhancement of henipavirus infection. *MBio.* 2015;6(2):e02427.
152. Arunkumar G, Devadiga S, McElroy AK, et al. Adaptive immune responses in humans during Nipah virus acute and convalescent phases of infection. *Clin Infect Dis.* 2019.

153. Chua KB. Nipah virus outbreak in Malaysia. *J Clin Virol.* 2003;26(3):265-275.
154. Taylor C, Playford EG, McBride WJ, et al. No evidence of prolonged Hendra virus shedding by 2 patients, Australia. *Emerg Infect Dis.* 2012;18(12):2025-2027.
155. Andrejeva J, Childs KS, Young DF, et al. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc Natl Acad Sci U S A.* 2004;101(49):17264-17269.
156. Levy DE, Marie IJ. RIGging an antiviral defense--it's in the CARDs. *Nat Immunol.* 2004;5(7):699-701.
157. Jensen S, Thomsen AR. Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion. *J Virol.* 2012;86(6):2900-2910.
158. Matsumoto M, Oshiumi H, Seya T. Antiviral responses induced by the TLR3 pathway. *Rev Med Virol.* 2011;21(2):67-77.
159. Parisien JP, Bamming D, Komuro A, et al. A shared interface mediates paramyxovirus interference with antiviral RNA helicases MDA5 and LGP2. *J Virol.* 2009;83(14):7252-7260.
160. Childs KS, Andrejeva J, Randall RE, et al. Mechanism of mda-5 Inhibition by paramyxovirus V proteins. *J Virol.* 2009;83(3):1465-1473.
161. Shaw ML, Garcia-Sastre A, Palese P, et al. Nipah virus V and W proteins have a common STAT1-binding domain yet inhibit STAT1 activation from the cytoplasmic and nuclear compartments, respectively. *J Virol.* 2004;78(11):5633-5641.
162. Sanchez-Aparicio MT, Feinman LJ, Garcia-Sastre A, et al. Paramyxovirus V Proteins Interact with the RIG-I/TRIM25 Regulatory Complex and Inhibit RIG-I Signaling. *J Virol.* 2018;92(6).
163. Shaw ML, Cardenas WB, Zamarin D, et al. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol.* 2005;79(10):6078-6088.
164. Aaronson DS, Horvath CM. A road map for those who don't know JAK-STAT. *Science.* 2002;296(5573):1653-1655.
165. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol.* 2005;5(5):375-386.
166. Rodriguez JJ, Wang LF, Horvath CM. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. *J Virol.* 2003;77(21):11842-11845.
167. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol.* 2002;76(22):11476-11483.
168. Rodriguez JJ, Cruz CD, Horvath CM. Identification of the nuclear export signal and STAT-binding domains of the Nipah virus V protein reveals mechanisms underlying interferon evasion. *J Virol.* 2004;78(10):5358-5367.
169. Ludlow LE, Lo MK, Rodriguez JJ, et al. Henipavirus V protein association with Polo-like kinase reveals functional overlap with STAT1 binding and interferon evasion. *J Virol.* 2008;82(13):6259-6271.
170. Smith KM, Tsimbalyuk S, Edwards MR, et al. Structural basis for importin alpha 3 specificity of W proteins in Hendra and Nipah viruses. *Nat Commun.* 2018;9(1):3703.
171. Sugai A, Sato H, Takayama I, et al. Nipah and Hendra Virus Nucleoproteins Inhibit Nuclear Accumulation of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT2 by Interfering with Their Complex Formation. *J Virol.* 2017;91(21).
172. Park MS, Shaw ML, Munoz-Jordan J, et al. Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus V, W, and C proteins. *J Virol.* 2003;77(2):1501-1511.
173. Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. *Antimicrob Agents Chemother.* 2006;50(5):1768-1772.

174. Virtue ER, Marsh GA, Wang LF. Interferon signaling remains functional during henipavirus infection of human cell lines. *J Virol.* 2011;85(8):4031-4034.
175. Virtue ER, Marsh GA, Baker ML, et al. Interferon production and signaling pathways are antagonized during henipavirus infection of fruit bat cell lines. *PLoS One.* 2011;6(7):e22488.
176. Rockx B. Recent developments in experimental animal models of Henipavirus infection. *Pathog Dis.* 2014;71(2):199-206.
177. Mathieu C, Guillaume V, Volchkova VA, et al. Nonstructural Nipah virus C protein regulates both the early host proinflammatory response and viral virulence. *J Virol.* 2012;86(19):10766-10775.
178. Chong HT, Kunjapan SR, Thayaparan T, et al. Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. *Can J Neurol Sci.* 2002;29(1):83-87.
179. Cong Y, Lentz MR, Lara A, et al. Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS Negl Trop Dis.* 2017;11(4):e0005532.
180. Pickering BS, Hardham JM, Smith G, et al. Protection against henipaviruses in swine requires both, cell-mediated and humoral immune response. *Vaccine.* 2016;34(40):4777-4786.
181. Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. *Vaccine.* 2016;34(26):2971-2975.
182. Gupta M, Lo MK, Spiropoulou CF. Activation and cell death in human dendritic cells infected with Nipah virus. *Virology.* 2013;441(1):49-56.
183. Berhane Y, Weingartl HM, Lopez J, et al. Bacterial infections in pigs experimentally infected with Nipah virus. *Transbound Emerg Dis.* 2008;55(3-4):165-174.
184. Leon AJ, Borisevich V, Boroumand N, et al. Host gene expression profiles in ferrets infected with genetically distinct henipavirus strains. *PLoS Negl Trop Dis.* 2018;12(3):e0006343.
185. Barclay AJ, Paton DJ. Hendra (equine morbillivirus). *Vet J.* 2000;160(3):169-176.
186. McCormack JG, Allworth AM, Selvey LA, et al. Transmissibility from horses to humans of a novel paramyxovirus, equine morbillivirus (EMV). *J Infect.* 1999;38(1):22-23.
187. Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis.* 2009;15(8):1229-1235.
188. Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect.* 2010;138(11):1630-1636.
189. Mounts AW, Kaur H, Parashar UD, et al. A cohort study of health care workers to assess nosocomial transmissibility of Nipah virus, Malaysia, 1999. *J Infect Dis.* 2001;183(5):810-813.
190. DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. *PLoS Negl Trop Dis.* 2013;7(1):e2024.
191. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep.* 2016;6:30916.
192. Rockx B, Winegar R, Freiberg AN. Recent progress in henipavirus research: molecular biology, genetic diversity, animal models. *Antiviral Res.* 2012;95(2):135-149.
193. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med.* 2011;3(105):105ra103.
194. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med.* 2014;6(242):242ra282.
195. Lieu KG, Marsh GA, Wang LF, et al. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral Res.* 2015;124:69-76.

196. Sarji SA, Abdullah BJ, Goh KJ, et al. MR imaging features of Nipah encephalitis. *AJR Am J Roentgenol.* 2000;175(2):437-442.
197. Wong SC, Ooi MH, Wong MN, et al. Late presentation of Nipah virus encephalitis and kinetics of the humoral immune response. *J Neurol Neurosurg Psychiatry.* 2001;71(4):552-554.
198. Chua KB, Lam SK, Tan CT, et al. High mortality in Nipah encephalitis is associated with presence of virus in cerebrospinal fluid. *Ann Neurol.* 2000;48(5):802-805.
199. Griffin DE, Lin WW, Nelson AN. Understanding the causes and consequences of measles virus persistence. *F1000Res.* 2018;7:237.
200. Watanabe S, Shirogane Y, Sato Y, et al. New Insights into Measles Virus Brain Infections. *Trends Microbiol.* 2019;27(2):164-175.
201. Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. *J Infect Dis.* 2000;181(5):1755-1759.
202. Field HE, Mackenzie JS, Daszak P. Henipaviruses: emerging paramyxoviruses associated with fruit bats. *Curr Top Microbiol Immunol.* 2007;315:133-159.
203. Luby SP. The pandemic potential of Nipah virus. *Antiviral Res.* 2013;100(1):38-43.
204. Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. *J Infect Dis.* 2000;181(5):1760-1763.
205. Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerg Infect Dis.* 2001;7(3):439-441.
206. Anderson DE, Islam A, Crameri G, et al. Isolation and Full-Genome Characterization of Nipah Viruses from Bats, Bangladesh. *Emerg Infect Dis.* 2019;25(1):166-170.
207. Epstein JH, Prakash V, Smith CS, et al. Henipavirus infection in fruit bats (Pteropus giganteus), India. *Emerg Infect Dis.* 2008;14(8):1309-1311.
208. Rahman SA, Hassan SS, Olival KJ, et al. Characterization of Nipah virus from naturally infected Pteropus vampyrus bats, Malaysia. *Emerg Infect Dis.* 2010;16(12):1990-1993.
209. Eaton BT, Broder CC, Middleton D, et al. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol.* 2006;4(1):23-35.
210. Williamson MM, Hooper PT, Selleck PW, et al. Experimental hendra virus infectionin pregnant guinea-pigs and fruit Bats (Pteropus poliocephalus). *J Comp Pathol.* 2000;122(2-3):201-207.
211. Selvey L, Taylor R, Arklay A, et al. Screening of bat carers for antibodies to equine morbillivirus. *Commun Dis Intell* 1996;20:477-478.
212. Khan MS, Hossain J, Gurley ES, et al. Use of infrared camera to understand bats' access to date palm sap: implications for preventing Nipah virus transmission. *Ecohealth.* 2010;7(4):517-525.
213. Chowdhury S, Khan SU, Crameri G, et al. Serological evidence of henipavirus exposure in cattle, goats and pigs in Bangladesh. *PLoS Negl Trop Dis.* 2014;8(11):e3302.
214. Montgomery JM, Hossain MJ, Gurley E, et al. Risk factors for Nipah virus encephalitis in Bangladesh. *Emerg Infect Dis.* 2008;14(10):1526-1532.
215. Mahalingam S, Herrero LJ, Playford EG, et al. Hendra virus: an emerging paramyxovirus in Australia. *Lancet Infect Dis.* 2012;12(10):799-807.
216. Hooper PT, Westbury HA, Russell GM. The lesions of experimental equine morbillivirus disease in cats and guinea pigs. *Vet Pathol.* 1997;34(4):323-329.
217. Murray K, Rogers R, Selvey L, et al. A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis.* 1995;1(1):31-33.
218. Arankalle VA, Bandyopadhyay BT, Ramdasi AY, et al. Genomic characterization of Nipah virus, West Bengal, India. *Emerg Infect Dis.* 2011;17(5):907-909.
219. Marsh GA, Todd S, Foord A, et al. Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. *Emerg Infect Dis.* 2010;16(11):1767-1769.

220. Chan YP, Chua KB, Koh CL, et al. Complete nucleotide sequences of Nipah virus isolates from Malaysia. *J Gen Virol.* 2001;82(Pt 9):2151-2155.
221. Wacharapluesadee S, Samseeneam P, Phermpool M, et al. Molecular characterization of Nipah virus from Pteropus hypomelanus in Southern Thailand. *Virol J.* 2016;13:53.
222. Peel AJ, Sargan DR, Baker KS, et al. Continent-wide panmixia of an African fruit bat facilitates transmission of potentially zoonotic viruses. *Nat Commun.* 2013;4:2770.
223. Weiss S, Nowak K, Fahr J, et al. Henipavirus-related sequences in fruit bat bushmeat, Republic of Congo. *Emerg Infect Dis.* 2012;18(9):1536-1537.
224. Kruger N, Hoffmann M, Drexler JF, et al. Attachment protein G of an African bat henipavirus is differentially restricted in chiropteran and nonchiropteran cells. *J Virol.* 2014;88(20):11973-11980.
225. Weis M, Behner L, Hoffmann M, et al. Characterization of African bat henipavirus GH-M74a glycoproteins. *J Gen Virol.* 2014;95(Pt 3):539-548.
226. Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. *Clin Infect Dis.* 2008;46(7):977-984.
227. Tan K-S, Sarji SA, Tan C-T, et al. Patients with asymptomatic Nipah virus infection may have abnormal cerebral MR imaging. *Neurol J Southeast Asia.* 2000;5:69-73.
228. Lim CC, Lee KE, Lee WL, et al. Nipah virus encephalitis: serial MR study of an emerging disease. *Radiology.* 2002;222(1):219-226.
229. Lee KE, Umapathi T, Tan CB, et al. The neurological manifestations of Nipah virus encephalitis, a novel paramyxovirus. *Ann Neurol.* 1999;46(3):428-432.
230. Lim CC, Lee WL, Leo YS, et al. Late clinical and magnetic resonance imaging follow up of Nipah virus infection. *J Neurol Neurosurg Psychiatry.* 2003;74(1):131-133.
231. Abdullah S, Chang LY, Rahmat K, et al. Late-onset Nipah virus encephalitis 11 years after the initial outbreak: A case report. *Neurol Asia.* 2012;17(1):71-74.
232. Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. *Curr Top Microbiol Immunol.* 2012;359:179-196.
233. Imada T, Abdul Rahman MA, Kashiwazaki Y, et al. Production and characterization of monoclonal antibodies against formalin-inactivated Nipah virus isolated from the lungs of a pig. *J Vet Med Sci.* 2004;66(1):81-83.
234. Tanimura N, Imada T, Kashiwazaki Y, et al. Monoclonal antibody-based immunohistochemical diagnosis of Malaysian Nipah virus infection in pigs. *J Comp Pathol.* 2004;131(2-3):199-206.
235. Tanimura N, Imada T, Kashiwazaki Y, et al. Reactivity of anti-Nipah virus monoclonal antibodies to formalin-fixed, paraffin-embedded lung tissues from experimental Nipah and Hendra virus infections. *J Vet Med Sci.* 2004;66(10):1263-1266.
236. White JR, Boyd V, Crameri GS, et al. Location of, immunogenicity of and relationships between neutralization epitopes on the attachment protein (G) of Hendra virus. *J Gen Virol.* 2005;86(Pt 10):2839-2848.
237. Smith IL, Halpin K, Warrilow D, et al. Development of a fluorogenic RT-PCR assay (TaqMan) for the detection of Hendra virus. *J Virol Methods.* 2001;98(1):33-40.
238. Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). *J Virol Methods.* 2004;120(2):229-237.
239. Feldman KS, Foord A, Heine HG, et al. Design and evaluation of consensus PCR assays for henipaviruses. *J Virol Methods.* 2009;161(1):52-57.
240. Eshaghi M, Tan WS, Mohidin TB, et al. Nipah virus glycoprotein: production in baculovirus and application in diagnosis. *Virus Res.* 2004;106(1):71-76.
241. Kashiwazaki Y, Na YN, Tanimura N, et al. A solid-phase blocking ELISA for detection of antibodies to Nipah virus. *J Virol Methods.* 2004;121(2):259-261.
242. Bossart KN, McEachern JA, Hickey AC, et al. Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. *J Virol Methods.* 2007;142(1-2):29-40.

243. Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. *J Virol Methods.* 2009;160(1-2):7-13.
244. Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. *J Virol Methods.* 2012;179(1):226-232.
245. Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. *J Virol Methods.* 2009;160(1-2):1-6.
246. Wright PJ, Crameri G, Eaton BT. RNA synthesis during infection by Hendra virus: an examination by quantitative real-time PCR of RNA accumulation, the effect of ribavirin and the attenuation of transcription. *Arch Virol.* 2005;150(3):521-532.
247. Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. *Ann Neurol.* 2001;49(6):810-813.
248. Pager CT, Wurth MA, Dutch RE. Subcellular localization and calcium and pH requirements for proteolytic processing of the Hendra virus fusion protein. *J Virol.* 2004;78(17):9154-9163.
249. Porotto M, Orefice G, Yokoyama CC, et al. Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy. *J Virol.* 2009;83(10):5148-5155.
250. Anonymous. *Hendra virus, human, equine - Australia (05): Queensland.* International Society for Infectious Diseases; 10-Sep 2009. 20090910.3189.
251. Warren TK, Jordan R, Lo MK, et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature.* 2016;531(7594):381-385.
252. Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. *Sci Rep.* 2017;7:43395.
253. Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. *J Gen Virol.* 2010;91(Pt 3):765-772.
254. Pallister J, Middleton D, Crameri G, et al. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. *J Virol.* 2009;83(22):11979-11982.
255. Eaton BT, Broder CC, Wang LF. Hendra and Nipah viruses: pathogenesis and therapeutics. *Curr Mol Med.* 2005;5(8):805-816.
256. Porotto M, Rockx B, Yokoyama CC, et al. Inhibition of Nipah virus infection in vivo: targeting an early stage of paramyxovirus fusion activation during viral entry. *PLoS Pathog.* 2010;6(10):e1001168.
257. Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. *Vaccine.* 2016;34(30):3525-3534.
258. Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. *J Virol.* 2004;78(2):834-840.
259. Weingartl HM, Berhane Y, Caswell JL, et al. Recombinant nipah virus vaccines protect pigs against challenge. *J Virol.* 2006;80(16):7929-7938.
260. Ploquin A, Szecsi J, Mathieu C, et al. Protection against henipavirus infection by use of recombinant adeno-associated virus-vector vaccines. *J Infect Dis.* 2013;207(3):469-478.
261. Yoneda M, Georges-Courbot MC, Ikeda F, et al. Recombinant measles virus vaccine expressing the Nipah virus glycoprotein protects against lethal Nipah virus challenge. *PLoS One.* 2013;8(3):e58414.
262. Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J.* 2013;10:353.
263. DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. *Vaccine.* 2014;32(22):2637-2644.

264. Lo MK, Bird BH, Chattopadhyay A, et al. Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters. *Antiviral Res.* 2014;101:26-29.
265. Prescott J, DeBuysscher BL, Feldmann F, et al. Single-dose live-attenuated vesicular stomatitis virus-based vaccine protects African green monkeys from Nipah virus disease. *Vaccine.* 2015;33(24):2823-2829.
266. DeBuysscher BL, Scott D, Thomas T, et al. Peri-exposure protection against Nipah virus disease using a single-dose recombinant vesicular stomatitis virus-based vaccine. *NPJ Vaccines.* 2016;1.
267. Bossart KN, Crameri G, Dimitrov AS, et al. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol.* 2005;79(11):6690-6702.
268. Mungall BA, Middleton D, Crameri G, et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol.* 2006;80(24):12293-12302.
269. Colgrave ML, Snelling HJ, Shiell BJ, et al. Site occupancy and glycan compositional analysis of two soluble recombinant forms of the attachment glycoprotein of Hendra virus. *Glycobiology.* 2012;22(4):572-584.
270. McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine.* 2008;26(31):3842-3852.
271. Pallister JA, Klein R, Arkinstall R, et al. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J.* 2013;10:237.
272. Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci Transl Med.* 2012;4(146):146ra107.
273. Mire CE, Geisbert JB, Agans KN, et al. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol.* 2014;88(9):4624-4631.
274. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis.* 2014;20(3):372-379.
275. Plotkin SA. Vaccines for epidemic infections and the role of CEPI. *Hum Vaccin Immunother.* 2017;13(12):2755-2762.
276. Guillaume V, Contamin H, Loth P, et al. Antibody prophylaxis and therapy against Nipah virus infection in hamsters. *J Virol.* 2006;80(4):1972-1978.
277. Zhu Z, Dimitrov AS, Bossart KN, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J Virol.* 2006;80(2):891-899.
278. Zhu Z, Bossart KN, Bishop KA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J Infect Dis.* 2008;197(6):846-853.
279. Xu K, Rockx B, Xie Y, et al. Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog.* 2013;9(10):e1003684.
280. Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. *Curr Opin Virol.* 2012;2(2):176-187.
281. WHO. 2018 annual review of the Blueprint list of priority diseases. WHO. https://www.who.int/blueprint/priority-diseases/en/. Published 2018. Accessed.
282. USDA. Henipavirus Gap Analysis Workshop Report. U.S. Department of Agriculture, Agricultural Research Service. http://go.usa.gov/xnHgR. Published 2018. Accessed.
283. CEPI. Coalition of Epidemic Preparedness Innovations: Vaccine Portfolio. https://cepi.net/research_dev/our-portfolio/. Published 2019. Accessed.

284. Schountz T, Baker ML, Butler J, et al. Immunological Control of Viral Infections in Bats and the Emergence of Viruses Highly Pathogenic to Humans. *Front Immunol.* 2017;8:1098.
285. Breed AC, Meers J, Sendow I, et al. The distribution of henipaviruses in Southeast Asia and Australasia: is Wallace's line a barrier to Nipah virus? *PLoS One.* 2013;8(4):e61316.
286. Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. *Emerg Infect Dis.* 2005;11(12):1949-1951.
287. Li Y, Wang J, Hickey AC, et al. Antibodies to Nipah or Nipah-like viruses in bats, China. *Emerg Infect Dis.* 2008;14(12):1974-1976.
288. Breed AC, Yu M, Barr JA, et al. Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua New Guinea. *Emerg Infect Dis.* 2010;16(12):1997-1999.
289. Sendow I, Field HE, Adjid A, et al. Screening for Nipah virus infection in West Kalimantan province, Indonesia. *Zoonoses Public Health.* 2010;57(7-8):499-503.
290. Wacharapluesadee S, Boongird K, Wanghongsa S, et al. A longitudinal study of the prevalence of Nipah virus in Pteropus lylei bats in Thailand: evidence for seasonal preference in disease transmission. *Vector Borne Zoonotic Dis.* 2010;10(2):183-190.
291. Plowright RK, Foley P, Field HE, et al. Urban habituation, ecological connectivity and epidemic dampening: the emergence of Hendra virus from flying foxes (Pteropus spp.). *Proc Biol Sci.* 2011;278(1725):3703-3712.
292. Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of nipah virus infection in bats, Vietnam. *Emerg Infect Dis.* 2012;18(3):536-537.
293. Yadav PD, Raut CG, Shete AM, et al. Detection of Nipah virus RNA in fruit bat (Pteropus giganteus) from India. *Am J Trop Med Hyg.* 2012;87(3):576-578.
294. Field H, de Jong CE, Halpin K, et al. Henipaviruses and fruit bats, Papua New Guinea. *Emerg Infect Dis.* 2013;19(4):670-671.
295. de Araujo J, Lo MK, Tamin A, et al. Antibodies Against Henipa-Like Viruses in Brazilian Bats. *Vector Borne Zoonotic Dis.* 2017;17(4):271-274.
296. Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. *PLoS One.* 2008;3(7):e2739.
297. Peel AJ, Baker KS, Crameri G, et al. Henipavirus neutralising antibodies in an isolated island population of African fruit bats. *PLoS One.* 2012;7(1):e30346.
298. Kessler MK, Becker DJ, Peel AJ, et al. Changing resource landscapes and spillover of henipaviruses. *Ann N Y Acad Sci.* 2018;1429(1):78-99.
299. Giles JR, Eby P, Parry H, et al. Environmental drivers of spatiotemporal foraging intensity in fruit bats and implications for Hendra virus ecology. *Sci Rep.* 2018;8(1):9555.
300. Plowright RK, Peel AJ, Streicker DG, et al. Transmission or Within-Host Dynamics Driving Pulses of Zoonotic Viruses in Reservoir-Host Populations. *PLoS Negl Trop Dis.* 2016;10(8):e0004796.
301. Wood JL, Leach M, Waldman L, et al. A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. *Philos Trans R Soc Lond B Biol Sci.* 2012;367(1604):2881-2892.
302. Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res.* 2013;100(1):8-13.
303. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia [see comments]. *Lancet.* 1999;354(9186):1257-1259.
304. AbuBakar S, Chang LY, Ali AR, et al. Isolation and molecular identification of Nipah virus from pigs. *Emerg Infect Dis.* 2004;10(12):2228-2230.
305. Chua KB, Lek Koh C, Hooi PS, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect.* 2002;4(2):145-151.
306. Anderson DE, Islam A, Crameri G, et al. Isolation and Full-Genome Characterization of Nipah Viruses from Bats, Bangladesh. *Emerg Infect Dis.* 2019;25(1):166-170.

307. Erbar S, Diederich S, Maisner A. Selective receptor expression restricts Nipah virus infection of endothelial cells. *Virol J.* 2008;5:142.
308. Pernet O, Wang YE, Lee B. Henipavirus receptor usage and tropism. *Curr Top Microbiol Immunol.* 2012;359:59-78.
309. Yoneda M. Nipah and Hendra virus infectious diseases. *Nihon Rinsho.* 2016;74(12):1973-1978.
310. Talekar A, Pessi A, Porotto M. Infection of primary neurons mediated by nipah virus envelope proteins: role of host target cells in antiviral action. *J Virol.* 2011;85(16):8422-8426.
311. Sawatsky B, Grolla A, Kuzenko N, et al. Inhibition of henipavirus infection by Nipah virus attachment glycoprotein occurs without cell-surface downregulation of ephrin-B2 or ephrin-B3. *J Gen Virol.* 2007;88(Pt 2):582-591.
312. Martinez-Gil L, Vera-Velasco NM, Mingarro I. Exploring the Human-Nipah Virus Protein-Protein Interactome. *J Virol.* 2017;91(23).
313. Aljofan M, Porotto M, Moscona A, et al. Development and validation of a chemiluminescent immunodetection assay amenable to high throughput screening of antiviral drugs for Nipah and Hendra virus. *J Virol Methods.* 2008;149(1):12-19.
314. Bender RR, Muth A, Schneider IC, et al. Receptor-Targeted Nipah Virus Glycoproteins Improve Cell-Type Selective Gene Delivery and Reveal a Preference for Membrane-Proximal Cell Attachment. *PLoS Pathog.* 2016;12(6):e1005641.
315. Khetawat D, Broder CC. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol J.* 2010;7:312.
316. Mathieu C, Guillaume V, Sabine A, et al. Lethal Nipah virus infection induces rapid overexpression of CXCL10. *PLoS One.* 2012;7(2):e32157.
317. Palomares K, Vigant F, Van Handel B, et al. Nipah virus envelope-pseudotyped lentiviruses efficiently target ephrinB2-positive stem cell populations in vitro and bypass the liver sink when administered in vivo. *J Virol.* 2013;87(4):2094-2108.
318. Escaffre O, Borisevich V, Carmical JR, et al. Henipavirus pathogenesis in human respiratory epithelial cells. *J Virol.* 2013;87(6):3284-3294.
319. Escaffre O, Borisevich V, Vergara LA, et al. Characterization of Nipah virus infection in a model of human airway epithelial cells cultured at an air-liquid interface. *J Gen Virol.* 2016;97(5):1077-1086.
320. Tanos T, Saibene AM, Pipolo C, et al. Isolation of putative stem cells present in human adult olfactory mucosa. *PLoS One.* 2017;12(7):e0181151.
321. Erbar S, Maisner A. Nipah virus infection and glycoprotein targeting in endothelial cells. *Virol J.* 2010;7:305.
322. Chua KB, Wong EM, Cropp BC, et al. Role of electron microscopy in Nipah virus outbreak investigation and control. *Med J Malaysia.* 2007;62(2):139-142.
323. Hudson AL, Weir C, Moon E, et al. Establishing a panel of chemo-resistant mesothelioma models for investigating chemo-resistance and identifying new treatments for mesothelioma. *Sci Rep.* 2014;4:6152.
324. Yoneda M, Fujita K, Sato H, et al. Reverse genetics of Nipah virus to probe viral pathogenicity. *Methods Mol Biol.* 2009;515:329-337.
325. Bossart KN, Tachedjian M, McEachern JA, et al. Functional studies of host-specific ephrin-B ligands as Henipavirus receptors. *Virology.* 2008;372(2):357-371.
326. Crameri G, Todd S, Grimley S, et al. Establishment, immortalisation and characterisation of pteropid bat cell lines. *PLoS One.* 2009;4(12):e8266.
327. Iehle C, Razafitrimo G, Razainirina J, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. *Emerg Infect Dis.* 2007;13(1):159-161.
328. Drexler JF, Corman VM, Gloza-Rausch F, et al. Henipavirus RNA in African bats. *PLoS One.* 2009;4(7):e6367.

329. Drexler JF, Corman VM, Muller MA, et al. Bats host major mammalian paramyxoviruses. *Nat Commun.* 2012;3:796.
330. Chua KB. A novel approach for collecting samples from fruit bats for isolation of infectious agents. *Microbes Infect.* 2003;5(6):487-490.
331. Olson JG, Rupprecht C, Rollin PE, et al. Antibodies to Nipah-like virus in bats (Pteropus lylei), Cambodia. *Emerg Infect Dis.* 2002;8(9):987-988.
332. Epstein JH, Anthony SJ, Islam A, et al. Nipah virus ecology and infection dynamics in its bat reservoir, *Pteropus medius*, in Bangladesh. *International Journal of Infectious Diseases.* 2016;53:20-21.
333. Mourya DT, Yadav P, Sudeep AB, et al. A spatial association between a Nipah virus outbreak in Kozhikode, Kerala, India and Nipah virus infection in Pteropus bats. *Clin Infect Dis.* 2018.

<Figure legends>

Figure 14.1. Syncytia induced in vero cells 24 hours after infection by Hendra virus (HeV) (A) and Nipah virus (NiV) (B). Methanol-fixed infected cells were labeled with rabbit monospecific antiserum to the HeV P protein and fluorescein-conjugated goat antirabbit immunoglobulin G. P protein is detected in extensive perinuclear ribonucleoprotein complexes (*small arrow*) and in discrete regularly shaped arrays (*large arrow*) distributed throughout the cytoplasm and believed to be sites of virus egress from the cell. Nuclei are indicated by *chevrons.*

Figure 14.2. A: Electron micrograph of Nipah virus (NiV)-infected Vero cells showing tubule-like structures, both in the cytoplasm and in a maturing virus particle (*arrows*). **B:** Electron micrograph of negatively stained Hendra virus (HeV) displaying the double fringe at the virus envelope (*small arrow*) and the herringbone nucleocapsids (*large arrow*). (Courtesy of Dr. Alex Hyatt, CSIRO Australian Animal Health Laboratory.)

Figure 14.3. Genome size and organization of Hendra virus in comparison with the type species in each of the other six genera in the family *Paramyxoviridae*. A: Genome length (in nucleotides) are given in brackets after each virus. Color coding: blue, proteins for the inner most nucleocapsid responsible for replication and transcription; green, envelope proteins. **B:** 3’UTR of certain genes in henipaviruses (N, P and G) are longer than their

counterparts from representative members of paramyxovirus genera that infect mammals. The 3'UTR length (number of nucleotides) of each gene from representative members of the indicated genera is compared separately in box-and-whisker plots (N, P, M, F, RBP, and RdRP). P values are calculated based on one-tailed t-test (*Henipavirus* vs all other genera); five virus species from *Henipavirus* (HeV, NC_001906; NiV, NC_002728; CedV, NC_025351.1; GhV, NC_025256.1; MojV, NC_025352.1), and three each from *Morbillivirus,* (MeV, NC_001498.1; CDV, NC_001921.1; FeMV, NC_039196.1), *Rubulavirus* (MuV, NC_002200.1; HPIV-2, NC_003443.1; PIV-5, NC_006430.1) and *Respirovirus* (SeV, NC_001552; HPIV-1, NC_003461; HPIV-3, NC_001796.2). Courtesy of Satoshi Ikegame and Christian Stevens, Icahn School of Medicine at Mount Sinai, New York.

Figure 14.4. The major proteins of Hendra virus and Nipah virus and their roles in the henipavirus life cycle. A: Viruses were purified and analyzed as described in Wang LF et al.(20) Major virus proteins and the molecular weights of standard marker proteins are shown on the right and left, respectively. Reproduced with permission from Wang LF et al, Molecular biology of Hendra and Nipah viruses. *Microbes Infect* 2001; 3:279-87**. B:** Henipavirus replication cycle is depicted. The virus receptor binding protein (RBP, deep blue lollipops) attaches the virus to the cell via its cognate receptor, typically ephrinB2/B3 **(1)**, and triggers virus-cell membrane fusion mediated by the viral fusion protein (F, filled orange trapezoids) **(2)**. Fusion delivers the nucleoprotein (N) encapsidated negative-sense virus RNA genome [vRNA(−), cyan] to the cytosol **(3).** The vRNA(-) serves as template for viral transcripts made by the transcriptase complex comprised of the phosphoprotein (P) and the large RNA-dependent RNA-polymerase (RdRP) (P-RdRP, transparent hexagons) **(4).** Viral transcripts are made following a 3′ to 5′ attenuation gradient. The vRNA(-) is also the template for making full-length antigenomic cRNA(+) **(5),** which in turn is a template for vRNA(−) synthesis during virus genome replication **(6).** Henipavirus genome replication

mediated by the N-P-RdRP replicase complex is similar to the details described for the *Paramyxoviridae* in chapter 13. However, some aspects of henipavirus assembly are relatively unique. The matrix protein (M, green octagons) needs to transit through the nucleus **(7)** in a ubiquitin-regulated manner **(8),** in order to traffic properly to the plasma membrane. This nuclear sojourn involves shuttling through subnuclear compartments such as nucleoli **(9).** Proper trafficking is required for M to orchestrate the assembly of the viral RNPs with the viral envelope glycoproteins into budding infectious virions bearing the vRNA(-) **(10).** Matrix also has non-structural functions such as antagonizing type-I IFN responses **(11);** see Fig. 14.8 and text for details. Additionally, in order for the henipavirus F protein to be cleaved and become fusion-competent, it needs to access cathepsins L/B in the endosomal compartment. To do this, F first reaches the plasma membrane via the secreory pathway in an uncleaved form (open orange trapezoids) **(12).** Subsequent endocytosis and recycling back to the plasma membrane **(12a)** is required to form infectious virions where cleaved fusion-competent F (filled orange trapezoids) **(12b)** is complexed with the RBP in its proper oligomeric state. Where this occurs along the vesicular trafficking pathway and/or the plasma membrane is unresolved **(13).** F protein cleavage is seldom complete and uncleaved F can be detected in budded virions **(14)** (Adapted from Aguilar HC and Lee B. Emerging paramyxoviruses: molecular mechanisms and antiviral strategies. *Expert Rev Mol Med* 2011;13:e6)

Figure 14.5. A structure-based phylogeny of paramyxoviral receptor binding proteins. Structure-based phylogenetic analysis on extant structures of all henipavirus RBPs and representative RBPs from at least one virus in other paramyxovirus genera for which structural data is available. RBPs are shown besides the tree and are rendered as surface representation (grey). All receptor-binding proteins, with the exception of the MojV-G, are shown in complex with their respective receptor molecules (yellow), with the receptor-

binding interface colored red. Analysis was performed and produced as described in Rissanen et al.(66) Paramyxoviral RBPs generally cluster according to their respective genera and cellular receptor usage with the exception MojV-RBP, which is almost equidistant to all structurally studied genera. Courtesy of Dr. Thomas Bowden, University of Oxford, U.K.

Figure 14.6. Nipah virus transmission. Major pathways of human Nipah virus infection are summarized for Malaysia (blue arrows), Bangladesh (red arrows), the Philippines (green arrows), and the latest outbreak in Kerala, India (orange arrows). (Adapted from Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. *Curr Opin Virol* 2017; 22:97-104)

Figure 14.7. Immunohistochemistry detection of Nipah virus (NiV) and Hendra virus (HeV) antigen in henipavirus-infected tissues. A-D: Hendra virus infected tissues. **A:** Staining of HeV antigen (N protein-specific rabbit antibody) within the wall of superficial arteriole in the sub-mucosa of the nasal cavity in a HeV-infected horse. **B:** Immmunolabeling (N protein-specific rabbit antibody) of the endothelium of medium caliber vessels and alveolar septae in lung of a HeV-infected African green monkey (AGM) (10x). **C:** Immunolabeling of HeV antigen (N protein-specific rabbit antibody) within neurons in the brainstem of a HeV-infected AGM (40x). **D:** Immunolabeling of HeV antigens (mouse anti-HeV polyclonal antibody) in neurons of acute HeV-infected human case (40x). **E:** Positive immunolabelling (using anti-Nipah virus rabbit serum) within the lung of a NiV-infected pig. Involvement of bronchial epithelium and airway debris is noted. **F:** Immunolabeling (N protein-specific antibody) of the endothelium of medium caliber vessels and alveolar septae in lung of a NiV-infected AGM (20x). **G:** Immunolabeling of NiV antigen (N protein-specific antibody) within neurons in the brainstem of a NiV-infected AGM (40x). **H:** Immunolabeling of NiV antigens (hyperimmune anti-NiV mouse ascitic fluid) in the cytoplasm and nuclei of neurons, and neuronal processes in the brain of acute NiV-infected human case (158x). A and E, courtesy of Dr. Deborah Middleton, CSIRO Australian Animal

Health Laboratory. B, C, F and G, courtesy of Dr. Karla Fenton, University of Texas Medical Branch at Galveston, Texas. D, courtesy of Dr. Kum Thong Wong, University of Malaya; H, from Wong et al.(136)

Figure 14.8. Ephrin-B2 and ephrin-B3 RNA-seq expression data across representative human non-disease tissues. Shown here are ephrin-B2 (upper) and ephrin-B3 (lower) expression data obtained from the Genotype-Tissue Expression (GTEx) Portal. The GTEx project is a public resource where up to 53 non-disease tissue sites obtained from nearly 1,000 individuals are subjected to RNA-seq by next-generation sequencing amongst other assays. Details on datasets can be found on https://gtexportal.org. Data from all available brain regions are shown. The non-brain tissues are chosen for illustrative purposes based on their relevance to henipavirus pathogenesis. Expression data in transcripts per million are plotted as box plots with the medians and 25th and 27th percentiles shown; points are displayed as outliers if they are above or below 1.5 times the interquartile range. 1a Artery – Aorta; 1b Artery – Coronary; 2a Brain – Amygdala; 2b Brain - Anterior cingulate cortex (BA24); 2c Brain - Caudate (basal ganglia); 2d Brain - Cerebellar Hemisphere; 2e Brain – Cerebellum; 2f Brain – Cortex; 2g Brain - Frontal Cortex (BA9); 2h Brain – Hippocampus; 2i Brain – Hypothalamus; 2j Brain - Nucleus accumbens (basal ganglia); 2k Brain - Putamen (basal ganglia); 2l Brain - Spinal cord (cervical c-1); 2m Brain - Substantia nigra; 3 Breast - Mammary Tissue; 4 Colon – Transverse; 5 Esophagus - Gastroesophageal Junction; Esophagus – Mucosa; 6 Heart - Left Ventricle; 7 Kidney – Cortex; 8 Liver; 9 Lung; 10 Minor Salivary Gland; 11 Spleen; 12 Whole Blood.

Figure 14.9. Innate immune evasion mechanisms of the Nipah virus N, P, V, W and M proteins. Left: The V and W proteins (green diamonds) block IFN-α/β expression by different mechanisms. Depicted are simplified versions of the RIG-I-like receptor (RLR) signaling (blue arrows) and TLR3 signaling (black arrows) pathways. Both lead to activation

of interferon regulatory factors 3 and 7 (IRF-3 and IRF-7) (dashed arrow). RIG-I, MDA5 and TLR3 can sense RNAs produced during viral infection. Activation of RIG-I or MDA5 leads to their interaction with the mitochondria-associated adapter protein MAVS (1). This leads to activation of the kinases IKKε and/or TBK-1 (2), which phosphorylate and activate IRF-3 and, when present, IRF-7 (3). For clarity, MAVS activation of NF-κB is not shown. Activated IRF-3 and IRF-7 translocate into and accumulate in the nucleus; both contribute to IFN-α/β gene transcription (4). TLR3 signals through adaptor protein TRIF to activate IKKε/TBK-1 (5). The cytoplasmic NiV and HeV V proteins interact with and inhibit MDA5, thereby inhibiting the induction of IFN-α/β expression early on in the pathway (a); however NiV V does not appear to inhibit TLR3 signaling. The nucleo-cytoplasmic shuttling NiV M protein (green hexagon), while it is in the cytoplasm, antagonizes induction of type-I IFNs by blocking IKKε activation (b). This prevents downstream phosphorylation and nuclear translocation of IRF3, which also leads to a failure of IFN-α/β induction. The nuclear W protein (green trapezoid) effectively blocks RLR and TLR3 activation of the IFN-β promoter by preventing the accumulation of phosphorylated IRF-3 (c). This function requires the nuclear localization of W. **Right:** The Nipah virus N, P, V and W proteins each block IFN signaling pathways at distinct steps (red arrows), ultimately leading to reduced expression of interferon- stimulated (antiviral) genes (ISGs). The P, V and W proteins can each inhibit the phosphorylation and activation of STAT1 and STAT2 in response to IFN-α/β (6) and block the phosphorylation and activation of STAT1 in response to IFN-γ. The inhibition of the IFN-α/β signaling pathway is depicted. The cytoplasmic P and V proteins interact with and inhibit STAT1 (d). The V protein also interacts with STAT2, in a STAT1-dependent manner. P and V prevent nuclear accumulation of pSTAT1 /pSTAT2 following IFN-α/β addition to cells and cause the STAT proteins to accumulate in a high molecular weight complex. The NiV and HeV N proteins (green oval) were recently reported to disrupt phospho-STAT1 complex formation (7), though not phosphorylation of STAT1 itself (e). Nonetheless, this N-mediated disruption of STAT complexes reduces nuclear accumulation of the activated STAT complexes required to stimulate ISG expression (8). The nuclear W protein relocalizes non-phosphorylated STAT1 from its typically cytoplasmic localization to the nucleus (f). STAT1 relocalized to the nucleus by W remains unphosphorylated and does not activate transcription of ISGs in response to IFN-α/β addition. (Adapted from Basler CF. Nipah and Hendra virus interactions with the innnate immune system. *Curr Top Microbiol Immunol* 2012; 359: 123-52)

Figure 14.10. Distribution of frugivorous bats belonging to various genera in the *Pteropodidae* family where evidence of henipavirus presence have been detected. The presence of henipavirus is graded into four levels with known outbreak (red), virus isolation (pink), molecular detection (blue), and antibody detection (green).

Figure 14.11: Phylogenetic tree based on RdRP proteins of selected henipaviruses and prototype species of the other genera in the family *Paramyxoviridae.* Tree was constructed by using Neighbor-Joining approach with 1,000 bootstrap replicates. Numbers at the nodes represent bootstrap values. Scale bar indicates amino acid substitutions per site. Sequences are labelled with the following ordination when all relative information is available for any given genome sequence: Virus/Country/Host/Year/Strain (GenBank accession number). For example, NiV/BD/Human/2010/Faridpur(JN808864) represents a Nipah virus isolated in Bangladesh from humans in 2010 with Faridpur as its isolate name and JN808864 as its Genbank accession number.

Figure 14.12. Chest radiographs of a Nipah virus (NiV) infected patient and Nipah virus (NiV) and Hendra virus (HeV) infected African green monkeys. A: Chest X-ray of patient infected with Nipah virus (Nipah-Bangladesh clade) from Kerala, India, 2018, at 6 days following onset of symptoms showing diffuse bilateral opacities covering the majority of the

lung fields, consistent with acute respiratory distress syndrome (ARDS). **B:** Top, comparison of pre-challenge to Nipah virus (Nipah-Malaysia clade) infected African green monkey at 11 days post-challenge showing congestion and pneumonia with infiltrates on the lung fields. Bottom, comparison of pre-challenge to Hendra virus infected African green monkey at 8 days post-challenge showing diffuse interstitial infiltrates and pulmonary consolidation and severe respiratory distress. Panel A, courtesy of Prof. Govindakarnavar Arunkumar, Manipal Centre for Virus Research, Manipal Academy of Higher Education (deemed to be University), Karnataka State, India and Dr. Suresh Kumar. E.K, Malabar Institute of Medical Sciences, Kerala State, India. Panel B, top, from Geisbert et al.(98), bottom, from Rockx et al.(103) (reproduced with permission).

Figure 14.13. Magnetic resonance imaging scans of patients with Nipah virus (NiV) or Hendra virus (HeV) encephalitis. A: Left, patient with NiV encephalitis (Nipah-Bangladesh clade) from Kerala, India, 2018, at 6 days following onset of symptoms showing cortical and subcortical hyperintense foci (arrow heads). Middle, patient with acute NiV encephalitis (Nipah-Malaysia clade) from Malaysia, 1999, showing multiple discrete hyperintense lesions in the grey and white matter. Right, patient with relapsed NiV encephalitis from Malaysia, 1999, showing confluent lesions involving primarily the cortical grey matter. **B:** Left, acute HeV encephalitis in a patient on day 18 of illness, showing cortical and subcortical hyperintense foci. Right, relapsed HeV encephalitis in a patient on day 14 following symptoms onset showing widespread multifocal neocortical involvement. Panel A, left (India), courtesy of Prof. Govindakarnavar Arunkumar, Manipal Centre for Virus Research, Manipal Academy of Higher Education (deemed to be University), Karnataka State, India and Dr. Suresh Kumar. E.K, Malabar Institute of Medical Sciences, Kerala State, India. A, middle and right (Malaysia) from Goh, et al.(108) B, left, from Playford et al.(8); right, from O'Sullivan et al.(3) (reproduced with permission)

Figure 14.14. Generalized understanding of infection and clinical features of human Nipah virus (NiV) and Hendra virus (HeV) infection. **A:** Transmission is generally accepted to occur via the oronasal route presumably by virus contaminated respiratory secretions or droplets from infected animals or people, or liquid/food (palm sap/fruit); **B:** The initial site of replication in humans is ill-defined but is thought to occur within the respiratory system and disease onset is characterized by fever, myalgia, shortness of breath, cough, which may progress to an acute respiratory distress syndrome (ARDS); **C:** Possible invasion into the central nervous system (CNS) via the olfactory bulb has also been suggested and has been demonstrated in animal models; **D:** Virus replication and hematogenous systemic spread occurs (cell-free or cell-associated viremia) and established infection is characterized by a widespread vasculitis with endothelial and smooth muscle cell tropism resulting in multinucleated syncytial cells; **E:** CNS infection and encephalitis results in altered sensorium, headache, vomiting, and seizures along with fever and other systemic symptoms; **F:** Infection of other organs in addition to lung and brain in human cases occurs and has been reported in kidney, heart, spleen and lymphoid tissues, and similar widespread infection has been demonstrated in multiple animal models.

Figure 14.15. Model of the Hendra virus soluble G glycoprotein subunit vaccine (HeV-sG) and its complex with the Hendra and Nipah virus neutralizing human monoclonal antibody m102.4. **A:** The HeV-sG glycoprotein subunit vaccine is composed of the entire ectodomain (amino acids 76-604) of the HeV G glycoprotein. HeV-sG is shown as dimer with one monomer colored green and the other cyan. The secondary structure elements of the two globular head domains are derived from the crystal structure of the HeV G head domain, the stalk regions of each G monomer (residues 77-136) are modeled, and N-linked glycosylation sites shown as gray spheres. The ephrin-binding face of the cyan globular head is facing forward with an overlay of the interacting ephrin-B2 G-H loop residues in yellow.

B: The HeV-sG dimer is modeled in complex with two m102.4 Fab antibody fragments. The two HeV-sG monomers are colored green and cyan as in panel A and rotated slightly to the right, and the two Fab molecules are shown with their heavy chains colored in magenta and light chain – in yellow, each binding one globular head domains of G. Modified from Broder et al.(302)

Figure 14.16. One Health Vaccine strategy. A: The known modes of henipavirus transmission in different countries. In Australia, all seven human cases were infected via contacting infected horses. A One Health vaccine strategy was developed for immunization of horses with the dual purposes of saving horses from lethal Hendra virus infection and prevention of transmission from bats to humans via horses. **B:** Location densities of Equivac® HeV vaccine administered to horses around Australia. As of 2018, more than 640,000 doses of Equivac® HeV have been administered to horses around Australia, equating to more than 153,000 unique horses vaccinated. Courtesy of Dr. Richard L'Estrange, Zoetis Inc.

<TABLES>

TABLE 14.1A. Summary of Hendra Virus Outbreaks in Horses in Australia

Year	Month	State[1]	Location	Total Cases[2]
1994	Aug	QLD	Mackay	2
1994	Sep	QLD	Hendra	20
1999	Jan	QLD	Cairns	1
2004	Oct	QLD	Cairns	1
	Dec	QLD	Townsville	1
2006	Jun	QLD	Peachester	1
	Oct	NSW	Murwillumbah	1
2007	Jun	QLD	Peachester	1
	Jul	QLD	Cairns	1
2008	Jun	QLD	Redlands	8
	Jul	QLD	Propserpine	4
2009	Jul	QLD	Cawarral	4
	Sep	QLD	Bowen	2
2010	May	QLD	Tewantin	1
	Jun	QLD	Beaudesert	1
		QLD	Boonah	3
		QLD	Logan	1
		NSW	Wollongbar	2

2011	Jul	QLD	Park Ridge	1
		NSW	Macksville	1
		QLD	Kuranda	1
		NSW	Lismore	1
		QLD	Hervey Bay	1
		QLD	Boondall	1
		QLD	Chinchilla	1
		NSW	Mulumbimby	1
	Aug	NSW	South Ballina	1
		NSW	South Balinna	2
		NSW	Mullumbinby	1
		QLD	Gold Coast Hinterland	1
		NSW	North Ballina	1
	Oct	QLD	Beachmere	3
2012	Jan	QLD	Townsville	1
	May	QLD	Rockhampton	1
		QLD	Ingham	1
	Jun	QLD	Mackay	1
	Jul	QLD	Rockhampton	3
		QLD	Cairns	1
	Sep	QLD	Port Douglas	1
	Oct	QLD	Ingham	1
2013	Jan	QLD	Mackay	1
	Feb	QLD	Atherton Tabelands	1
	Jun	NSW	Macksville	1
		QLD	Brisbane Valley	1
	Jul	QLD	Gold Coast Hinterland	1
		NSW	Macksville	1
		NSW	Kempsey	2
2014	Mar	QLD	Bundaberg	1
	Jun	QLD	Beenleigh	1
		NSW	Murvillumbah	1
	Jul	QLD	Gladstone	1
2015	Jun	NSW	Murwillumbah	1
	Jul	QLD	Atherton Tableland	1
	Spt	NSW	Lismore	1
2016	Dec	NSW	Casino	1
2017	May	QLD	Gold Cost Hinterland	1
	Jun	NSW	Lismore	1
	Aug	NSW	Murwillumbah	1
		NSW	Lismore	1
2018	Sep	NSW	Tween Heads	1
Total				***103***

[1] QLD = Queensland; NSW = New South Wales

[2] All cases were deceased

TABLE 14.1B. Summary of Hendra Virus Outbreaks in Humans

Year	Month	Country	Location	No. cases	No. deaths	Case Fatality Rate
1994	Aug	Australia	Mackay	1	1	100%
1994	Sep		Hendra	2	1	50%
2004	Oct		Cairns	1	1	100%
2008	Jul		Redlands	2	1	60%
2009	Aug		Cararral	1	0	0%
			Total	***7***	***4***	***57%***

Table 14.1C. Summary of Nipah Virus Outbreaks in Humans

Year	Month	Country	Location	No. cases	No. deaths	NiV Clade (M/B)		Case Fatality Rate
1998	Spt-Apr 1999	Malaysia	Started from Nipah near Ipoh	265	105	M		40%
1999	Mar	Singapore	Singapore	11	1	M		9%
2001	Feb	India	Siliguri	66	45		B	68%
2001	Apr-May	Bangladesh	Meherpur	13	9		B	69%
2003	Jan		Naogaon	12	8		B	67%
2004	Jan		Raibari	31	23		B	74%
	Apr		Faridpur	36	27		B	75%
2005	Jan-Mar		Tangail	12	11		B	92%
2007	Jan-Feb		Thakurgaon	7	3		B	43%
	Mar		Kushtia	8	5		B	63%
	Apr		Pabna, Natore, Naogaon	3	1		B	33%
	Apr	India	Nadia	5	5		B	100%
2008	Feb	Bangladesh	Manikgonj	4	4		B	100%
	Apr		Rajbari	7	5		B	71%
2009	Jan		Gaibandha, Rangpur, Nilphamari	3	0		B	0%
			Rajbari	1	1		B	100%
2010	Feb-Mar		Faridpur, Rajbari, Gopalgonj, Kurigram	17	15		B	88%
2011	Jan-Feb		Lalmohirhat, Dinajpur, Comilla, Nilphamari, Faridpur,	44	40		B	91%

			Rajbari					
2012	Jan		Joypurhat	12	10		B	83%
2013	Jan-Apr		Pabna, Natore, Naogaon, Gaibandha, Manikganj	24	21			88%
2014	Jan-Feb		13 Districts	18	9		B	50%
2014	Mar-May	The Philippines	Senator Ninoy Aquino	17	9	M		53%
2015	Jan-Feb	Bangladesh	Nilphamari, Ponchoghor, Faridpur, Magura, Naugaon, Rajbari	9	6		B	67%
2018	May	India	Kerala	23	21		B	94%
				293	***115***	***M***		***39%***
				355	***269***		***B***	***76%***
			Total	***648***	***384***	***M***	***B***	***59%***

TABLE 14.2. Summary of Henipaviruses Isolated from Different Species and Geographic Locations

		Isolation details			
Virus	**Isolate name and number**	**Year**	**Country**	**Host species/tissue**	**Reference**
Hendra	Horse-1 VR-1	1994	Australia	Horse/spleen, lung Human/lung, liver, kidney, spleen	(2)
	Bat-1-1 Bat-1-2 Bat-2	1996	Australia	Grey-headed flying fox (*Pteropus poliocephalus*)/uterine fluid Grey-headed flying fox (*P. poliocephalus*)/fetus Black flying fox (*Pteropus alecto*)/fetal lung	(6)
	Murwillumbah Clifton Beach Peachester Redlands Proserpine	2006 2007 2008	Australia	Horse/lung Horse/lung Horse/blood Horse/lung Horse/lung	(219)
Nipah	PKL EKK	1999	Malaysia	Human/cerebral spinal fluid Human/cerebral spinal fluid	(11,303)

	WWS			Human/cerebral spinal fluid	
	UMMC1	1999	Malaysia	Human/cerebral spinal fluid	(220)
	UMMC2			Human/throat secretion	
	UM-0128	1999	Malaysia	Human	(304)
	VRI-0626			Pig/lung	
	VRI-1413			Pig/lung	
	VRI-2794			Pig/lung	
	B13/6-18	2000	Malaysia	Bat/pooled urine	(305)
	B13/6-43			Bat/pooled urine	
	JA13/6-4			Bat/partially eaten jambu air fruit	
	Rajbari-1	2004	Bangladesh	Human/oropharyngeal	(33)
	Rajbari-2			Human/cerebral spinal fluid	
	Faridpur			Human/urine	
	Rajshahi			Human/urine	
	CSUR381	2004	Cambodia	Flying fox (*Pteropus lylei*)/urine	(89)
	CSUR382			Flying fox (*Pteropus lylei*)/urine	
	Raypur-31	2013	Bangladesh	Flying fox (*Pteropus medius*)/urine	(306)
	Raypur-36			Flying fox (*Pteropus medius*)/urine	
	Raypur-38			Flying fox (*Pteropus medius*)/urine	
	Raypur-40			Flying fox (*Pteropus medius*)/urine	
	Raypur-41			Flying fox (*Pteropus medius*)/urine	
	Raypur-42			Flying fox (*Pteropus medius*)/urine	
	Raypur-43			Flying fox (*Pteropus medius*)/urine	
	Raypur-46			Flying fox (*Pteropus medius*)/urine	
	Raypur-47				
	Sylhet-47				

				Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine	

Table 14.3. Henipavirus Cell Tropism and Receptor Expression

Cell line/ Primary cells	Species/Cell Type	Virus	EFNB2[a]	EFNB3[a]	Infection Permissive[b]	Reference[c]
HUMAN						
HeLa-CCL2	Human Cervical Cancer	NiV HeV	+	-	+	(62) (44)
HeLa-USU	Human Cervical Cancer	NiV HeV	-	-	-	(62)
HeLa-USU-EFNB2	HeLa-USU stably expressing EFNB2	HeV CedV	+ +	- -	+ +	(28) (53)
HeLa-USU-EFNB3	HeLa-USU stably expressing EFNB3	HeV CedV	- -	+ +	+ -	(28) (53)
Vero (various subclones)	African green monke kidney epithelial	NiV HeV CedV GhV	++	+	+	(2) (11) (62) (307) (28) (308)
293T	Human embryonic kidney fibroblast		+	+	+	(309) (310) (311) (312)
A549	Human lung adenocarcinoma	NiV HeV	+	+	+	(313) (87) (49)
PCI 13	Human head and neck carcinoma	NiV HeV	+	+	+	(62) (267)
U373-MG	Human astroglioma	NiV	+	++	+	(62) (267) (150)
U87-MG	Human glioblastoma	NiV GhV	+	+	+	(314) (65)

						(308) (315)
HBMEC (Primary)	Human brain microvascular endothelial	NiV	+	-	+	(136) (307)
HUVEC (Primary)	Human umbilical cord endothelial	NiV HeV	+	+	+	(316) (43)
HuNSC Neurons/ Astrocytes (Primary)	Human neural stem cells derived neurons and astrovytes	NiV, HeV	+	+	+	(317) (43)
NHBE (Primary)	(Normal) Human bronchial epithelial	NiV, HeV	+	+	+	(318) (319)
SAEC (Primary)	Human small airway epithleilial	NiV HeV	+	+	+	(318) (319)
Hu PBL (Primary)	Human peripheral blood lymphocytes	NiV	(+/-)[d]	(+/-)[d]	-	(150)
HuMono/ Mac/DCs (Primary)	Human monocytes, macrophages, dendritic cells	NiV	Mono/ Mac (+/-)[d] DC (+/-)[d]	Mono/ Mac (+/-)[d] DC (-)	Mono (-) Mac (-) DC (+/-)	(150,182)
HuOE (Primary)	Human olfactory epithelial	NiV HeV	(?)	+	+	(142) (320)
Cell line/ Primary cells	**Species/Cell Type**	**Virus**	**EFNB2[a]**	**EFNB3[a]**	**Infection Permissive[b]**	**References[c]**
NON-HUMAN						
BHK-21	Baby hamster kidney fibroblast	NiV	+	(?)	+	(44) (313)
CHO	Chinese Hamster Ovary	NiV HeV	-	-	-	(63) (64) (44)
CHO-B2	CHO stably expressing ephrin-B2	NiV HeV	+	-	+	(63) (64)
CHO-B3	CHO stably expressing ephrin-B3	NiV HeV	-	+	+	(63) (64)

CRFK	Cat (Feline) kidney epithelial	NiV HeV	+	+	+	(311)
PBMEC (Primary)	Porcine brain microvascular endothelial	NiV	+	+	+	(307) (321)
PAEC (Primary)	Pig Aorta Endothelial	NiV	-	-	-	(307)
PK13	Pig kidney fibroblast	NiV	-	-	-	(63) (322)
Rat cortical neurons	Rat cortical neurons (Primary)	NiV	+	+	+	(63) (310)
4/4RM4	Rat pleural mesothelial	NiV	+	ND	+	(44) (323)
L2	Rat lung epithelial	NiV	+	ND	+	(44) (324)
208f	Rat embryonic fibroblast	NiV	+	ND	-	(44) (324)
P815	Mouse mast cell	NiV	+	ND	-	(44) (324)
MyEnd	Mouse myocardial endothelial	NiV	(?)	(?)	-	(307)
PaLuT02	Immortalized *P. alecto* (fruit bat) lung cells	NiV HeV	+	+	+	(175) (325)
PaKi	Primary *P. alecto* (fruit bat) kidney cells	HeV	+	+	+	(175) (325)
PaBrH04	Immortalized *P. alecto* (fruit bat) brain cells	NiV HeV	+	+	+	(326) (325)
MMEC	Mouse microvascular endothelium	NiV HeV	(?)	(?)	(+/-)[d]	(313)

[a] Expression inferred from (1) transcriptional data (microarray, next-generation sequencing, RT-PCR, etc.) provided in the cited references, or (2) indepedently verified through the Gene Expression Omnibus (GEO) Database (https://www.ncbi.nlm.nih.gov/geo/), or (3) competition with soluble ephrinB ligands or interference with cognate henipavirus receptor binding proteins. [b] For clarity and uniformity, only infection-permissive (not fusion-permissive) data are indicated; infection includes use of live virus and/or pseudotyped virus. [c] References cited support expression, infection data, or cell-type origin where relevant, and are representative, not comprehensive. [d] Indicates >10-100-

fold below positive control when quantitative data is avialable. ND, not determined; (?), not specifically mentioned.

TABLE 14.4. Detection of Nipah or Nipah-related Virus Infection in Bats (Geographic surveillance studies)

Bat Species	Method and country of detection			Reference
	Serology	PCR	Isolation	
Suborder: Yinpterochiroptera				
Cynopterus brachyotis	Malaysia			(205)
Cynopterus sphinx	Vietnam			(292)
Eidolon dupreanum	Madgascar			(327)
Eidolon helvum	Ghana, Zambia, Malawi, Tanzania, Uganda, Rio Muni, Bioko, Príncipe, Saõ Tomé, Annobón, Cameroon	Ghana, Gabon, Democratic Republic of Congo, Central African Republic,		(296); (328); (329); (222); (19)
Eonycteris spelaea	Malaysia			(205)
Epomophorus gambianus	Ghana	Democratic Republic of Congo, Gabon, Republic of Congo		(296), (329)
Hipposideros armiger	China			(287)
Hipposideros larvatus	Thailand	Thailand		(286)
Hipposideros pomona	China			(287)
Hypsignathus monstrosus	Ghana	Republic of Congo, Central African Republic,		(296), (329)
Myonycteris torquata		Democratic Republic of Congo, Gabon, Republic of Congo		(329)
Rhinolophus affinis	China			(287)
Rhinolophus	China			(287)

sinicus				
Rousettus leschenaulti	China, Vietnam			(287); (292)
Rousettus aegyptiacus		Gabon		(329)
Pteropus giganteus	Bangladesh, India			(207)
Pteropus hypomelanus	Malaysia, Thailand	Thailand	Malaysia	(205); (221); (330)
Pteropus lylei	Cambodia, Thailand	Thailand	Cambodia	(89,290,331)
Pteropus medius		Bangladesh, India	Bangladesh	(332); (306); (333)
Pteropus rufus	Madgascar			(327)
Pteropus vampyrus	Malaysia, Thailand			(205); (286)
Suborder: Yangochiroptera				
Carolia perspicillta		Costa Rica		(329)
Miniopterus spp.	China			(287)
Myotis daubentoni	China			(287)
Myotis ricketti	China			(287)
Pteronotus parnellii		Costa Rica		(329)
Scotophilus kuhlii	Malaysia			(205)

TABLE 14.5. Summary of Advanced Henipavirus Vaccination Development Initiatives Evaluated in Animal Challenge Models

Platform	Viral antigen target or immunogen	Animal challenge model
Active vaccination Recombinant vaccinia virus Recombinant canarypox virus Recombinant VSV Recombinant AAV Recombinant measles	Nipah F and/or G glycoprotein Nipah F and/or G glycoprotein Nipah F and/or G glycoprotein Nipah G glycoprotein Nipah G glycoprotein Hendra soluble G glycoprotein	Hamster[a] (NiV) Pig[b] (NiV) Ferret[c] (NiV), Hamster[d] (NiV), nonhuman primate[e] (NiV) Hamster[f] (NiV, HeV) Hamster[g], Nonhuman primate (NiV, HeV) Cat[h] (NiV), Ferret[i] (HeV, NiV), nonhuman primate[j] (HeV, NiV) Horse[k]

virus Recombinant subunit		(HeV)
Passive immunization Human monoclonal antibody m102.4	Hendra / Nipah G glycoprotein	Ferret[l] (NiV) Nonhuman primate[m] (HeV, NiV)

[a]Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vaccinia viruses were protected against disease following intraperitoneal challenge with 10^3 PFU of NiV.(258)

[b]Pigs immunized with NiV F and/or G glycoprotein encoding recombinant canarypox viruses were protected against intranasal challenge with $2.5x10^5$ PFU of NiV.(259).

[c]Ferrets immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intranasal challenge with $5x10^3$ PFU of NiV.(262)

[d]Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intraperitoneal challenge with 10^5 $TCID_{50}$ of NiV;(264) or $6.8x10^4$ $TCID_{50}$ of NiV.(263)

[e]African green monkeys immunized with a NiV G encoding recombinant VSV vector were protected against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV.(265,266)

[f]Hamsters immunized with a NiV G encoding recombinant adeno-associated virus (AAV) vector were protected against lethal intraperitoneal with 10^4 PFU of NiV.(260)

[g]Hamsters and African green monkeys immunized with a NiV G encoding recombinant measles virus vector were protected against lethal intraperitoneal challenge with 10^3 $TCID_{50}$ of NiV (hamsters) or 10^5 $TCID_{50}$ of NiV (AGMs).(261)

[h]Hendra virus soluble G glycoprotein (HeV-sG) used to immunize cats protects against lethal subcutaneous (500 $TCID_{50}$)(268) or oronasal ($5x10^4$ $TCID_{50}$) NiV challenge.(270)

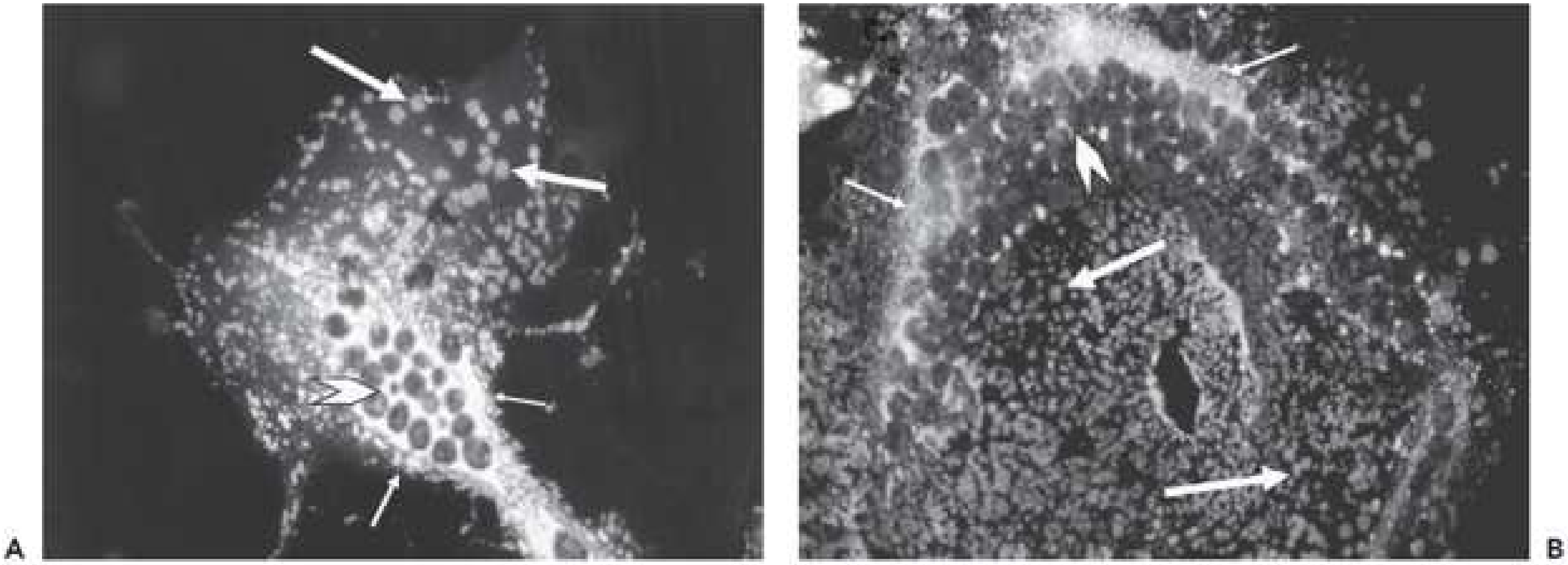
[i]HeV-sG used to immunize ferrets protects against lethal oronasal challenge with $5x10^3$ $TCID_{50}$ of HeV(106) or $5x10^3$ $TCID_{50}$ of NiV challenge.(271)

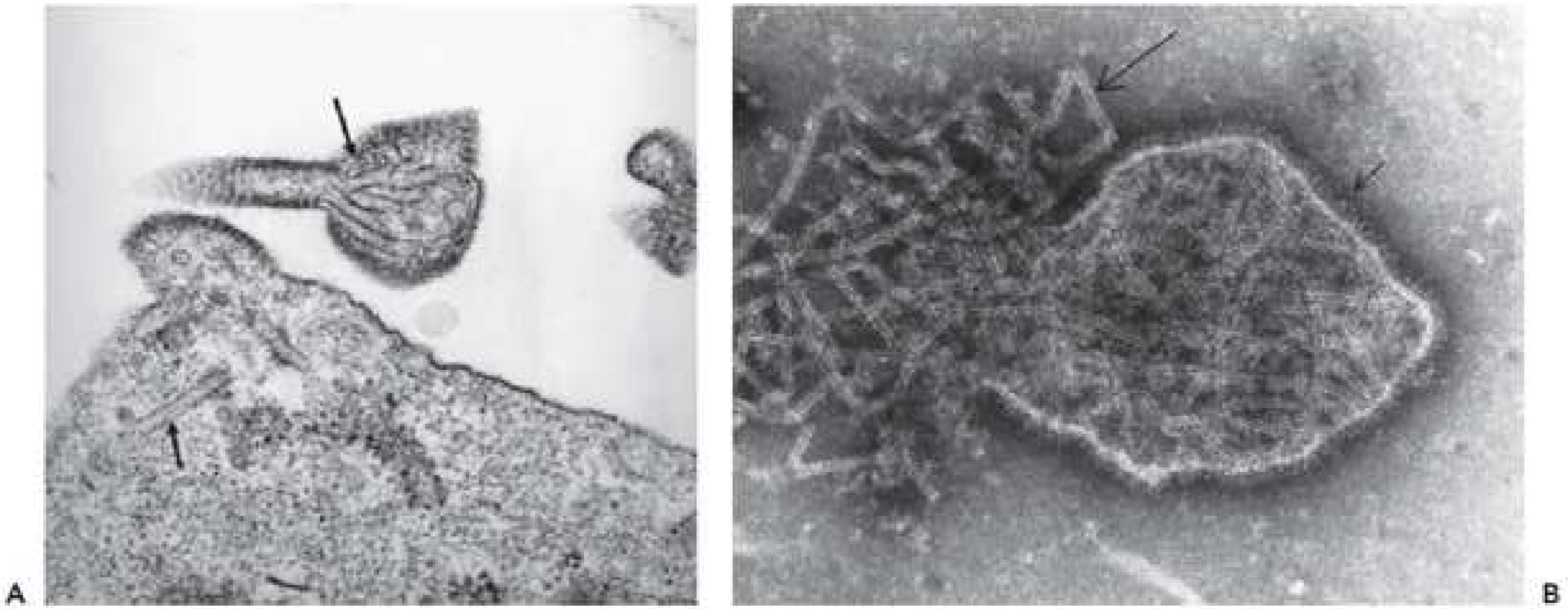
[j]HeV-sG used to immunize African green monkeys protects against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV(272) or $5x10^5$ PFU of HeV.(273)

[k]HeV-sG used to immunize horses protects against lethal oronasal challenge with $2x10^6$ $TCID_{50}$ of HeV(274).

[l]A NiV and HeV cross-reactive G glycoprotein specific neutralizing human mAb (m102.4) protects ferrets against lethal oronasal challenge with $5x10^3$ $TCID_{50}$ of NiV(97) or $5x10^3$ $TCID_{50}$ of HeV (J. Pallister and C. Broder, unpublished) by post-exposure infusion.

[m]Human mAb m102.4 protects African green monkeys by post-exposure infusion following lethal intratracheal challenge with $4x10^5$ $TCID_{50}$ of HeV(193) or lethal intratracheal challenge with $5x10^5$ PFU of NiV.(191,194)


A
B


A
B

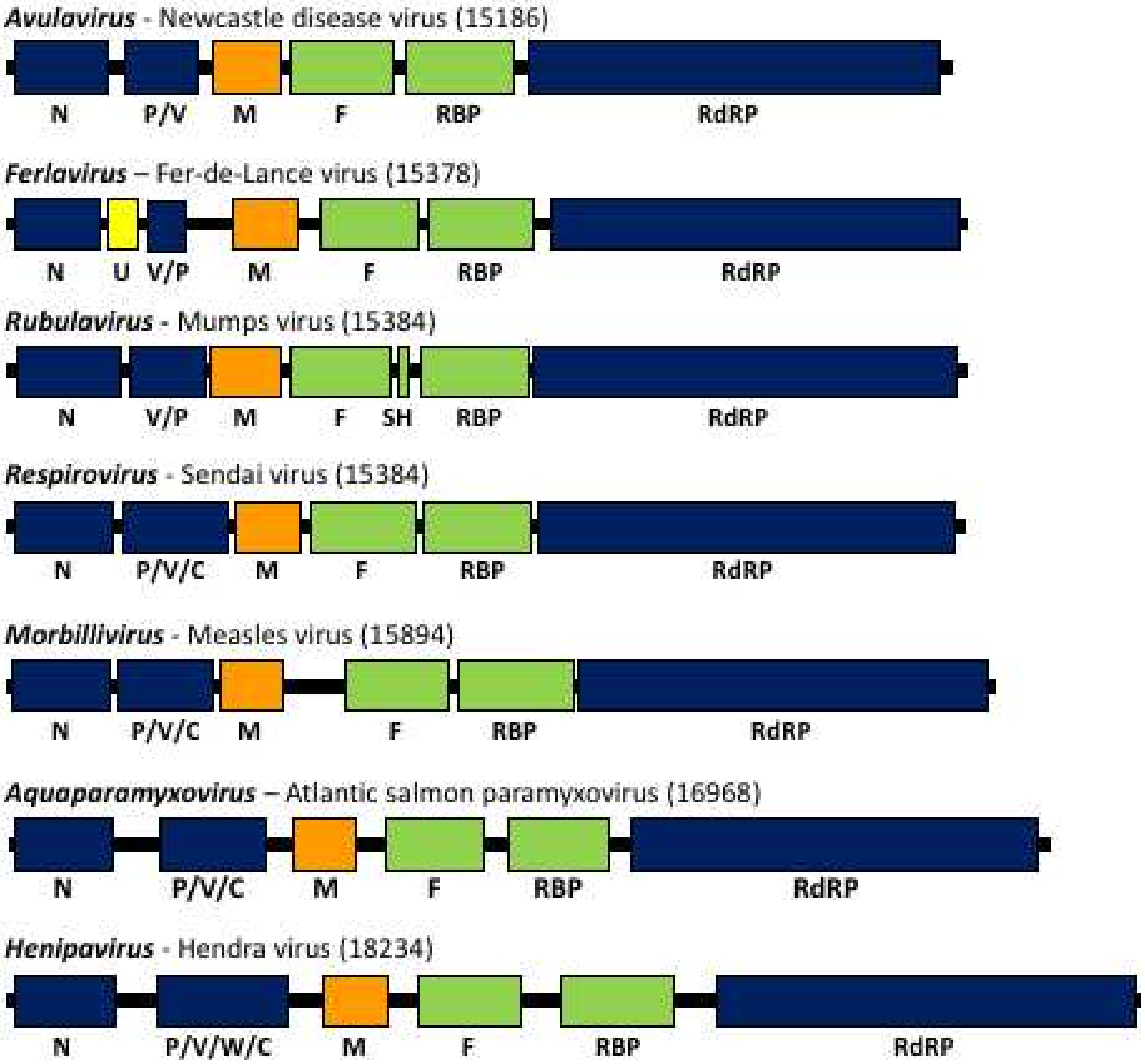
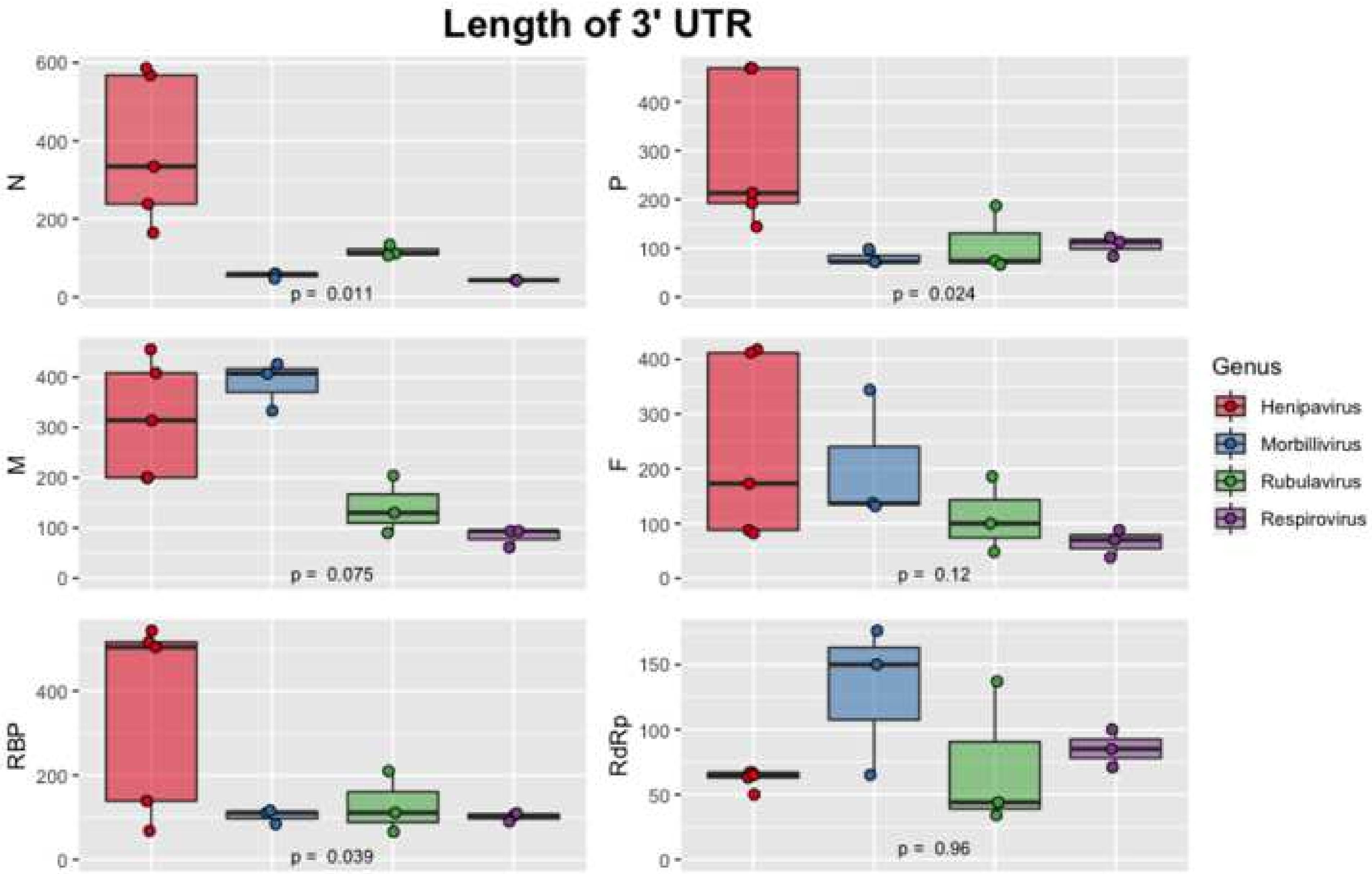

Avulavirus - Newcastle disease virus (15186)
N
P/V
M
F
RBP
RdRP
Ferlavirus – Fer-de-Lance virus (15378)
N
U
V/P
M
F
RBP
RdRP
Rubulavirus - Mumps virus (15384)
N
V/P
M
F
SH
RBP
RdRP
Respirovirus - Sendai virus (15384)
N
P/V/C
M
F
RBP
RdRP
Morbillivirus - Measles virus (15894)
N
P/V/C
M
F
RBP
RdRP
Aquaparamyxovirus – Atlantic salmon paramyxovirus (16968)
N
P/V/C
M
F
RBP
RdRP
Henipavirus - Hendra virus (18234)
N
P/V/W/C
M
F
RBP
RdRP

Figure 14.3B


Length of 3' UTR
N
0
200
400
600
p = 0.011
P
0
100
200
300
400
p = 0.024
M
0
100
200
300
400
p = 0.075
F
0
100
200
300
400
p = 0.12
RBP
0
200
400
p = 0.039
RdRp
0
50
100
150
p = 0.96
Genus
Henipavirus
Morbillivirus
Rubulavirus
Respirovirus

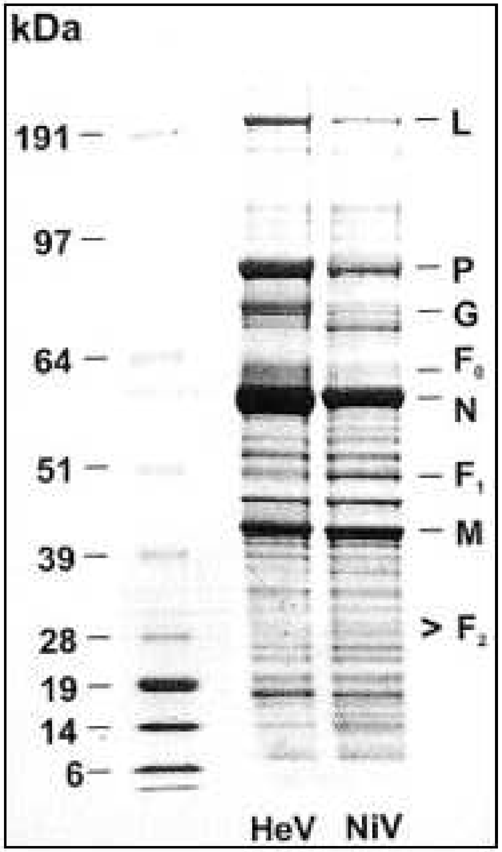
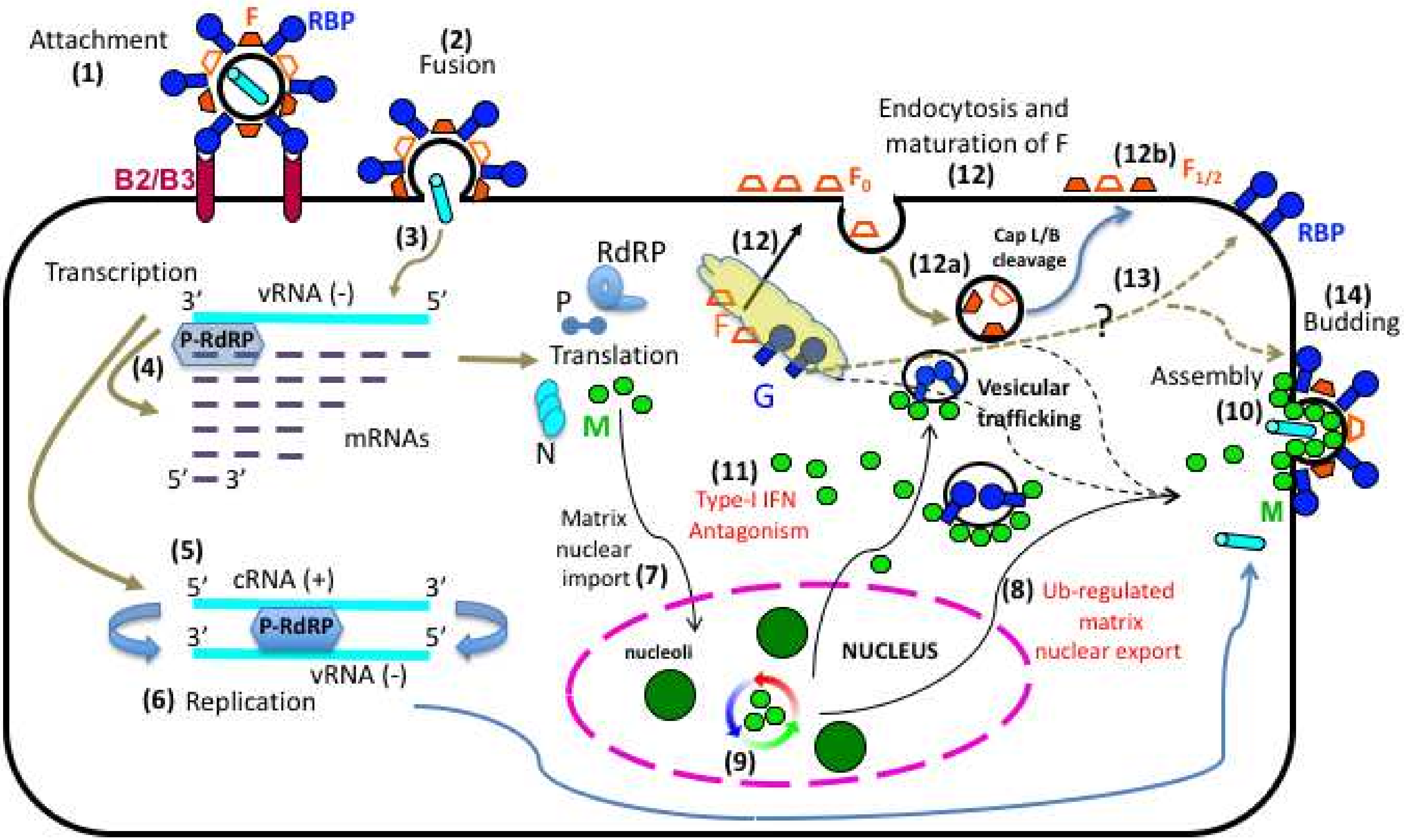

kDa
191
97
64
51
39
28
19
14
6
L
P
G
F0
N
F1
M
> F2
HeV
NiV

Figure 14.4B


Attachment
(1)
F
RBP
(2)
Fusion
B2/B3
(3)
Endocytosis and
maturation of F
(12)
F_0
(12b)
$F_{1/2}$
RBP
Transcription
3′
vRNA (-)
5′
P-RdRP
(4)
mRNAs
5′ 3′
RdRP
P
Translation
N
M
(12)
F
G
(12a)
Cap L/B
cleavage
(13)
?
(14)
Budding
Vesicular
trafficking
Assembly
(10)
(11)
Type-I IFN
Antagonism
Matrix
nuclear
import (7)
M
(5)
5′
cRNA (+)
3′
3′
P-RdRP
5′
vRNA (-)
(6) Replication
nucleoli
NUCLEUS
(9)
(8) Ub-regulated
matrix
nuclear export

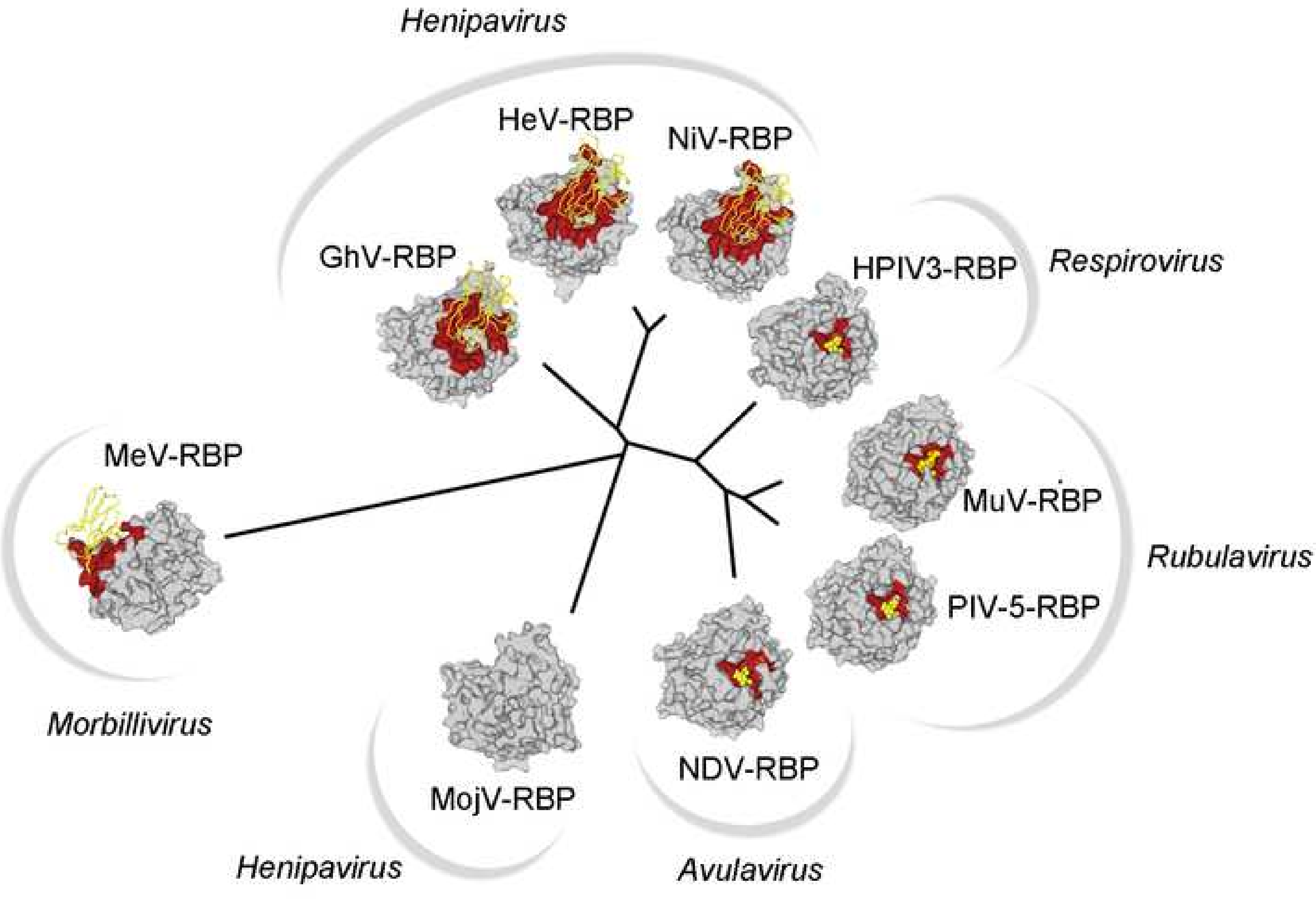
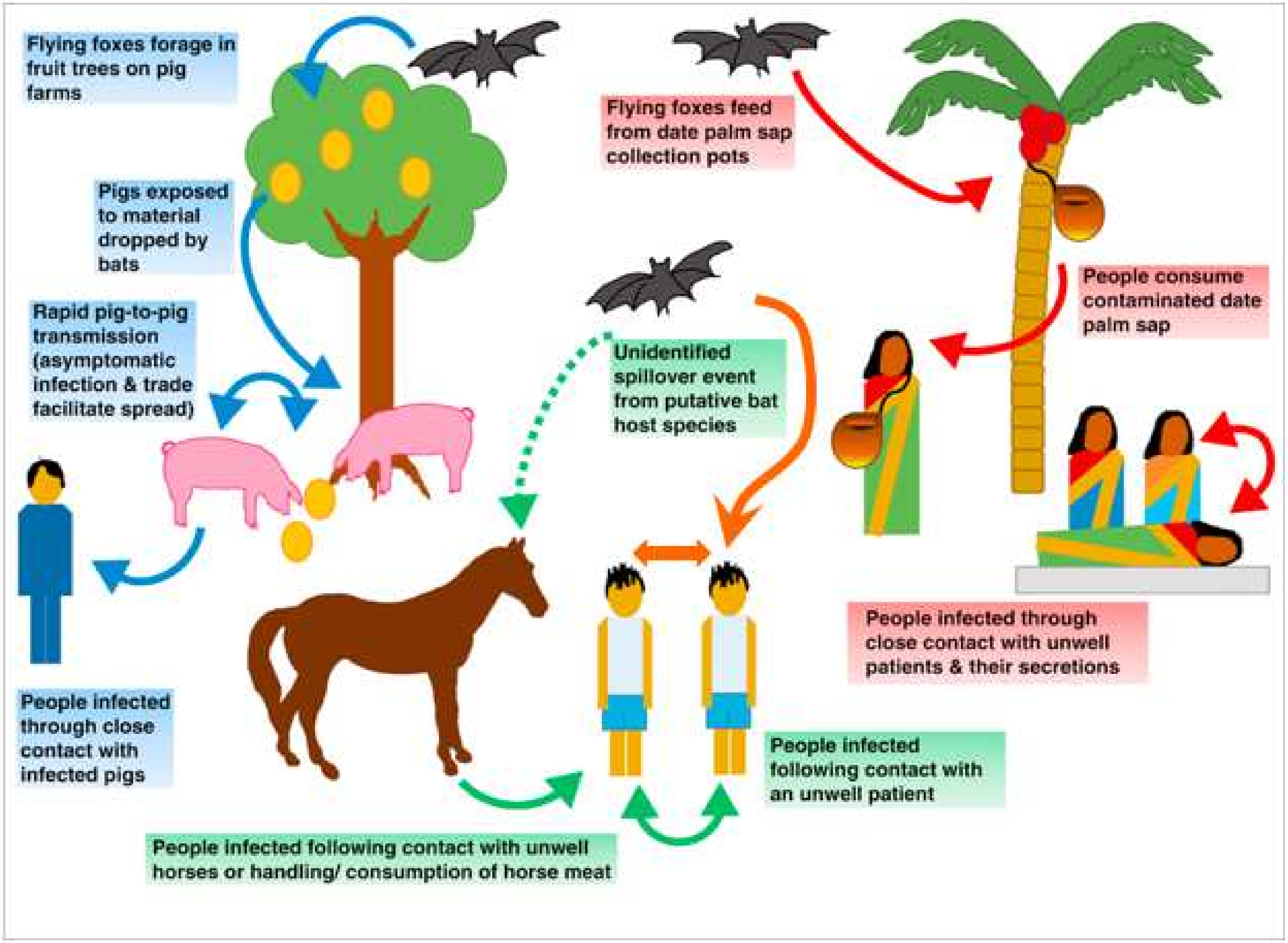
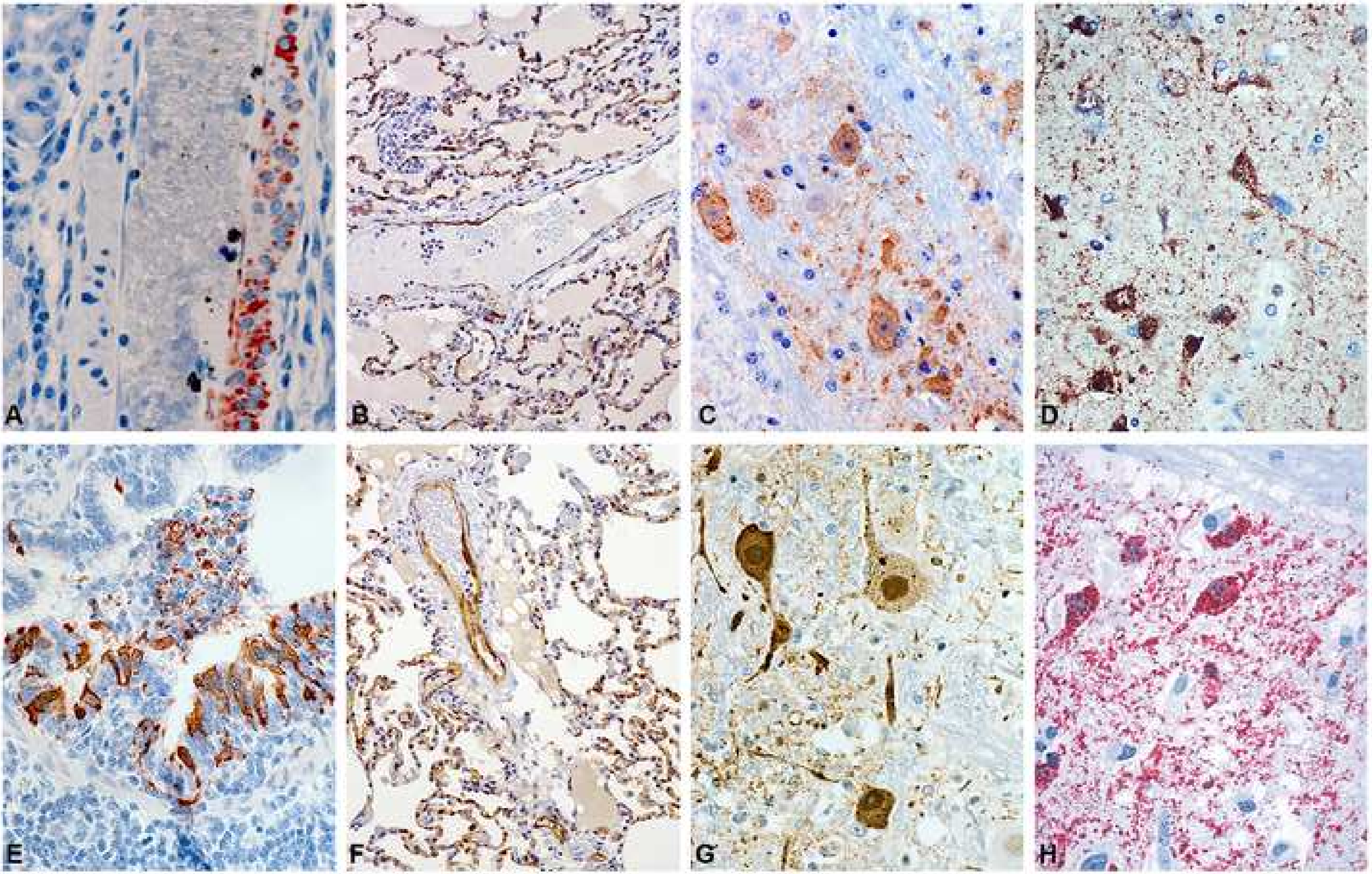
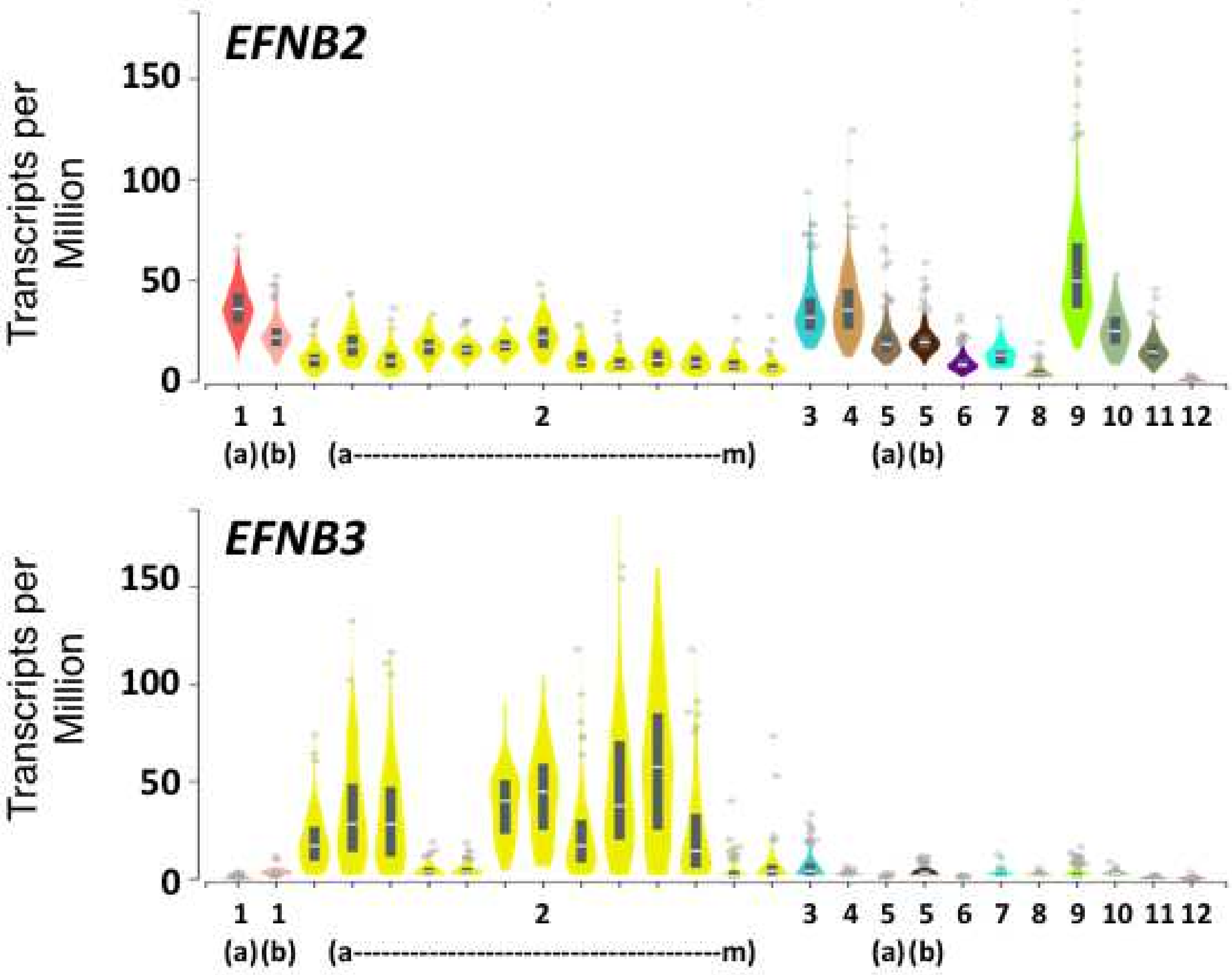

Henipavirus
HeV-RBP
NiV-RBP
GhV-RBP
HPIV3-RBP
Respirovirus
MeV-RBP
MuV-RBP
Rubulavirus
PIV-5-RBP
Morbillivirus
NDV-RBP
MojV-RBP
Henipavirus
Avulavirus

Figure 14.6


Flying foxes forage in fruit trees on pig farms
Pigs exposed to material dropped by bats
Rapid pig-to-pig transmission (asymptomatic infection & trade facilitate spread)
People infected through close contact with infected pigs
Flying foxes feed from date palm sap collection pots
People consume contaminated date palm sap
Unidentified spillover event from putative bat host species
People infected through close contact with unwell patients & their secretions
People infected following contact with an unwell patient
People infected following contact with unwell horses or handling/ consumption of horse meat


A
B
C
D
E
F
G
H

Click here to access/download;Figure;Figure 14.8_Ephrin-B2 & B3.GTEx.tiff



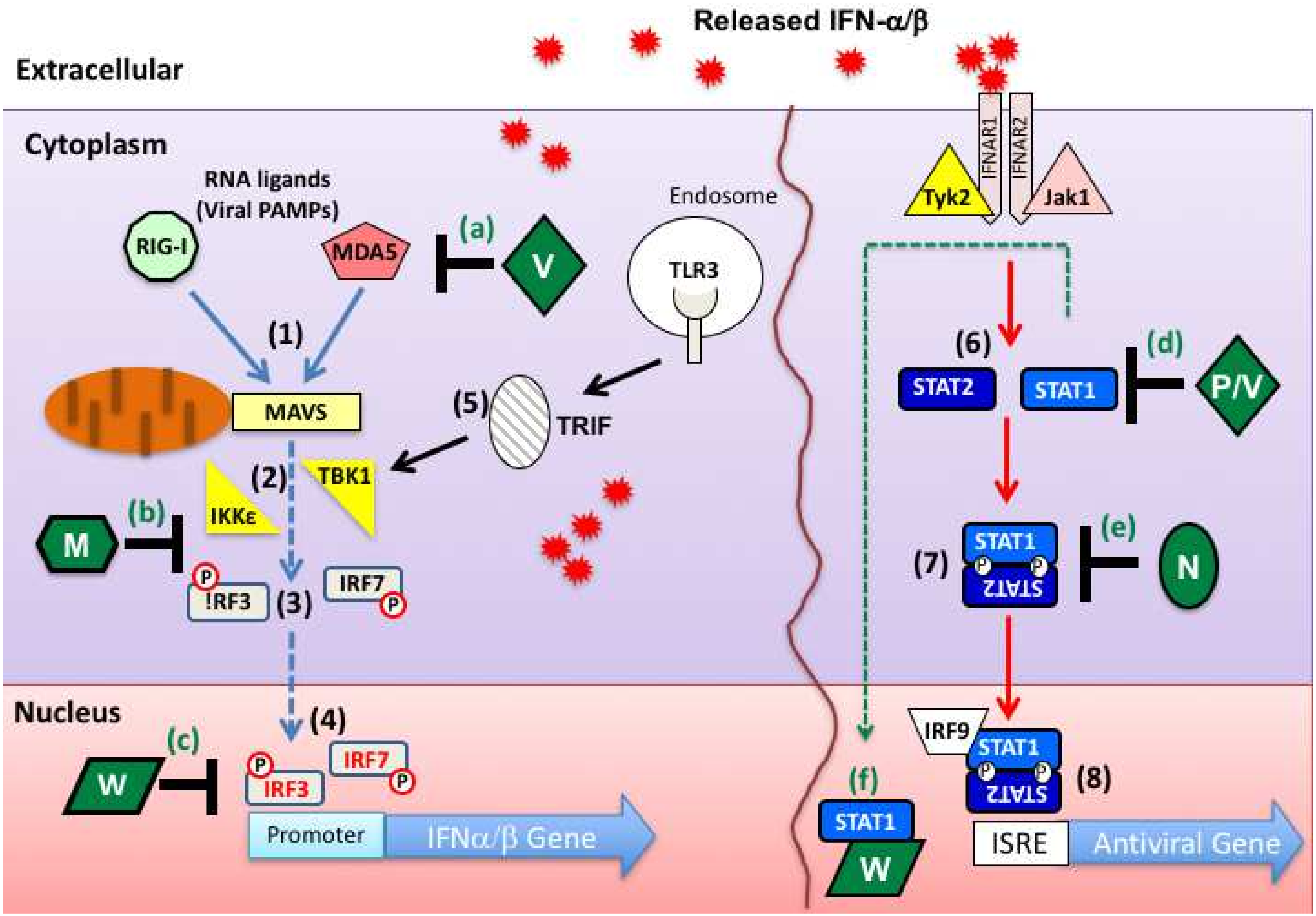

Released IFN-α/β
Extracellular
Cytoplasm
RNA ligands
(Viral PAMPs)
RIG-I
MDA5
(a)
V
Endosome
TLR3
(1)
MAVS
(5)
TRIF
(2)
TBK1
IKKε
(b)
M
P
IRF3
(3)
IRF7
P
Nucleus
(c)
W
(4)
P
IRF3
IRF7
P
Promoter
IFNα/β Gene
IFNAR1
IFNAR2
Tyk2
Jak1
(6)
STAT2
STAT1
(d)
P/V
(7)
STAT1
P
P
STAT2
(e)
N
IRF9
STAT1
P
P
STAT2
(8)
(f)
STAT1
W
ISRE
Antiviral Gene

Figure 14.10

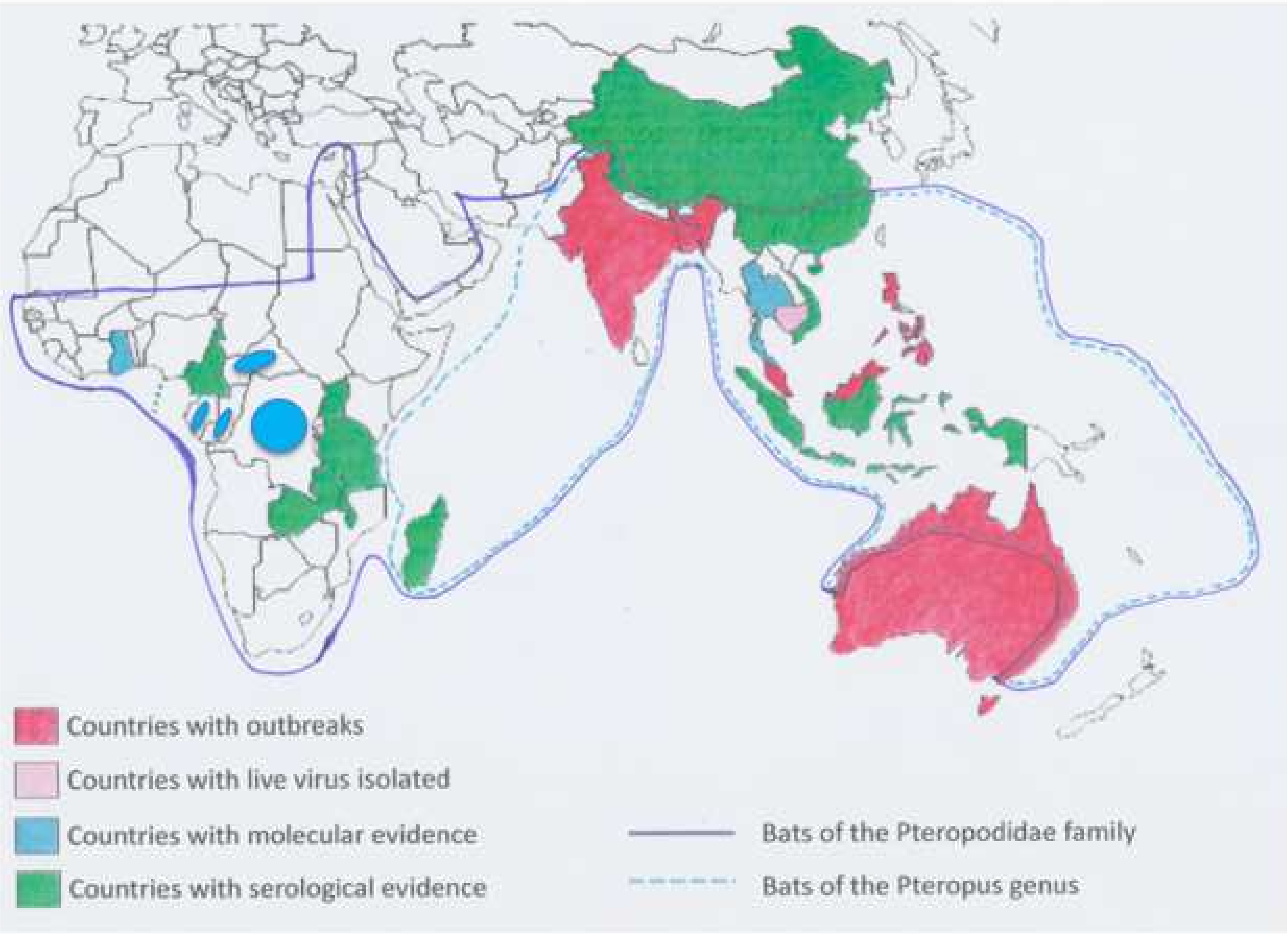

🟥 Countries with outbreaks
Countries with live virus isolated
Countries with molecular evidence
🟩 Countries with serological evidence
Bats of the Pteropodidae family
Bats of the Pteropus genus

Figure 14.11

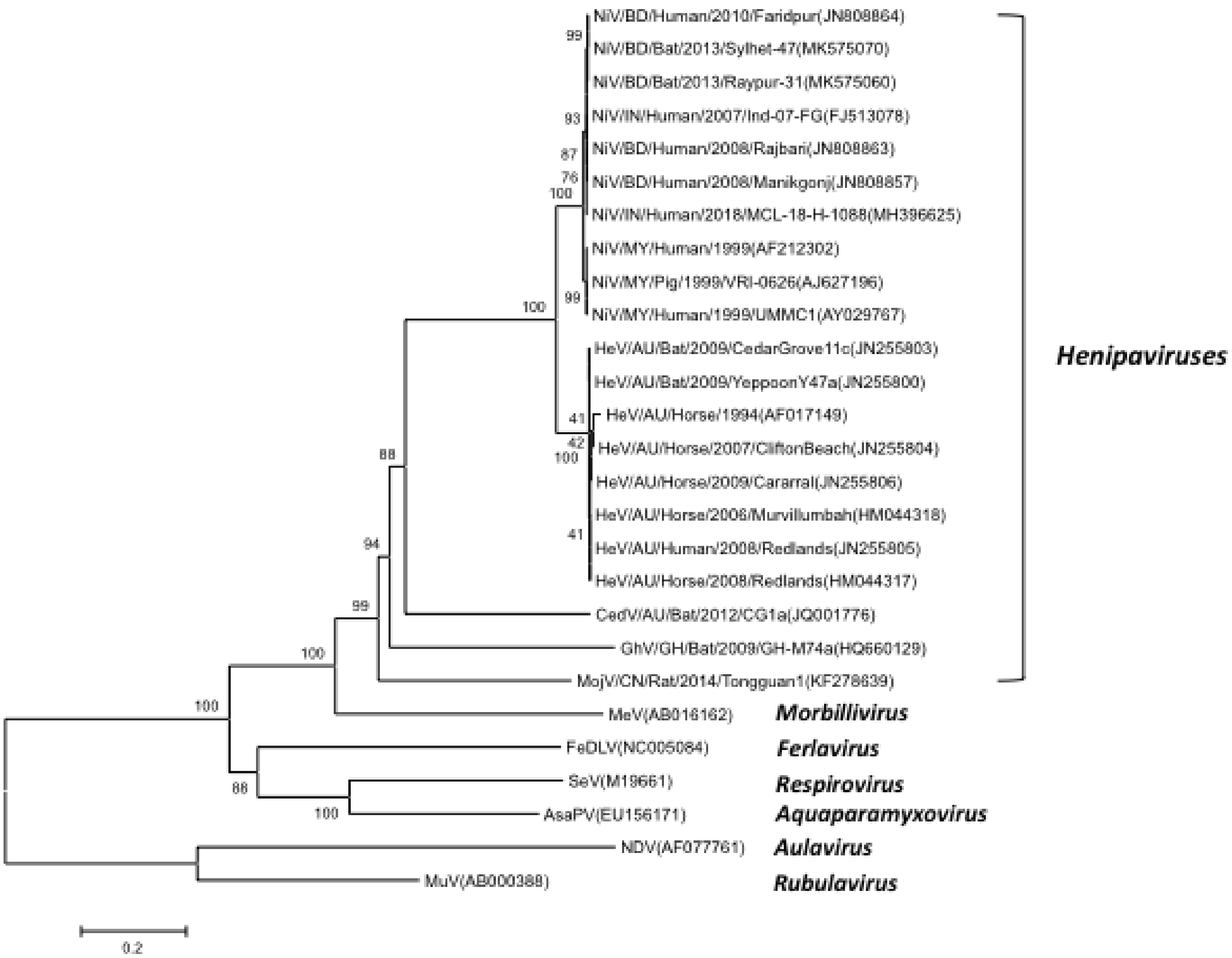
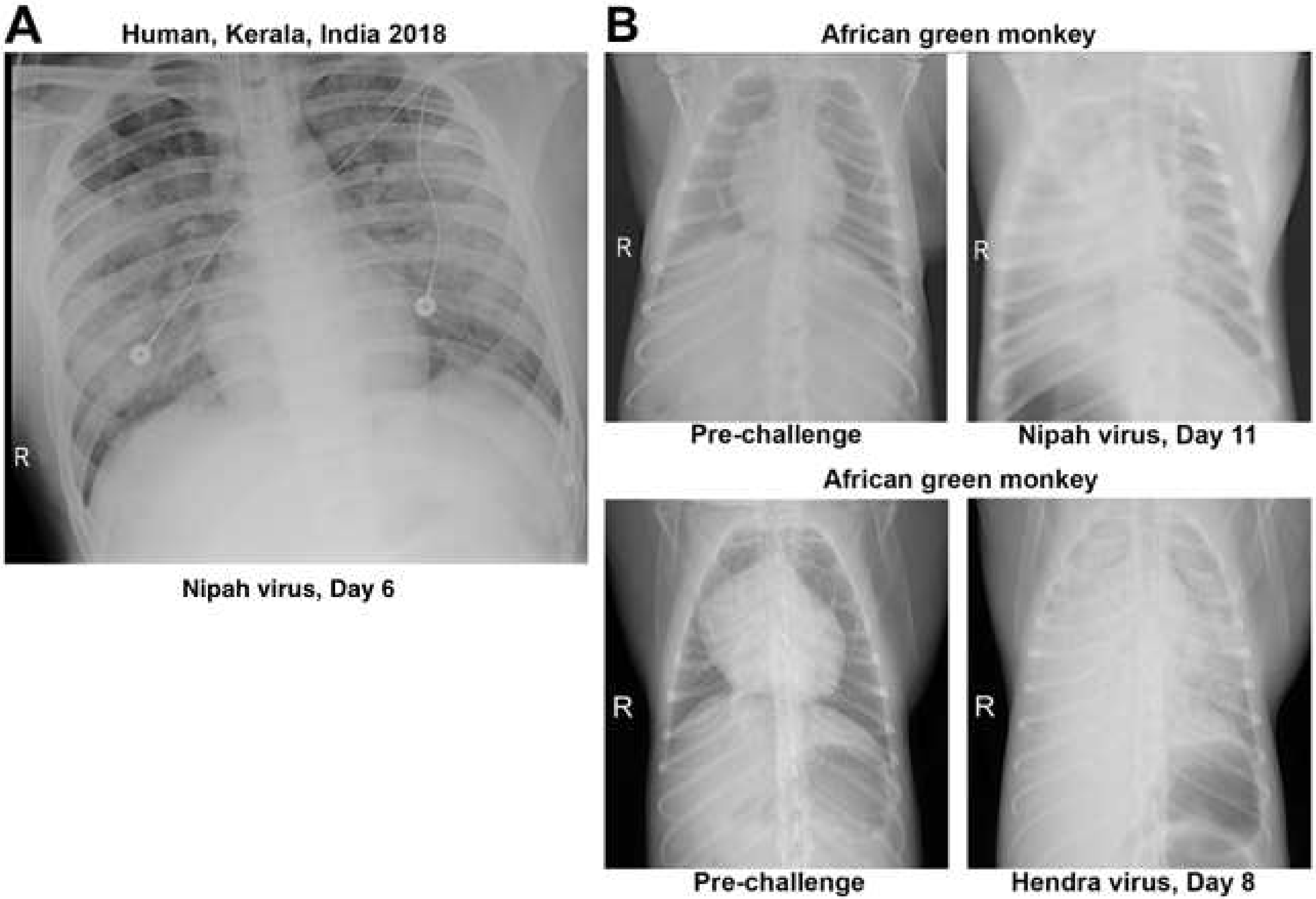

NiV/BD/Human/2010/Faridpur(JN808864)
NiV/BD/Bat/2013/Sylhet-47(MK575070)
NiV/BD/Bat/2013/Raypur-31(MK575060)
NiV/IN/Human/2007/Ind-07-FG(FJ513078)
NiV/BD/Human/2008/Rajbari(JN808863)
NiV/BD/Human/2008/Manikgonj(JN808857)
NiV/IN/Human/2018/MCL-18-H-1088(MH396625)
NiV/MY/Human/1999(AF212302)
NiV/MY/Pig/1999/VRI-0626(AJ627196)
NiV/MY/Human/1999/UMMC1(AY029767)
HeV/AU/Bat/2009/CedarGrove11c(JN255803)
HeV/AU/Bat/2009/YeppoonY47a(JN255800)
HeV/AU/Horse/1994(AF017149)
HeV/AU/Horse/2007/CliftonBeach(JN255804)
HeV/AU/Horse/2009/Cararral(JN255806)
HeV/AU/Horse/2006/Murvillumbah(HM044318)
HeV/AU/Human/2008/Redlands(JN255805)
HeV/AU/Horse/2008/Redlands(HM044317)
CedV/AU/Bat/2012/CG1a(JQ001776)
GhV/GH/Bat/2009/GH-M74a(HQ660129)
MojV/CN/Rat/2014/Tongguan1(KF278639)
MeV(AB016162)
FeDLV(NC005084)
SeV(M19661)
AsaPV(EU156171)
NDV(AF077761)
MuV(AB000388)
Henipaviruses
Morbillivirus
Ferlavirus
Respirovirus
Aquaparamyxovirus
Aulavirus
Rubulavirus
99
93
87
76
100
99
100
41
42
100
41
88
94
99
100
100
88
100
0.2

Figure 14.12


A
Human, Kerala, India 2018
R
Nipah virus, Day 6
B
African green monkey
R
Pre-challenge
R
Nipah virus, Day 11
African green monkey
R
Pre-challenge
R
Hendra virus, Day 8

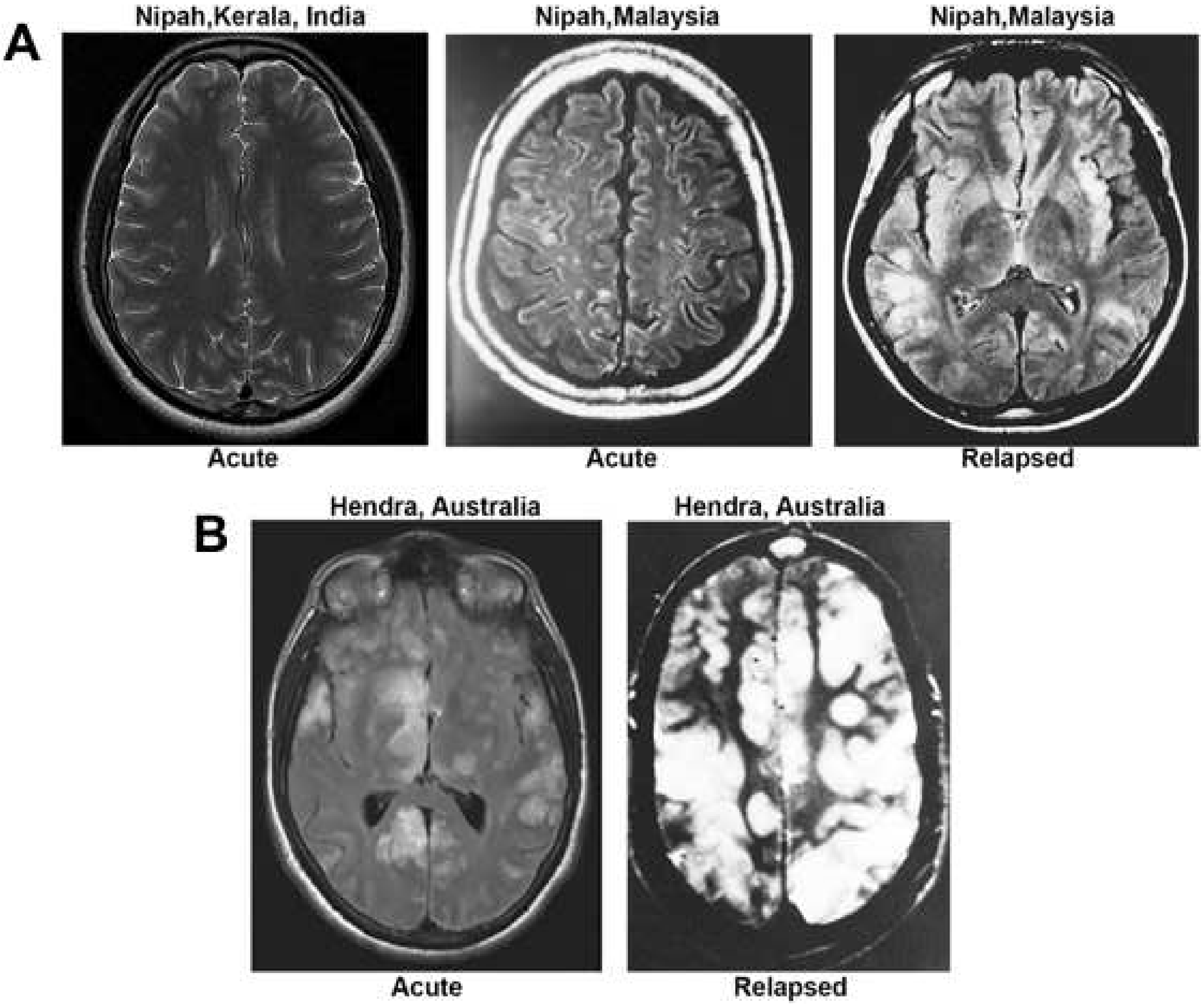

A
Nipah,Kerala, India
Nipah,Malaysia
Nipah,Malaysia
Acute
Acute
Relapsed
B
Hendra, Australia
Hendra, Australia
Acute
Relapsed

Figure 14.14

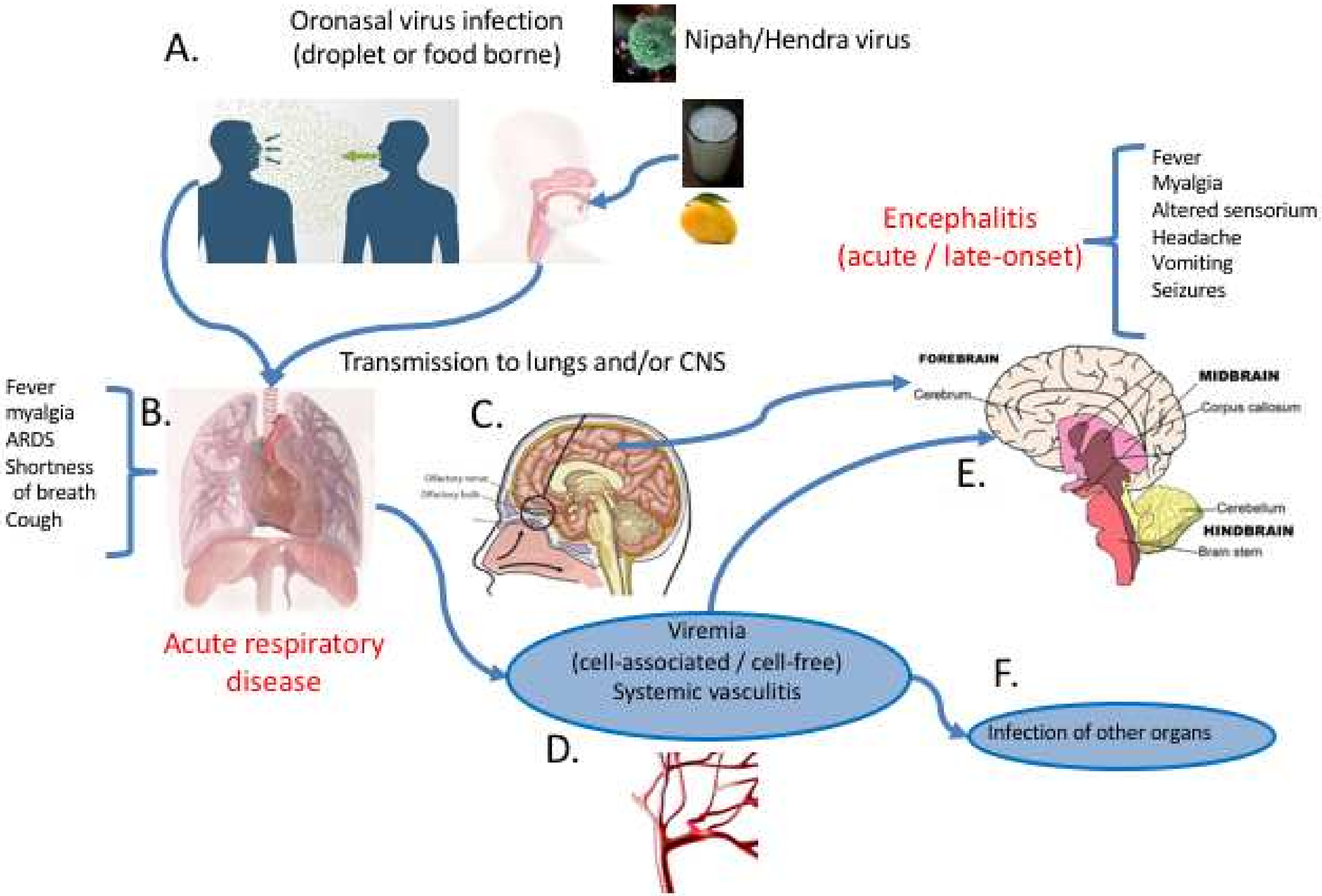


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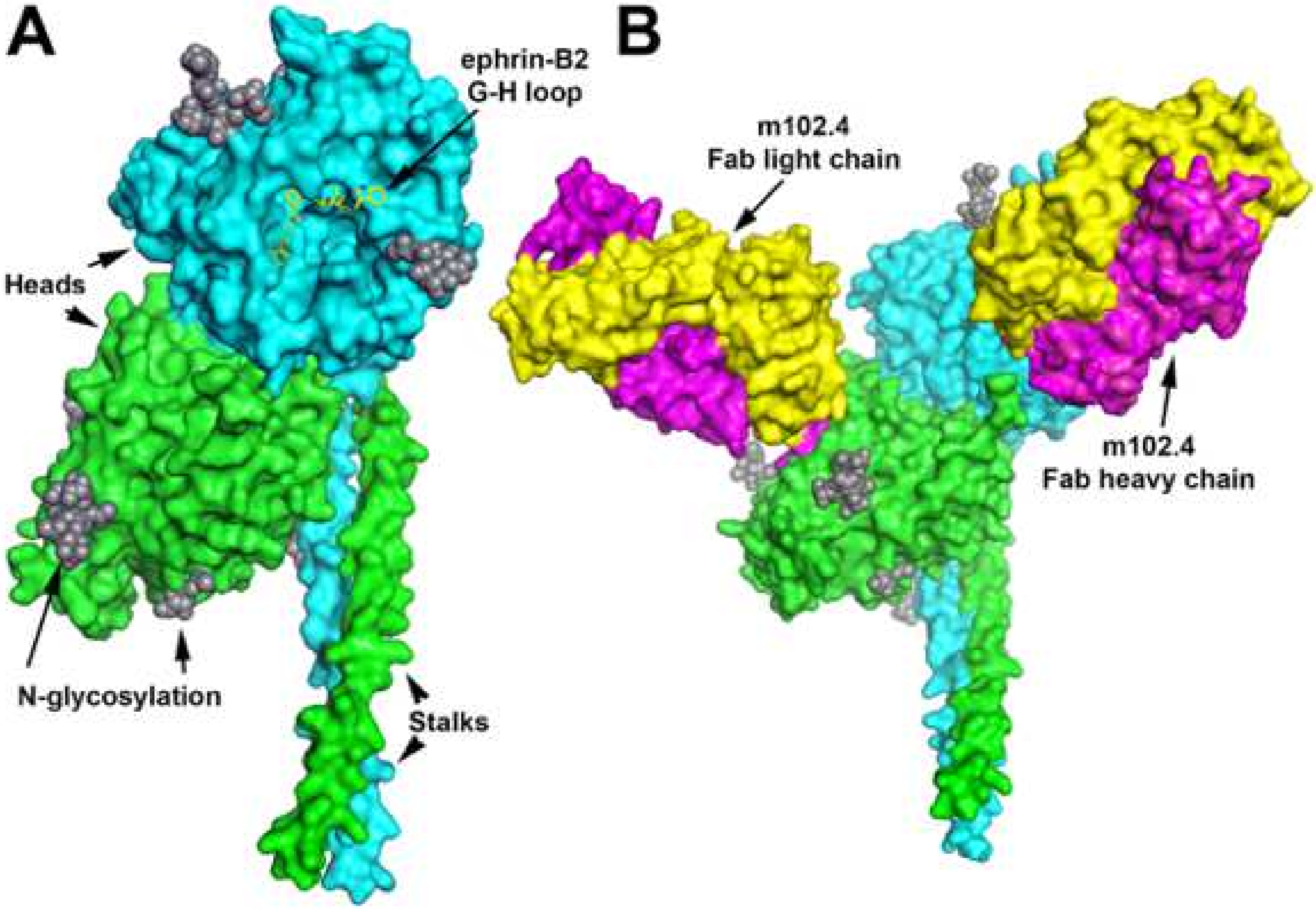
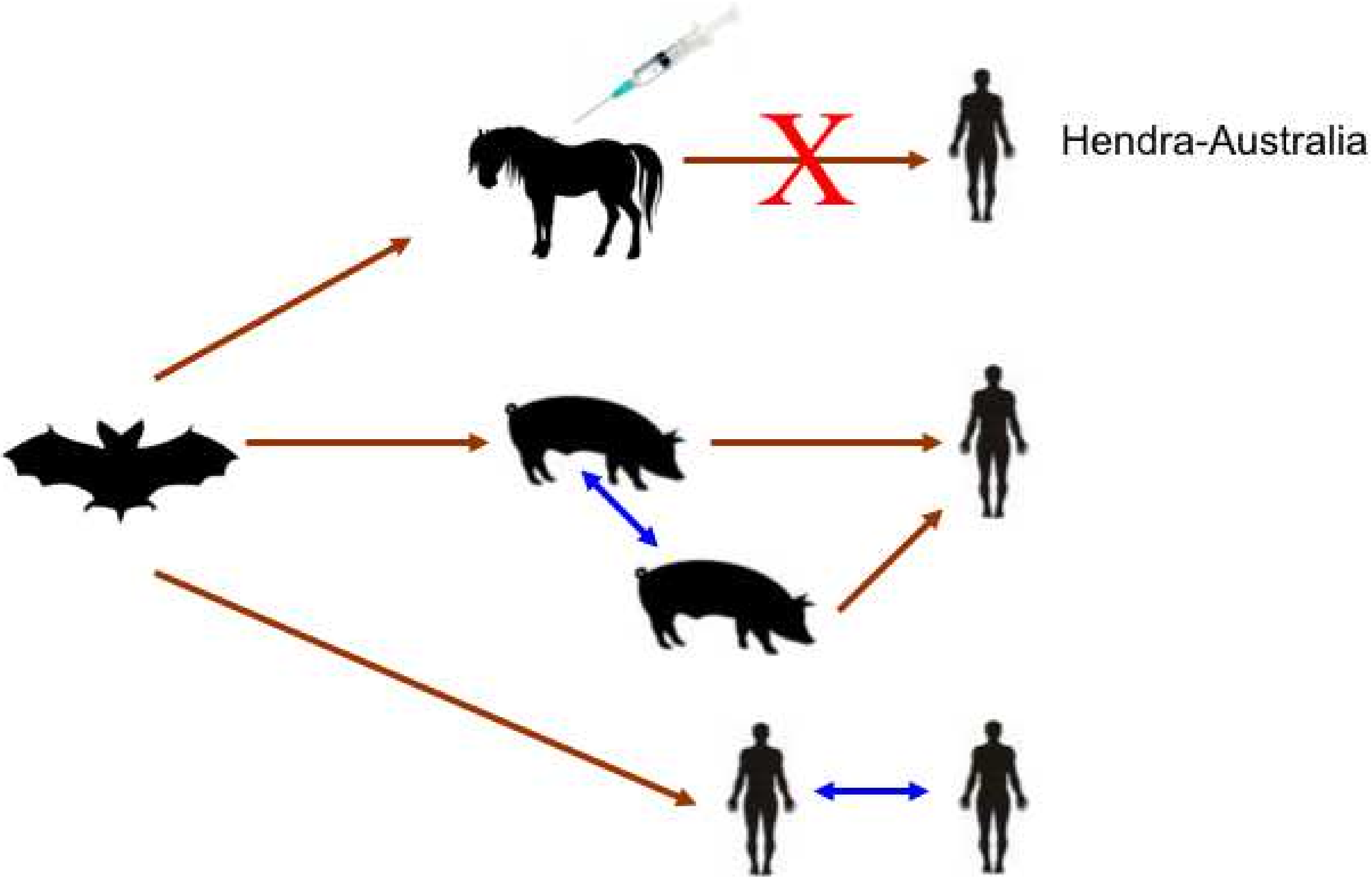
Figure 14.15


A
ephrin-B2
G-H loop
Heads
N-glycosylation
Stalks
B
m102.4
Fab light chain
m102.4
Fab heavy chain



This figure (14.16A) NEEDS Professional artistic rendering?

Figure 14.16B

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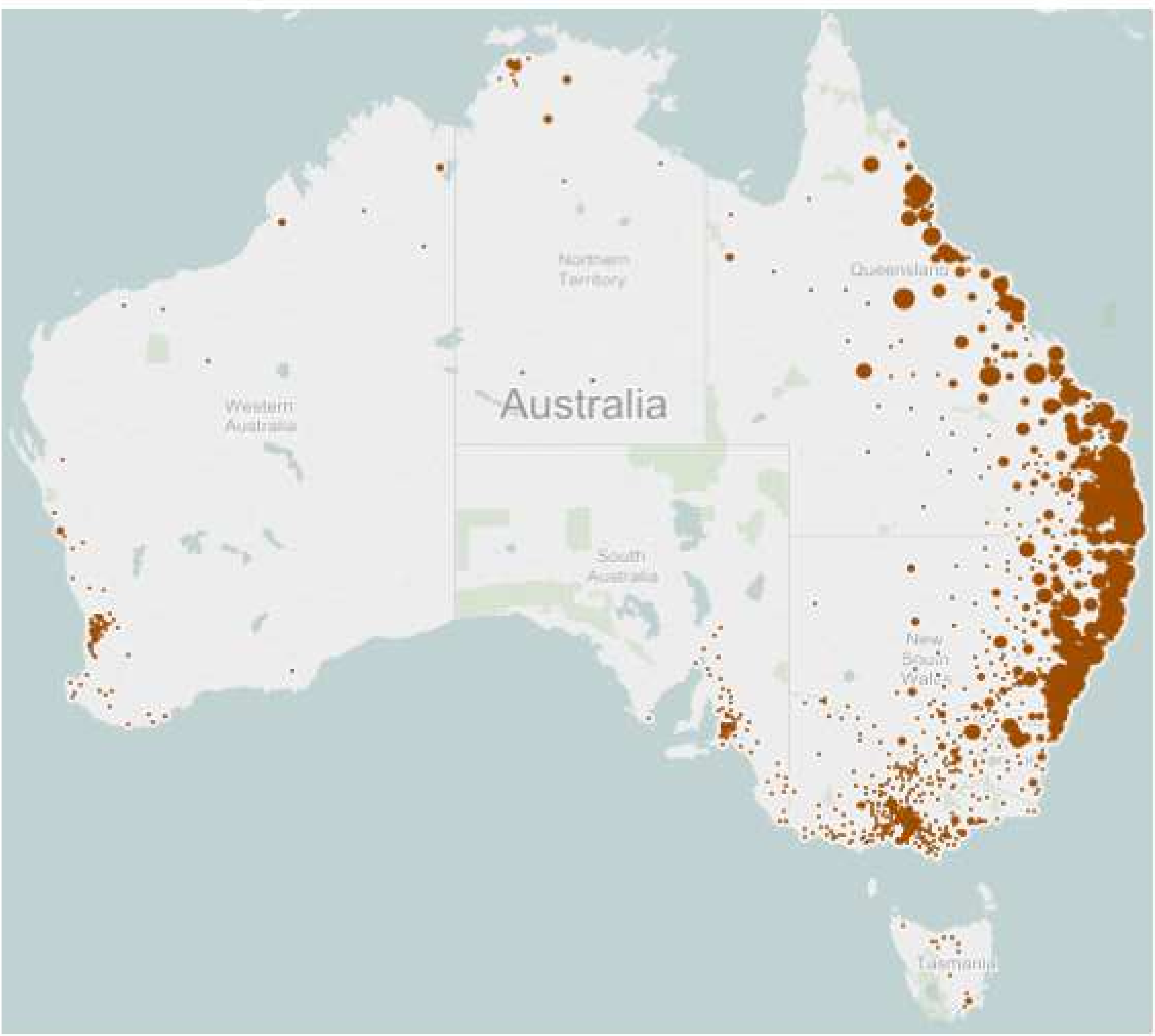

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Original figure numbers	figure 1
Title of the book	Fields Virology
Publisher of the book	Wolters Kluwer
Author of the book	Multiple authors; Edited by Sean Whelan, Peter Howley, David Knipe
Expected publication date	Dec 2019
Estimated size of the book (number of pages)	2500
Requestor Location	Icahn School of Medicine at Mount Sinai One Gustave L Levy Place Department of Microbiology

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Development of an Acute and Highly Pathogenic Nonhuman Primate Model of Nipah Virus Infection

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Abstract

Nipah virus (NiV) is an enigmatic emerging pathogen that causes severe and often fatal neurologic and/or respiratory disease in both animals and humans. Amongst people, case fatality rates range between 40 and 75 percent and there are no vaccines or treatments approved for human use. Guinea pigs, hamsters, cats, ferrets, pigs and most recently squirrel monkeys (New World monkey) have been evaluated as animal models of human NiV infection, and with the exception of the ferret, no model recapitulates all aspects of NiV-mediated disease seen in humans. To identify a more viable nonhuman primate (NHP) model, we examined the pathogenesis of NiV in African green monkeys (AGM). Exposure of eight monkeys to NiV produced a severe systemic infection in all eight animals with seven of the animals succumbing to infection. Viral RNA was detected in the plasma of challenged animals and occurred in two of three subjects as a peak between days 7 and 21, providing the first clear demonstration of plasma-associated viremia in NiV experimentally infected animals and suggested a progressive infection that seeded multiple organs simultaneously from the initial site of virus replication. Unlike the cat, hamster and squirrel monkey models of NiV infection, severe respiratory pathology, neurological disease and generalized vasculitis all manifested in NiV-infected AGMs, providing an accurate reflection of what is observed in NiV-infected humans. Our findings demonstrate the first consistent and highly pathogenic NHP model of NiV infection, providing a new and critical platform in the evaluation and licensure of either passive and active immunization or therapeutic strategies for human use.

Citation: Geisbert TW, Daddario-DiCaprio KM, Hickey AC, Smith MA, Chan Y-P, et al. (2010) Development of an Acute and Highly Pathogenic Nonhuman Primate Model of Nipah Virus Infection. PLoS ONE 5(5): e10690. doi:10.1371/journal.pone.0010690

Editor: Steven Jacobson, National Institutes of Health, United States of America

Received March 8, 2010; **Accepted** April 26, 2010; **Published** May 18, 2010

Funding: This study was supported in part by the Department of Health and Human Services, National Institutes of Health, grants AI082121 and AI057159 to TWG; DE018339, DE019397 and AI071812 to JJM, and AI054715 and AI077995 to CCB. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

Nipah virus (NiV) is a recently recognized viral zoonotic pathogen of the family *Paramyxoviridae* that is distinguished by its ability to cause fatal disease in both animal and human hosts. NiV along with Hendra virus (HeV) comprise the new genus *Henipavirus* (reviewed [1,2]). NiV was first identified during an outbreak of severe encephalitis in Malaysia and Singapore in 1998–99 that involved hundreds of people and more than 100 deaths, with pigs serving as the intermediate amplifying host [3,4]. Since 1998 there have been more than a dozen recognized occurrences of human NiV infection, primarily in Bangladesh and India (reviewed [5]) with the two most recent outbreaks in March 2008 [6] and January 2010 [7]. In the majority of subsequent spillover events, the mortality rate among humans has been higher (~75%) along with evidence of multiple rounds of person-to-person transmission [8].

Several species of fruit bats of the *Pteropus* genus (flying foxes) appear to be the principle natural reservoirs of both NiV and HeV (reviewed [5]) but serological evidence of NiV or Nipah-like virus infection has recently been reported in several additional frugivorous and insectivorous bats [9,10]. NiV has been isolated from bat urine and partially eaten fruit [11,12] and direct transmission of NiV from flying foxes to humans from contaminated food sources has been suggested [13,14]. The Centers for Disease Control and Prevention (CDC) and the National Institute of Allergy and Infectious Diseases (NIAID) have classified NiV as a select agent with the potential for causing significant morbidity and mortality in humans and major economic and public health impacts. Consequently, work with live virus requires Biosafety Level 4 (BSL-4) containment.

Host cell infection by NiV requires two membrane-anchored envelope glycoproteins; the attachment glycoprotein (G) which binds receptor and the fusion (F) glycoprotein which facilitates virus-host cell membrane fusion [2]. NiV G lacks hemagglutinin and neuraminidase activities and F is a typical class I fusion glycoprotein [15]. Recently, ephrin-B2 and ephrin-B3 ligands

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Portion	figure/table
Number of figures/tables	1
Include high res file(s) delivery	no
Format	print and electronic
Will you be translating?	no
Circulation	2080
Distributing to	Worldwide
Title of new book	Fields Virology
Lead author	Multiple authors; Edited by Sean Whelan, Peter Howley, David Knipe
Publisher	Wolters Kluwer
Expected publication date	Dec 2019
Expected number of pages	2500
Portions	Figure 1, page 1232
Requestor Location	Christopher Broder Department of Microbiolgy B4-106 4301 Jones Bridge Road BETHESDA, MD 20814 United States Attn: Christopher Broder
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Page created: February 04, 2010

Page last updated: January 20, 2017

Page last reviewed: January 20, 2017

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Expected publication date	Dec 2019
Estimated size of the book (number of pages)	2500

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Antiviral Research
Volume 100, Issue 1, October 2013, Pages 8-13

Review

A treatment for and vaccine against the deadly Hendra and Nipah viruses

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Highlights

- Advanced antivirals against Nipah and Hendra are a Hendra-sG subunit vaccine and a human monoclonal antibody, m102.4.
- The Hendra-sG vaccine was launched in Australia for equine use and is the first BSL-4 agent vaccine for public use.

- m102.4 has been used in people on a compassionate use basis in Australia and is presently in pre-clinical development.
- A structural model of the Hendra-sG vaccine and its complex with the neutralizing human monoclonal antibody is shown.

Abstract

Hendra virus and Nipah virus are bat-borne paramyxoviruses that are the prototypic members of the genus *Henipavirus*. The henipaviruses emerged in the 1990s, spilling over from their natural bat hosts and causing serious disease outbreaks in humans and livestock. Hendra virus emerged in Australia and since 1994 there have been 7 human infections with 4 case fatalities. Nipah virus first appeared in Malaysia and subsequent outbreaks have occurred in Bangladesh and India. In total, there have been an estimated 582 human cases of Nipah virus and of these, 54% were fatal. Their broad species tropism and ability to cause fatal respiratory and/or neurologic disease in humans and animals make them important transboundary biological threats. Recent experimental findings in animals have demonstrated that a human monoclonal antibody targeting the viral G glycoprotein is an effective post-exposure treatment against Hendra and Nipah virus infection. In addition, a subunit vaccine based on the G glycoprotein of Hendra virus affords protection against Hendra and Nipah virus challenge. The vaccine has been developed for use in horses in Australia and is the first vaccine against a Biosafety Level-4 (BSL-4) agent to be licensed and commercially deployed. Together, these advances offer viable approaches to address Hendra and Nipah virus infection of livestock and people.

Previous Next

Keywords

Hendra virus; Nipah virus; Vaccine; Monoclonal antibody; G glycoprotein; Horse

1. Introduction

Hendra virus and Nipah virus are recently recognized bat-borne paramyxoviruses, each of which have repeatedly emerged causing significant morbidity and mortality in both animal and human populations since the mid to late 1990's. Hendra virus was isolated in Australia from fatal cases of severe respiratory disease in horses and one person in the Brisbane suburb of Hendra in September, 1994, and was shown to be distantly related to measles virus and other morbilliviruses (Murray et al., 1995). The same virus had also caused fatal infections in horses a month prior in Mackay, Australia, but this emergence was only recognized when one individual who was unknowingly exposed to the infected horses at that time developed a recrudescence of fatal meningoencephalitis 13 months later (O'Sullivan et al., 1997, Wong et al., 2009). Hendra virus' close relative, Nipah virus, emerged in peninsular Malaysia in 1998–99, in a large outbreak of respiratory disease in pigs along with numerous cases of encephalitis among pig farmers, eventually resulting in more than 100 human fatalities. Genetic and serological studies revealed the relatedness of this new virus to Hendra virus (Chua et al., 2000). Hendra virus and Nipah virus now represent the prototype species of the new genus *Henipavirus* within the paramyxovirus family (Wang et al., 2013).

Since their discovery, both Hendra virus and Nipah virus have continued to repeatedly cause spillover events into animals and/or people. Hendra virus infection among horses in Australia has occurred annually since 2006 and in total there have now been 7 human cases of which 4 have been fatal (Anonymous, 2009b, Playford et al., 2010). In all 7 human cases, Hendra virus was transmitted from infected horses to humans. Of note, in 2011 from the months of June to October, a significant increase in the number of Hendra virus spillovers occurred with 18 separate episodes of infection in horses in Australia, including the first recognized case of infection in a dog (reviewed in (Broder, 2012)). There were 8 cases of Hendra virus spillovers into horses in 2012 (Anonymous, 2012b) and a further two cases of Hendra virus infection in horses in early 2013 (Anonymous, 2013b). In all, a total of 42 Hendra virus spillover events have occurred since 1994 and 28 of these have occurred in just the past 2 years. Likewise, following the Malaysian outbreak in 1998, nearly annual outbreaks of Nipah virus infection, occurring primarily in Bangladesh but also India have occurred since 2001. The most recent outbreak occurred in early 2013, with apparently 10 fatalities of 12 cases (Anonymous, 2013c). Compared to the original Malaysian outbreak, these Nipah virus spillovers have been smaller in case number, however the fatality rates in people overall have been notably higher, ranging from 75–100%. Importantly, direct transmission of Nipah virus from bats to humans and significant human-to-human transmission have also been documented during outbreaks in India and

Bangladesh. The epidemiological details of the spillovers of both Hendra virus and Nipah virus into people since their emergence and recognition have recently been reviewed and summarized in detail (Luby and Gurley, 2012). There have been an estimated 582 cases of Nipah virus infection with 315 human fatalities (Anonymous, 2013c, Luby and Gurley, 2012, Luby et al., 2009, Pallister et al., 2011a).

2. The henipavirus transboundary threat

The natural reservoir hosts of Hendra virus and Nipah virus are several species of pteropid fruit bats among which they are not known to cause disease (Halpin et al., 2011). However, Hendra and Nipah viruses possess an exceptionally broad species tropism and both natural and experimental infections have demonstrated their capacity to cause disease which can often be fatal in horses, pigs, cats, dogs, ferrets, hamsters, guinea pigs, monkeys, and humans, spanning 6 mammalian Orders (reviewed in (Geisbert et al., 2012)). In disease susceptible animal hosts and people, Nipah virus and Hendra virus cause a systemic infection that is characterized as a wide-spread vasculitis and endothelial cell tropism. Though this pathology is not unique to these henipaviruses, an understanding of Hendra and Nipah virus cellular tropism on the molecular level has provided an explanation to this disease feature which includes the appearance of syncytia, thrombosis, ischemia and necrosis, with parenchymal cell infection and associated pathology in many major organ systems, and prominently in the brain and lung (reviewed in (Weingartl et al., 2009, Wong and Ong, 2011)). The major involvement of the lung and brain in Hendra and Nipah virus infection often manifests as an acute severe respiratory syndrome, encephalitis or a combination of both. Disturbingly however, infection in people can also have longer term consequences, and in addition to an acute symptomatic infection, Hendra and Nipah virus infection can also take a protracted course following recovery from an initial infection. Individuals in these cases can later undergo a recrudescence of virus replication in the central nervous system (CNS) causing a relapse of encephalitis, a process that was first noted in the second fatal case of Hendra virus human infection (O’Sullivan et al., 1997, Wong et al., 2009). Quite remarkably, relapsed-encephalitis caused by Nipah virus has been reported in people from several months to as long as 11 years following infection (Abdullah et al., 2012) (reviewed in (Wong, 2010)). How the henipaviruses survive immune-mediated clearance and can later cause a recrudescence of replication in the CNS is unknown, but this virological feature clearly has important implications for anti-henipavirus therapeutics development.

Given the virulence of Hendra and Nipah virus and the increase in their spillover occurrences over the past decade, strategies to mitigate the risk of Hendra and Nipah virus exposure have become paramount. Both Hendra virus and Nipah virus reside in large wild bat populations, which make controlling virus in the reservoir host or influencing the reservoir host population dynamics difficult to impossible. In extreme instances, bat culling has been proposed to minimize exposure; however, the ecological importance of bats as a whole makes this an unrealistic option. In Malaysia and Australia efforts have been made to reduce livestock interactions with bats; for example, restricting livestock access to areas under fruit trees, covering water and feed containers to prevent contamination and not placing water and feed under fruit trees (Anonymous, 2013a). However, the significant numbers of fruit trees and roosting flying foxes on or near properties containing livestock makes complete separation of the wildlife and livestock populations near impossible. In Bangladesh, measures have been employed to prevent flying foxes access to date palm sap collectors in hopes of preventing contamination with Nipah virus (Luby and Gurley, 2012). Unfortunately, Nipah outbreaks continue to occur every year reflecting the difficulty of implementing a new practice culturally to prevent such a disease that is still considered to be rare. Developing vaccines and antiviral therapies for Hendra and Nipah virus are also viable alternatives for mitigating disease risk. As livestock have been identified as intermediate hosts for both Hendra and Nipah virus, antiviral therapies seem less attractive given the size of horses and pigs and the significant costs associated with producing large quantities of any possible drug. Conversely, vaccination of livestock populations is a highly attractive mitigation strategy since both disease in the target species as well as secondary transmission of virus to humans would be prevented. In areas such as Bangladesh, where no intermediate host has been definitively identified, there is a real need for the development of effective therapies and vaccine strategies to prevent infection. Similarly, for individuals who have potential occupational exposure to Hendra and Nipah virus infection, such as pig farmers and equine veterinarians, therapeutic agents and/or a vaccine to prevent infection would significantly reduce morbidity and mortality associated with Hendra and Nipah viruses.

Hendra and Nipah virus attach to host cell-surface displayed ephrin-B2 or -B3 proteins and infect host cells by the coordinated activity of their attachment (G) and fusion (F) glycoproteins (reviewed in (Aguilar and Iorio, 2012, Lee and Ataman, 2011)). The G glycoprotein monomer consists of a stalk and globular head (Fig. 1) and the atomic structures of both the Nipah and Hendra virus G glycoprotein's globular head domain have been determined alone and in complex with ephrin proteins (reviewed in (Xu et al., 2012a)). The F glycoprotein mediates the membrane fusion process between the viral and host cell membranes by a Class I fusion mechanism that is initiated following the G glycoprotein engagement of ephrin receptor (Lee and Ataman, 2011). The susceptible host species and associated cellular tropism and pathology of Hendra and Nipah virus has in large part been explained by their use of the highly conserved ephrin-B2 and -B3 proteins as entry receptors (reviewed in (Pernet et al., 2012, Wong and Ong, 2011)). In addition and of importance to countermeasure development, the henipavirus G and F envelope glycoprotein spikes are major targets of virus-neutralizing antibodies and as discussed below, the development of potential vaccines have largely focused on these important structural components of the virion (reviewed in (Broder, 2010)).

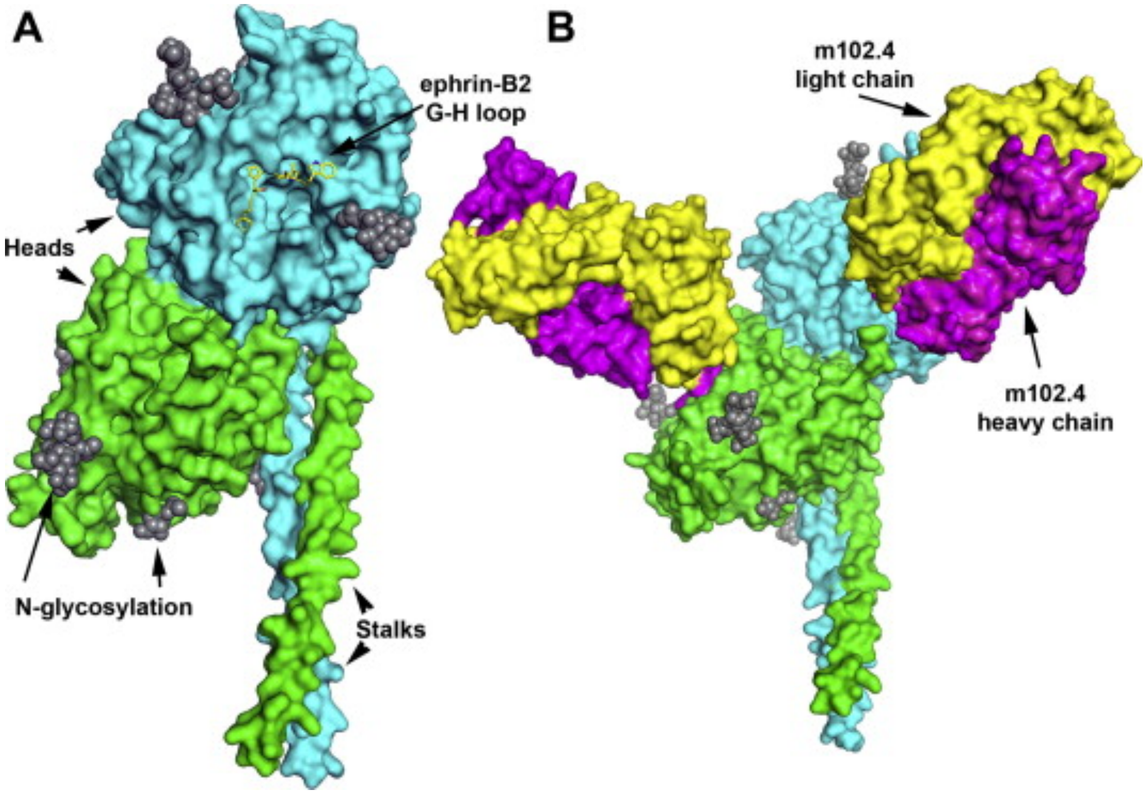

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Fig. 1. Model of the Hendra virus soluble G glycoprotein subunit vaccine (HeV-sG) and its complex with the henipavirus-neutralizing human monoclonal antibody m1024. (A) The HeV-sG glycoprotein subunit vaccine is composed of the entire ectodomain (amino acids 76–604) of the HeV G glycoprotein. Here, HeV-sG is shown as dimer with one monomer colored green and the other cyan. The secondary structure elements of the two globular head domains are derived from the crystal structure of the HeV G head domain (Colgrave et al., 2011, Xu et al., 2012b), and the stalk regions of each G monomer (residues 77–136) are modeled (Kelley and Sternberg, 2009). N-linked glycosylation sites shown as gray spheres. The ephrin-binding face of the cyan globular head is facing forward with an overlay of the interacting ephrin-B2 G-H loop residues

in yellow. (B) The HeV-sG dimer shown in complex with two m102.4 Fab antibody fragments. The two HeV-sG monomers are colored green and cyan as in panel A and rotated slightly to the right. The two Fab m102.4 molecules are shown with their heavy chains colored in magenta and light chain – in yellow, each binding one globular head domains of G. (Xu, K. et al., submitted). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

The development of medical countermeasures for use in humans is a time-consuming process, especially for highly pathogenic BSL-4 agents like Hendra and Nipah virus where human efficacy trials are not feasible. Demonstrated efficacy in two animal models of disease is required to support possible licensure. In recent years monoclonal antibodies (mAbs) have attracted considerable attention as viable antiviral and antibacterial therapies, and the Food and Drug Administration (FDA) has approved both humanized and fully human monoclonal antibody (mAb) for use in preventing or treating infectious diseases in humans (Dolgin, 2013, Zhu et al., 2013). The development of human monoclonal antibodies (humAbs) against Hendra and Nipah virus infection has been highly successful and as discussed below, a viable post-exposure mAb therapy is currently in development. In addition, a recombinant subunit vaccine candidate has been successfully trialed in several animal challenge models of Hendra and Nipah virus infection, and has recently been deployed as an effective equine vaccine in Australia; potentially breaking the chain of Hendra virus transmission and a practical cost effective way to mitigate human Hendra virus infection.

3. Antiviral treatment

There are no approved or licensed therapeutics for treating henipavirus infection or disease in people, and antiviral approaches against the henipaviruses that have been tested in animal models are few (reviewed in (Broder, 2012)). Ribavirin is a well-known first line treatment strategy for suspected viral infections of unknown etiology. Ribavirin exhibits antiviral activity against a wide variety of both RNA and some DNA viruses (Sidwell et al., 1972) and is an accepted or approved treatment for several viral infections including respiratory syncytial virus and arenaviral hemorrhagic-fevers (reviewed in (Snell, 2001)). *In vitro* studies have shown that ribavirin is effective against both Hendra and Nipah virus replication (Aljofan et al., 2009, Wright et al., 2005). Also, the anti-malarial drug chloroquine was shown earlier to block the critical proteolytic processing needed for the maturation and function of the Hendra virus F glycoprotein (Pager et al., 2004), and not surprisingly cholorquine was later shown to inhibit

Nipah and Hendra virus infection in cell culture (Porotto et al., 2009).

An open label ribavirin treatment trial was carried out during the outbreak of Nipah virus in Malaysia in 1998 and was reported to reduce mortality by 36% in treated patients when compared to those patients who presented before ribavirin availability or who refused treatment (Chong et al., 2001). Of the recorded human Hendra virus cases, three individuals were treated with ribavirin, and of these, two succumbed to disease and one survived (Playford et al., 2010). Chloroquine was administered along with ribavirin to one HeV-infected individual in 2009 (Anonymous, 2009c) with no apparent clinical benefit. Three additional people received ribavirin treatment in combination with chloroquine after suspected exposure to Hendra virus contaminated secretions from infected horses. While all three individuals survived, infection was not confirmed and therefore it remains unknown whether the treatment had any effect (Anonymous, 2009a). In the absence of other therapies, ribavirin may be an option for treatment of henipavirus infections. However, more recent animal studies have revealed no therapeutic benefit of either drug. Two studies in hamsters and one study in nonhuman primates (African green monkey (AGM)) showed that ribavirin treatment only delayed but did not prevent death after Nipah or Hendra virus infection (Freiberg et al., 2010, Georges-Courbot et al., 2006, Rockx et al., 2010) and AGMs treated with ribavirin following Hendra virus infection had marked increases of neurological symptoms. Similarly, chloroquine was unable to prevent Nipah infection or disease in ferrets (Pallister et al., 2009).

4. A passive immunotherapy for people

In contrast, passive immunotherapy with polyclonal or monoclonal antibody specific for the viral envelope glycoproteins has proved successful from initial proof-of-concept findings from several studies carried out in hamsters (Guillaume et al., 2004, Guillaume et al., 2006). Presently, the only reported and effective post-exposure therapy against Hendra or Nipah virus infection and one that could likely be approved in the near future for use in people has been a human monoclonal antibody (mAb) known as m102.4 which was isolated from a recombinant naïve human phage-displayed Fab library (Zhu et al., 2008).

The m102.4 mAb has exceptionally potent neutralizing activity against both Nipah and Hendra viruses and its epitope maps to the ephrin receptor binding site (Fig. 1). Testing of m102.4 has confirmed its neutralization activity against several isolates; NiV-Malaysia, HeV-1994, HeV-Redlands, NiV-Bangladesh (Bossart et al., 2009). Effective post-exposure efficacy with m102.4 has now been demonstrated in both ferrets and nonhuman primates (African green monkey

(AGM)) infected with either Hendra virus or Nipah virus (Table 1). The successful m102.4 passive immunotherapy in the AGM was recently reported in a study designed to reflect a possible real life scenario requiring mAb as a post-exposure treatment, and was a follow-up from the initial successful m102.4 post-exposure therapy carried out in ferrets (Bossart et al., 2009). Fourteen monkeys were challenged intratracheally with Hendra virus and 12 animals were infused twice with a 100 mg dose (~20 mg/kg) of m102.4 beginning at 10 h, 24 h or 72 h p.i. with the second infusion ~48 h later. All 12 animals that received m102.4 survived infection; whereas the untreated control subjects succumbed to severe systemic disease by day 8 (Bossart et al., 2011). There was no evidence of Hendra virus mediated pathology in any of the m102.4-treated animals and no infectious Hendra virus could be recovered from any tissues from any m102.4-treated subjects.

Table 1. Evaluation of post-exposure therapy with the henipavirus G glycoprotein-specific human monoclonal antibody m102.4 against lethal henipavirus challenge in laboratory animals.

Virus	Animal model	Experimental design and results[a]	Reference
Hendra	AGM	A 10, 24, or 72 h post-exposure use of m102.4 could protect against a 10-fold lethal intratracheal virus challenge	(Bossart et al., 2011)
	Ferret	A 10 or 24 h post-exposure dose of m102.4 could protect against a 10-fold lethal oronasal virus challenge	(Pallister J., unpublished)
Nipah	Ferret	A 10 h post-exposure dose of m102.4 could protect against a 10-fold lethal oronasal virus challenge	(Bossart et al., 2009)
	AGM	A 24, 72, or 120 h post-exposure use of m102.4 could protect against a 10-fold lethal intratracheal virus challenge	(Geisbert T., unpublished)

[a] The administration of m102.4 mAb was performed by infusion in all studies with the exception of one ferret in one study were it had to be administered by intraperitoneal injection (Bossart et al., 2009). All studies to date have reported that all animals receiving m102.4 post-exposure therapy have survived virus challenge, some with varying levels of mild to moderate illness.

In May of 2010, an instance of possible Hendra virus infection in two individuals was reported on the Sunshine Coast, north of Brisbane, Australia. Both individuals had extensive close contact with a horse just prior to and during the development of clinical illness in the animal. Following a diagnosis of Hendra virus infection in the horse, both individuals were considered to have had high-risk exposure to Hendra virus (Anonymous, 2010). A request was made by Australian health authorities to obtain m102.4 as a possible compassionate use therapeutic option even though clinical trials in human had not been undertaken and safety data of the mAb in humans was lacking. These two individuals were administered the m102.4 mAb (Miles, 2010). Both individuals ultimately did not develop detectable Hendra virus infection but whether this was due to the mAb therapy could not be determined. In 2010, the cell line expressing the human m102.4 mAb was provided to the Queensland Government, Queensland Health, to allow health authorities to manufacture m102.4 for its potential use on a compassionate basis in future cases of high-risk human exposure. In 2012, a third asymptomatic individual who experienced high-risk Hendra virus exposure was also given m102.4 mAb therapy (Anonymous, 2012a, Guest, 2012). There have been no adverse effects observed or reported in these cases.

5. A one-health solution – vaccination

Initial immunization strategies using the henipavirus G or F viral glycoproteins were first evaluated using recombinant vaccinia viruses providing evidence that complete protection from disease was achievable by eliciting an immune response to the Nipah virus envelope glycoproteins (Guillaume et al., 2004). Other studies using recombinant canarypox-based vaccine candidates for potential use in pigs have also been carried out (Weingartl et al., 2006). To date, the most widely evaluated henipavirus vaccine antigen has been a subunit, consisting of a recombinant soluble and oligomeric form of the G glycoprotein (sG) of Hendra virus (HeV-sG) (Bossart et al., 2005). The HeV-sG subunit vaccine (Fig. 1) is a secreted version of the molecule in which the transmembrane and cytoplasmic tail domains have been deleted from the coding sequence. HeV-sG is produced in mammalian cell culture expression systems and is properly N-linked glycosylated and retains many native characteristics including its oligomerization into dimers and tetramers, ability to bind ephrin receptors and elicit potent cross-reactive (Hendra and Nipah virus) neutralizing antibody responses (reviewed in (Broder et al., 2012)) Table 2.

Table 2. Evaluation of the protective efficacy of henipavirus sG vaccines against lethal henipavirus challenge in laboratory animals.

Virus	Animal model	Experimental design and results[a]	Reference
Hendra	Ferret	Hendra-sG used to immunize followed by 10-fold lethal oronasal virus challenge	(Pallister et al., 2011b)
	AGM	Hendra-sG used to immunize followed by 10-fold lethal intratracheal virus challenge	(Bossart et al., 2012)
	Horse	Hendra-sG is used to immunize horses followed by lethal oronasal virus challenge	(Balzer, 2011) (Middleton D., et al., submitted)
Nipah	Cat	Hendra-sG or Nipah-sG used to immunize followed by 10-fold lethal subcutaneous virus challenge; Hendra-sG used to immunize followed by 100-fold lethal oronasal virus challenge	(McEachern et al., 2008, Mungall et al., 2006)
	Ferret	Hendra-sG used to immunize followed by 10-fold lethal oronasal virus challenge	(Pallister J., unpublished)
	AGM	Hendra-sG used to immunize followed by 10-fold lethal intratracheal virus challenge	(Geisbert T., unpublished)

[a] All studies to date have reported that all Hendra-sG immunized animals can be completely protected from infection and disease following either a Hendra or Nipah virus challenge. No evidence of virus replication or shedding has been reported in the majority of challenged subjects.

Studies showing the HeV-sG subunit immunogen as a successful vaccine against lethal Hendra virus or Nipah virus challenge have been carried out in the cat (McEachern et al., 2008, Mungall et al., 2006), ferret (Pallister et al., 2011b) and nonhuman primates (Bossart et al., 2012) (Table 2), and details of the results from these studies have been reviewed elsewhere (Broder et al., 2012). The success of the HeV-sG vaccine-mediated protection observed in multiple animal challenge models led to the consideration of the HeV-sG as a safe and effective vaccine for horses against Hendra virus infection in Australia following a human fatality in 2009 and the human exposure cases in 2010 discussed above. The adopted equine vaccination strategy was to both prevent infection in horses and thus ameliorate the risk of Hendra virus transmission to people. A series of horse HeV-sG vaccination and Hendra virus challenge studies have been carried out in Australia; at the high containment biological safety level 4 (BSL-4) facilities of the Animal Health Laboratories (AAHL), Commonwealth Scientific and Industrial Research Organisation (CSIRO), in Geelong. The development of HeV-sG as an equine vaccine against Hendra virus was a collaborative research program between the Uniformed Services University of the Health Sciences, the Henry M. Jackson Foundation, the AAHL and Pfizer Animal Health (now Zoetis, Inc.). Findings from these initial studies were reported at Australian Veterinary Association, Annual Conference in Adelaide, in May 2011 (Balzer, 2011). The HeV-sG subunit glycoprotein was used to vaccinate horses (a 2 dose regime with a 3 week interval) and both a high and a low dose of HeV-sG antigen was examined. Following a high dose oronasal challenge with Hendra virus, all vaccinated horses remained clinically disease-free, and there was no evidence of virus replication or virus shedding in any of the immunized horses. On November 1, 2012, the vaccine called Equivac HeV® was released for use in Australia, and it is the first vaccine licensed and commercially deployed against a BSL-4 agent and currently is the only licensed prophylactic treatment for henipaviruses.

6. Concluding remarks

The Nipah virus and Hendra virus are zoonotic paramyxoviruses that can infect and cause lethal disease across a broad range of vertebrate species including humans. They are present in a variety of bat reservoirs, can be isolated and propagated and because of their associated high morbidity and mortality they pose a risk from natural outbreaks, laboratory accidents or deliberate misuse. For all of these reasons, the development of effective prevention and treatment strategies has been pursued. Over the past decade a considerable amount of research has focused on the henipavirus envelope glycoproteins and their roles in the virus attachment and infection process. These efforts have now led to the development and testing of both passive and active immunization strategies applicable to both human and animal use. Presently, a cross-reactive human mAb (m102.4) has been demonstrated as an exceptionally efficacious post-exposure therapy in protecting both ferrets and nonhuman primates from lethal henipavirus disease, and its effectiveness led to its application in people as a compassionate use post-exposure prophylaxis in Australia. Also, as an active vaccination strategy for preventing Hendra virus infection and disease in horses in Australia and thus blocking potential transmission to people, a recombinant subunit vaccine, HeV-sG, which has been shown to provide protection against henipavirus challenge in cats, ferrets, monkeys and now horses, has been licensed and deployed for use in Australia.

To date, henipavirus antivirals have only been deployed in Australia in the fight against Hendra virus. As Nipah virus causes significantly more instances of human disease, increased efforts are needed to advance Nipah-targeted countermeasures in endemic regions. Animal models have demonstrated that both the HeV-sG vaccine and the m102.4 human antibody can prevent both Nipah virus infection and/or disease. Efforts are currently under way to develop HeV-sG for human use as well as for use in pigs. However, the cost of the vaccine per animal and uptake of the vaccine in the absence of repeated outbreaks or disease will be critical factors influencing the feasibility of its application in Southeast Asia. The pre-clinical development of recombinant HeV-sG for use in people is only a first step and the acquisition of further support for manufacture and clinical trials will certainly be challenging. Clinical trials will also be needed for the m102.4 human antibody therapy, and both the United States and Australia are developing the m102.4 antibody for human use as a Nipah and Hendra virus countermeasure. Nipah virus has not occurred in Malaysia since 1998 and requests for compassionate use of the m102.4 antibody in India or Bangladesh following high-risk Nipah virus exposure or cases of infection have not occurred and may be difficult to orchestrate. Whether the antibody could be pre-positioned in Nipah virus endemic areas will largely depend on international cooperation and financial support.

Acknowledgements

The views expressed in the manuscript are solely those of the authors, and they do not represent official views or opinions of the Department of Defense or The Uniformed Services University of the Health Sciences. CCB, KNB and TWG are supported in part by grants from the United States, Department of Health and Human Services, National Institutes of Health (NIH). ZZ and DSD are supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

Recommended articles Citing articles (31)

References

Abdullah et al., 2012 S. Abdullah, L.Y. Chang, K. Rahmat, K.T. Goh, C.T. Tan

Late-onset Nipah virus encephalitis 11 years after the initial outbreak: a case report

Neurol. Asia, 17 (2012), pp. 71-74

View Record in Scopus Google Scholar

Aguilar and Iorio, 2012 H.C. Aguilar, R.M. Iorio

Henipavirus membrane fusion and viral entry

Curr. Top. Microbiol. Immunol., 359 (2012), pp. 79-94

CrossRef View Record in Scopus Google Scholar

Aljofan et al., 2009 M. Aljofan, S. Saubern, A.G. Meyer, G. Marsh, J. Meers, B.A. Mungall

Characteristics of Nipah virus and Hendra virus replication in different cell lines and their suitability for antiviral screening

Virus Res., 142 (2009), pp. 92-99

Article Download PDF View Record in Scopus Google Scholar

Anonymous, 2009a Anonymous, 2009a. Hendra virus, human, equine – Australia (02): Queensland, Pro-med. International Society for Infectious Diseases. August 21, archive no. 20090826.2998. Available at www.promedmail.org.

Google Scholar

Anonymous, 2009b Anonymous, 2009b. Hendra virus, human, equine – Australia (04): Queensland, fatal, Pro-med. International Society for Infectious Diseases. September 3, archive no. 20090903.3098. Available at www.promedmail.org.

Google Scholar

Anonymous, 2009c Anonymous, 2009c. Hendra virus, human, equine – Australia (05): Queensland, Pro-med. International Society for Infectious Diseases. September 10, archive no. 20090910.3189. Available at www.promedmail.org.

Google Scholar

Anonymous, 2010 Anonymous, 2010. Hendra virus, equine – Australia (05): Queensland: human exposure. Pro-MED. International Society for Infectious Diseases. May 27, archive no. 20100527.1761. Available at www.promedmail.org.

Google Scholar

Anonymous, 2012a Anonymous, 2012a. Hendra virus, equine – Australia (08) Queensland: possible human case. Pro-med. International Society for Infectious Diseases. July 19, archive no. 20120720.1208397. Available at www.promedmail.org.
Google Scholar

Anonymous, 2012b Anonymous, 2012b. Hendra virus, equine – Australia (12): Queensland: vaccine. Pro-med. International Society for Infectious Diseases. November 3, archive no. 20121104.1390394. Available at www.promedmail.org.
Google Scholar

Anonymous, 2013a Anonymous, 2013a. Hendra virus. The State of Queensland, Department of Agriculture, Fisheries and Forestry, Queensland Government. Available at www.daff.qld.gov.au/4790_20801.htm.
Google Scholar

Anonymous, 2013b Anonymous, 2013b. Hendra virus, equine – Australia: (02) Queensland. Pro-med. International Society for Infectious Diseases. February 22, archive no. 20130224.1557005. Available at www.promedmail.org.
Google Scholar

Anonymous, 2013c Anonymous, 2013c. Nipah encephalitis, human – Bangladesh (03), Pro-med. International Society for Infectious Diseases. February 5, archive no. 20130205.1530748. Available at www.promedmail.org.
Google Scholar

Balzer, 2011 M. Balzer
Hendra vaccine success announced
Aust. Vet. J., 89 (2011), pp. N2-3
Google Scholar

Bossart et al., 2005 K.N. Bossart, G. Crameri, A.S. Dimitrov, B.A. Mungall, Y.R. Feng, J.R. Patch, A. Choudhary, L.F. Wang, B.T. Eaton, C.C. Broder
Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble g glycoprotein of hendra virus
J. Virol., 79 (2005), pp. 6690-6702
CrossRef View Record in Scopus Google Scholar

Bossart et al., 2009 K.N. Bossart, Z. Zhu, D. Middleton, J. Klippel, G. Crameri, J. Bingham, J.A.

McEachern, D. Green, T.J. Hancock, Y.P. Chan, A.C. Hickey, D.S. Dimitrov, L.F. Wang, C.C. Broder
A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute nipah virus infection
PLoS Pathog., 5 (2009), p. e1000642
CrossRef Google Scholar

Bossart et al., 2011 K.N. Bossart, T.W. Geisbert, H. Feldmann, Z. Zhu, F. Feldmann, J.B. Geisbert, L. Yan, Y.R. Feng, D. Brining, D. Scott, Y. Wang, A.S. Dimitrov, J. Callison, Y.P. Chan, A.C. Hickey, D.S. Dimitrov, C.C. Broder, B. Rockx
A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge
Sci. Transl. Med., 3 (2011)
105ra103
Google Scholar

Bossart et al., 2012 K.N. Bossart, B. Rockx, F. Feldmann, D. Brining, D. Scott, R. Lacasse, J.B. Geisbert, Y.R. Feng, Y.P. Chan, A.C. Hickey, C.C. Broder, H. Feldmann, T.W. Geisbert
A hendra virus g glycoprotein subunit vaccine protects african green monkeys from nipah virus challenge
Sci. Transl. Med., 4 (2012)
146ra107
Google Scholar

Broder, 2010 C.C. Broder
Therapeutics and Vaccines Against Hendra and Nipah Viruses
M.M. Levine, G. Dougan, M.F. Good, M.A. Liu, G.J. Nabel, J.P. Nataro, R. Rappuoli (Eds.), New Generation Vaccines (4 ed.), Informa Healthcare USA, New York (2010), pp. 885-894
View Record in Scopus Google Scholar

Broder, 2012 C.C. Broder
Henipavirus outbreaks to antivirals: the current status of potential therapeutics
Curr. Opin. Virol., 2 (2012), pp. 176-187
Article Download PDF View Record in Scopus Google Scholar

Broder et al., 2012 C.C. Broder, T.W. Geisbert, K. Xu, D.B. Nikolov, L.F. Wang, D. Middleton, J. Pallister, K.N. Bossart

Immunization strategies against henipaviruses
Curr. Top. Microbiol. Immunol., 359 (2012), pp. 197-223
CrossRef View Record in Scopus Google Scholar

Chong et al., 2001 H.T. Chong, A. Kamarulzaman, C.T. Tan, K.J. Goh, T. Thayaparan, S.R. Kunjapan, N.K. Chew, K.B. Chua, S.K. Lam
Treatment of acute Nipah encephalitis with ribavirin
Ann. Neurol., 49 (2001), pp. 810-813
CrossRef View Record in Scopus Google Scholar

Chua et al., 2000 K.B. Chua, W.J. Bellini, P.A. Rota, B.H. Harcourt, A. Tamin, S.K. Lam, T.G. Ksiazek, P.E. Rollin, S.R. Zaki, W. Shieh, C.S. Goldsmith, D.J. Gubler, J.T. Roehrig, B. Eaton, A.R. Gould, J. Olson, H. Field, P. Daniels, A.E. Ling, C.J. Peters, L.J. Anderson, B.W. Mahy
Nipah virus: a recently emergent deadly paramyxovirus
Science, 288 (2000), pp. 1432-1435
Article Download PDF CrossRef View Record in Scopus Google Scholar

Colgrave et al., 2011 M.L. Colgrave, H.J. Snelling, B.J. Shiell, Y.R. Feng, Y.P. Chan, K.N. Bossart, K. Xu, D.B. Nikolov, C.C. Broder, W.P. Michalski
Site occupancy and glycan compositional analysis of two soluble recombinant forms of the attachment glycoprotein of Hendra virus
Glycobiology, 22 (2011), pp. 572-584
Google Scholar

Dolgin, 2013 E. Dolgin
Animal rule for drug approval creates a jungle of confusion
Nat. Med., 19 (2013), pp. 118-119
CrossRef View Record in Scopus Google Scholar

Freiberg et al., 2010 A.N. Freiberg, M.N. Worthy, B. Lee, M.R. Holbrook
Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection
J. Gen. Virol., 91 (2010), pp. 765-772
CrossRef View Record in Scopus Google Scholar

Geisbert et al., 2012 T.W. Geisbert, H. Feldmann, C.C. Broder

Animal challenge models of henipavirus infection and pathogenesis
Curr. Top. Microbiol. Immunol., 359 (2012), pp. 153-177
CrossRef View Record in Scopus Google Scholar

Georges-Courbot et al., 2006 M.C. Georges-Courbot, H. Contamin, C. Faure, P. Loth, S. Baize, P. Leyssen, J. Neyts, V. Deubel
Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection
Antimicrob. Agents Chemother., 50 (2006), pp. 1768-1772
CrossRef View Record in Scopus Google Scholar

Guest, 2012 A. Guest
Experimental Therapy for Hendra Virus Patient, The World Today
ABC News (Australian Broadcasting Corporation) (2012)
Google Scholar

Guillaume et al., 2004 V. Guillaume, H. Contamin, P. Loth, M.C. Georges-Courbot, A. Lefeuvre, P. Marianneau, K.B. Chua, S.K. Lam, R. Buckland, V. Deubel, T.F. Wild
Nipah virus: vaccination and passive protection studies in a hamster model
J. Virol., 78 (2004), pp. 834-840
CrossRef View Record in Scopus Google Scholar

Guillaume et al., 2006 V. Guillaume, H. Contamin, P. Loth, I. Grosjean, M.C. Courbot, V. Deubel, R. Buckland, T.F. Wild
Antibody prophylaxis and therapy against Nipah virus infection in Hamsters
J. Virol., 80 (2006), pp. 1972-1978
CrossRef View Record in Scopus Google Scholar

Halpin et al., 2011 K. Halpin, A.D. Hyatt, R. Fogarty, D. Middleton, J. Bingham, J.H. Epstein, S.A. Rahman, T. Hughes, C. Smith, H.E. Field, P. Daszak, H. The
Pteropid bats are confirmed as the reservoir hosts of henipaviruses: a comprehensive experimental study of virus transmission
Am. J. Trop. Med. Hyg., 85 (2011), pp. 946-951
CrossRef View Record in Scopus Google Scholar

Kelley and Sternberg, 2009 L.A. Kelley, M.J. Sternberg
Protein structure prediction on the Web: a case study using the Phyre server

Nat. Protoc., 4 (2009), pp. 363-371
CrossRef View Record in Scopus Google Scholar

Lee and Ataman, 2011 B. Lee, Z.A. Ataman
Modes of paramyxovirus fusion: a Henipavirus perspective
Trends Microbiol., 19 (2011), pp. 389-399
Article Download PDF View Record in Scopus Google Scholar

Luby and Gurley, 2012 S.P. Luby, E.S. Gurley
Epidemiology of henipavirus disease in humans
Curr. Top. Microbiol. Immunol., 359 (2012), pp. 25-40
CrossRef View Record in Scopus Google Scholar

Luby et al., 2009 S.P. Luby, M.J. Hossain, E.S. Gurley, B.N. Ahmed, S. Banu, S.U. Khan, N. Homaira, P.A. Rota, P.E. Rollin, J.A. Comer, E. Kenah, T.G. Ksiazek, M. Rahman
Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007
Emerg. Infect. Dis., 15 (2009), pp. 1229-1235
CrossRef View Record in Scopus Google Scholar

McEachern et al., 2008 J.A. McEachern, J. Bingham, G. Crameri, D.J. Green, T.J. Hancock, D. Middleton, Y.R. Feng, C.C. Broder, L.F. Wang, K.N. Bossart
A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats
Vaccine, 26 (2008), pp. 3842-3852
Article Download PDF View Record in Scopus Google Scholar

Miles, 2010 J. Miles
Hendra Therapy for Mum, Daughter, The Courier-Mail.com.au
Queensland Newspapers Pty Ltd., Queensland 4001, Australia (2010)
Google Scholar

Mungall et al., 2006 B.A. Mungall, D. Middleton, G. Crameri, J. Bingham, K. Halpin, G. Russell, D. Green, J. McEachern, L.I. Pritchard, B.T. Eaton, L.F. Wang, K.N. Bossart, C.C. Broder
Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine
J. Virol., 80 (2006), pp. 12293-12302

CrossRef View Record in Scopus Google Scholar

Murray et al., 1995 K. Murray, P. Selleck, P. Hooper, A. Hyatt, A. Gould, L. Gleeson, H. Westbury, L. Hiley, L. Selvey, B. Rodwell, *et al.*
A morbillivirus that caused fatal disease in horses and humans
Science, 268 (1995), pp. 94-97
View Record in Scopus Google Scholar

O'Sullivan et al., 1997 J.D. O'Sullivan, A.M. Allworth, D.L. Paterson, T.M. Snow, R. Boots, L.J. Gleeson, A.R. Gould, A.D. Hyatt, J. Bradfield
Fatal encephalitis due to novel paramyxovirus transmitted from horses
Lancet, 349 (1997), pp. 93-95
Article Download PDF View Record in Scopus Google Scholar

Pager et al., 2004 C.T. Pager, M.A. Wurth, R.E. Dutch
Subcellular localization and calcium and pH requirements for proteolytic processing of the Hendra virus fusion protein
J. Virol., 78 (2004), pp. 9154-9163
CrossRef View Record in Scopus Google Scholar

Pallister et al., 2009 J. Pallister, D. Middleton, G. Crameri, M. Yamada, R. Klein, T.J. Hancock, A. Foord, B. Shiell, W. Michalski, C.C. Broder, L.F. Wang
Chloroquine administration does not prevent Nipah virus infection and disease in ferrets
J. Virol., 83 (2009), pp. 11979-11982
CrossRef View Record in Scopus Google Scholar

Pallister et al., 2011a J. Pallister, D. Middleton, C.C. Broder, L.F. Wang
Henipavirus vaccine development
J. Bioterr. Biodef. (2011), p. S1:005, 10.4172/2157-2526.S1-005
Google Scholar

Pallister et al., 2011b J. Pallister, D. Middleton, L.F. Wang, R. Klein, J. Haining, R. Robinson, M. Yamada, J. White, J. Payne, Y.R. Feng, Y.P. Chan, C.C. Broder
A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge
Vaccine, 29 (2011), pp. 5623-5630

Article Download PDF View Record in Scopus Google Scholar

Pernet et al., 2012 O. Pernet, Y.E. Wang, B. Lee
Henipavirus receptor usage and tropism
Curr. Top. Microbiol. Immunol., 359 (2012), pp. 59-78
CrossRef View Record in Scopus Google Scholar

Playford et al., 2010 E.G. Playford, B. McCall, G. Smith, V. Slinko, G. Allen, I. Smith, F. Moore, C. Taylor, Y.H. Kung, H. Field
Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008
Emerg. Infect. Dis., 16 (2010), pp. 219-223
CrossRef View Record in Scopus Google Scholar

Porotto et al., 2009 M. Porotto, G. Orefice, C.C. Yokoyama, B.A. Mungall, R. Realubit, M.L. Sganga, M. Aljofan, M. Whitt, F. Glickman, A. Moscona
Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy
J. Virol., 83 (2009), pp. 5148-5155
CrossRef View Record in Scopus Google Scholar

Rockx et al., 2010 B. Rockx, K.N. Bossart, F. Feldmann, J.B. Geisbert, A.C. Hickey, D. Brining, J. Callison, D. Safronetz, A. Marzi, L. Kercher, D. Long, C.C. Broder, H. Feldmann, T.W. Geisbert
A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment
J. Virol., 84 (2010), pp. 9831-9839
CrossRef View Record in Scopus Google Scholar

Sidwell et al., 1972 R.W. Sidwell, J.H. Huffman, G.P. Khare, L.B. Allen, J.T. Witkowski, R.K. Robins
Broad-spectrum antiviral activity of Virazole: 1-beta-D-ribofuranosyl-1,2,4-triazole-3-carboxamide
Science, 177 (1972), pp. 705-706
View Record in Scopus Google Scholar

Snell, 2001 N.J. Snell
Ribavirin–current status of a broad spectrum antiviral agent

Expert Opin. Pharmacother., 2 (2001), pp. 1317-1324
CrossRef View Record in Scopus Google Scholar

Wang et al., 2013 L.-F. Wang, J.S. Mackenzie, C.C. Broder
Henipaviruses
D.M. Knipe, P.M. Howley (Eds.), Fields Virology, Lippincott Williams & Wilkins, Philadelphia (2013), pp. 1070-1085
View Record in Scopus Google Scholar

Weingartl et al., 2006 H.M. Weingartl, Y. Berhane, J.L. Caswell, S. Loosmore, J.C. Audonnet, J.A. Roth, M. Czub
Recombinant nipah virus vaccines protect pigs against challenge
J. Virol., 80 (2006), pp. 7929-7938
CrossRef View Record in Scopus Google Scholar

Weingartl et al., 2009 H.M. Weingartl, Y. Berhane, M. Czub
Animal models of henipavirus infection: a review
Vet. J., 181 (2009), pp. 211-220
Article Download PDF View Record in Scopus Google Scholar

Wong, 2010 K.T. Wong
Emerging epidemic viral encephalitides with a special focus on henipaviruses
Acta Neuropathol., 120 (2010), pp. 317-325
CrossRef View Record in Scopus Google Scholar

Wong and Ong, 2011 K.T. Wong, K.C. Ong
Pathology of acute henipavirus infection in humans and animals
Pathol. Res. Int., 2011 (2011), p. 567248
View Record in Scopus Google Scholar

Wong et al., 2009 K.T. Wong, T. Robertson, B.B. Ong, J.W. Chong, K.C. Yaiw, L.F. Wang, A.J. Ansford, A. Tannenberg
Human Hendra virus infection causes acute and relapsing encephalitis
Neuropathol. Appl. Neurobiol., 35 (2009), pp. 296-305
CrossRef View Record in Scopus Google Scholar

Wright et al., 2005 P.J. Wright, G. Crameri, B.T. Eaton
RNA synthesis during infection by Hendra virus: an examination by quantitative real-

time PCR of RNA accumulation, the effect of ribavirin and the attenuation of transcription
Arch. Virol., 150 (2005), pp. 521-532
CrossRef View Record in Scopus Google Scholar

Xu et al., 2012a K. Xu, C.C. Broder, D.B. Nikolov
Ephrin-B2 and ephrin-B3 as functional henipavirus receptors
Semin. Cell Dev. Biol., 23 (2012), pp. 116-123
Article Download PDF View Record in Scopus Google Scholar

Xu et al., 2012b K. Xu, Y.P. Chan, K.R. Rajashankar, D. Khetawat, L. Yan, M.V. Kolev, C.C. Broder, D.B. Nikolov
New insights into the hendra virus attachment and entry process from structures of the virus g glycoprotein and its complex with ephrin-b2
PLoS One, 7 (2012), p. e48742
CrossRef Google Scholar

Zhu et al., 2008 Z. Zhu, K.N. Bossart, K.A. Bishop, G. Crameri, A.S. Dimitrov, J.A. McEachern, Y. Feng, D. Middleton, L.F. Wang, C.C. Broder, D.S. Dimitrov
Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody
J. Infect. Dis., 197 (2008), pp. 846-853
CrossRef View Record in Scopus Google Scholar

Zhu et al., 2013 Z. Zhu, P. Prabakaran, W. Chen, C.C. Broder, R. Gong, D.S. Dimitrov
Human monoclonal antibodies as candidate therapeutics against emerging viruses and HIV-1
Virol. Sin., 28 (2013), pp. 71-80
CrossRef View Record in Scopus Google Scholar

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Tiff File		Figure No.	Description
Yes	1	Fig. 14.1	Syncytia induced in vero cells 24 hours after infection by Hendra virus (HeV) (A) and Nipah virus (NiV) (B).
Yes	2	Fig. 14.2	EM of NiV infected cells and HeV particle
Yes	3	Fig. 14.3A	Genome size and organization of Hendra virus in comparison with the type species in each of the other six genera in the family *Paramyxoviridae.*
Yes	3	Fig. 14.3B	3'UTR of certain genes in henipaviruses (N, P and G) are longer than their counterparts from representative members of paramyxovirus genera that infects mammals.
Yes	4	Fig. 14.4A	The major proteins of Hendra virus and Nipah virus
Yes	4	Fig. 14.4B	Henipavirus replication cycle.
Yes	5	Fig. 14.5	A structure-based phylogeny of paramyxoviral receptor binding proteins.
Yes	6	Fig. 14.6	Nipah virus transmission pathways.

Yes	7	**Fig. 14.7**	Immunohistochemistry detection of Nipah virus (NiV) and Hendra virus (HeV) antigen in henipavirus-infected tissues
Yes	8	**Fig. 14.8**	Ephrin-B2 and ephrin-B3 RNA-seq expression data across representative human non-disease tissues.
DELETE		**Fig. 14.8**	Mapping sequence conservation onto henipavirus attachment glycoproteins
Yes	9	**Fig. 14.9**	Innate immune evasion mechanisms of the Nipah virus P, V, W, and M proteins
DELETE		**Fig. 14.10**	Potential brain MRI to illustrate relaspinfg encephalitis when discussing persistence in text
Yes	10	**Fig. 14.10**	Distribution of bats in the *Pteropodidae* family and *Pteropus* genus and locations of henipavirus presence known to date.
Yes	11	**Fig. 14.11**	Phylogenetic tree based on L-proteins of selective henipaviruses and type species of the other six genera in the family *Paramyxoviridae.*
	12	**Fig. 14.12A** **Fig. 14.12B**	Chest radiographs of a Nipah virus (NiV) infected patient (A) and Nipah virus (NiV) and Hendra virus (HeV) infected African green monkeys (B).
	13	**Fig. 14.13A** **Fig. 14.13B**	Magnetic resonance imaging scans of patients with Nipah virus (NiV)(A) or Hendra virus (HeV)(B) encephalitis.
	14	**Fig. 14.14A-F**	Generalized understanding of infection and clinical features of human Nipah virus (NiV) and Hendra virus (HeV) infection
	15	**Fig. 14.15A** **Fig. 14.15B**	Model of the Hendra virus soluble G glycoprotein subunit vaccine (HeV-sG) (A) and its complex with the Hendra and Nipah virus neutralizing human monoclonal antibody m102.4 (B).

16	**Fig. 14.16A**	**One Health Vaccine strategy. A:** The known modes of henipavirus transmission in different countries. In Australia, all seven human cases were infected via contacting infected horses. A One Health vaccine strategy was developed for immunization of horses with the dual purposes of saving horses from lethal Hendra virus infection and prevention of transmission from bats to humans via horses.
16	**Fig. 14.16B**	**One Health Vaccine strategy. B:** Location densities of Equivac® HeV vaccine administered to horses around Australia, courtesy of Dr. Richard L'Estrange, Zoetis Inc.

Txt Pgs	
1.8	**TABLE 14.1A Summary of Hendra Virus Outbreaks in H**
	TABLE 14.1B. Summary of Hendra Virus Outbreaks in
1	**TABLE 14.1C. Summary of Nipah Virus Outbreaks in Hu**

1.5	**TABLE 14.2. Summary of Henipaviruses Isolated from**

3	**TABLE 14.3. Henipavirus Cell Tropism and Receptor Ex**

1.5	**TABLE 14.4. Detection of Nipah or Nipah-related Virus**

1.2	**TABLE 14.5 Summary of Advanced Henipavirus Vaccin**

10

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Chapter 14

<TITLE>14 Henipaviruses

Benhur Lee
Christopher C. Broder
Lin-Fa Wang

History
Infectious Agent
    Classification
    Propagation in Cell Culture and Cytopathic Effect
    Virus Morphology
    Genome Length and Organization
    Virus Proteins and Their Properties
    Host Range
Pathogenesis and Pathology (BHL)
    Entry into the host
    Site of primary replication and virus spread
    Cell and Tissue Tropism
    Immune Response
    Release from host and transmission
    Virulence
    Persistence
Epidemiology
    Age
    Morbidity and Mortality
    Origin and Spread of Epidemics
    Genetic Diversity
Clinical Features
    Incubation Period
    Acute Clinical Features
    Outcome of Infection
Diagnosis

Prevention and Control
    Treatment
    Vaccines and Passive Immunotherapy
    Peptide Inhibitors
Perspective and Geographic Considerations

<H1>History

Hendra virus (HeV), the first known member of the genus *Henipavirus* in the family *Paramyxoviridae*, came to light in September 1994 as the causative agent of a sudden outbreak of acute respiratory disease in thoroughbred horses at a stable in Brisbane, Australia. A total of 21 horses and 2 humans (a horse trainer and a stable hand) became infected. The horse trainer and 14 horses died, and 7 horses with mild or subclinical infection were euthanized.[1,2] A virus was isolated, initially called *Equine Morbillivirus* but later re-named *Hendra virus* after the Brisbane suburb where the outbreak occurred. A second person died from HeV infection 13 months after the Brisbane outbreak, a farmer from Mackay, nearly 1000 km north of Brisbane. Unlike the first case, however, the man succumbed to encephalitis caused by HeV infection.[3] A forensic retrospective investigation found that the farmer had suffered a mild meningitic illness 14 months earlier after assisting at the necropsy of two horses that had died of severe respiratory distress—later found to have been caused by HeV[4]—and that he became infected at that time. After initial serologic evidence suggested that fruit bats (flying foxes) of the genus *Pteropus* in the suborder Yinpterochiroptera were the reservoir hosts.[5] HeV was isolated from two species of flying fox.[6] In total, there have been 60 recognized occurrences of HeV in Australia between 1994–2018, with at least one occurrence per year since 2006 (Table 14.1A-online).[7] Every occurrence of HeV has involved horses as the initial infected host, causing lethal respiratory disease and encephalitis, along with a total of seven human cases arising from exposure to infected horses, among which four have been fatal and the most recent in 2009 (Table 14.1B-online).[8]

Nipah virus (NiV), the second known member of the genus *Henipavirus*, emerged as the cause of an outbreak of disease in pigs and humans in Peninsular Malaysia in 1998 through 1999. The epidemic started in Perak State as clusters of cases of encephalitis among pig farmers. It was initially believed to be caused by Japanese encephalitis virus; however, various features of the outbreak, including a high proportion of cases in direct contact with pigs and illness and deaths in pigs, differed from those expected with Japanese encephalitis.[9] Indeed, respiratory illness and encephalitis in pigs preceded human cases in the same district.[10] The epidemic spread south to the intensive pig-farming areas of Negeri Sembilan in December 1998 and subsequently peaked between February and April 1999. More than 1 million pigs were destroyed to halt the spread of the epidemic, and by late May, 265 human cases of acute encephalitis with 105 deaths were recorded.[9,11] A cluster of 11 cases with 1 death occurred among abattoir workers in Singapore.[12] In early March 1999, a virus was isolated from the cerebrospinal fluid (CSF) of a patient with encephalitis and identified as the etiologic agent.[9,11] Named *Nipah virus* after the village from which the patient had come, it was shown to be closely related to HeV. NiV was subsequently isolated from the urine of Malaysian flying foxes.[13] A highly related NiV emerged in Bangladesh in 2001,[14] and outbreaks of NiV-related encephalitis have occurred in people from that country almost every year since, along with three reports of NiV encephalitis in India[15,16], albeit in Bangladesh adjacent regions. However, the most recent outbreak of NiV occurred in 2018 in Kerala (India), more than 2,500 km south-west of NiV-endemic Bangladesh counties. This outbreak claimed 21 lives out of 23 infected human cases.[17] In 2014, a NiV outbreak occurred in the Province of Sultan Kudarat, the Phillipines, which resulted in the death of 9 humans from 11 which showed an acute encephalitis syndrome.[18] The human case fatality rate of these NiV outbreaks averages around 60% (Table 14.1C). So far human NiV oubreaks have been documented in a total of five countries: Malaysia, Singapore, Bangladesh, India and the Philippines. Sero-epidemiological evidence for henipavirus spillover events from bats into high-risk human populations have also been reported in Cameroon.[19]

<TAB14.1C>

<H1>Infectious Agent

<H2>Classification

When HeV was first isolated in 1994, partial sequencing of the matrix gene (M) revealed that it most closely resembled members of the genus *Morbillivirus* in the subfamily *Paramyxovirinae.*[2] Subsequent characterization of the full-length genome, however, revealed that many of the genetic features of HeV were unique among paramyxoviruses and that the virus did not fit within any of the three genera existing at that time, *Morbillivirus, Respirovirus*, and *Rubulavirus.*[20,21] When NiV was isolated in 1999, it was initially described as Hendra-like on the basis of its strong reactivity with anti-HeV antibodies. Later it was shown that sera raised against HeV were able to neutralize NiV and vice versa, and that both viruses shared a high degree of similarity in genome organization and protein size and sequence.[11,22-24] In 2002, the genus *Henipavirus* was created to accommodate these novel paramyxoviruses, and HeV was designated the type species.[25] Since 1994, there have been 38 isolations of HeV or NiV from humans, bats, horses, and pigs over a wide geographic area and spanning a period of approximately 20 years (Table 14.2-online). The susceptibility of humans, the virulence of the viruses, and absence of therapeutics and vaccines led to classification of HeV and NiV as biosafety level 4 (BSL4) pathogens. In the latest report from the paramyxovirus study group under the order Mononegavirales, the genus *Henipavirus* has been expanded to include three new species,[26,27] they are *Cedar henipavirus* (Cedar virus, CedV) isolated from bats in Australia, [28] *Ghanaian bat henipavirus* (Ghana virus, GhV) detected in bats from Ghana, [29] and *Mojiang henipavirus* (Mòjiāng, MojV) detected in rats in China.[30] These three new species are highly divergent from NiV and HeV, and only CedV has been isolated. GhV and MojV are only known from sequence information. *Henipavirus* is now classified as one of the seven ICTV-approved genera in the family *Paramyxoviridae*.

Commented [BHL1]: Nomenclature will change pending official ICTV ratification in March 2019

<H2>Propagation in Cell Culture and Cytopathic Effect

The ultrastructural characteristics of henipavirus-infected cells resemble those found in cells infected by other members of the *Paramyxoviridae.* Shared features include generation of large syncytia and the presence of viral nucleocapsids in cytoplasmic inclusion bodies and underlying electron-dense areas of the plasma membrane.[31,32] In Vero cells, NiV-induced syncytia are significantly larger than those generated by HeV and nuclei and nucleocapsids are frequently located at the cell periphery, compared with HeV-induced syncytia, where they tend to be more centrally located or distributed randomly throughout the cytoplasm (Fig. 14.1). Henipavirus-infected cells also contain structures that are not seen with other paramyxoviruses—specifically a network of membrane-like reticular structures in the cytoplasm and long tubules that appear to be continuous with the plasma membrane in NiV-infected cells. Tubules can also be observed in NiV virions (Fig. 14.2A). *In situ* hybridization suggests that these reticular structures contain viral RNA and may play a role in viral transcription.[31]

<FIG14.1><FIG14.2>

<H2>Virus Morphology

Henipavirus particles are pleomorphic, varying from spherical to filamentous and ranging in size from 40 to 1,900 nm. [2,31,32] Nucleocapsids have a diameter of 18 to 19 nm with an average pitch of 5 nm. When examined by electron microscopy (EM), HeV has a unique double-fringed appearance, caused by the presence of surface projections 15 ± 1 nm and 8 ± 1 nm in length (see Fig. 14.2B). Approximately 95% of virions contain the double fringe, and the remaining 5% display a uniform fringe length of 15 ± 1 nm. Unlike HeV, NiV possesses a single layer of surface projections with an average length of 17 ± 1 nm, and NiV particles released into the culture medium are difficult to image because they are routinely penetrated by negative stains. This suggests that the viruses may differ in the physical nature of their envelope.[32]

<H2>Genome Length and Organization

In the family *Paramyxoviridae*, the genome length of all characterized viruses is divisible by six, an observation caused by the requirement of each N protein in the viral ribonucleoprotein to bind 6 nucleotide (nt) residues (see Chapter 13). This is also true for HeV and NiV despite their much larger genome sizes.[24] The genomes of the Malaysian (MY) and Bangladesh (BD) strains of NiV differ by 6 nt because of a 6-nt increase in the 3′ untranslated region of the *F* gene in NiV-BD.[33] A minigenome replicon study confirms that NiV complies with the rule of six.[34] When the complete genome sequence of HeV was determined, its length (18,234 nt) was more than 2,700 nt, or 15% longer than the genomes of all other paramyxoviruses known at that time.[21] The size of NiV genomes at 18,246 nt to 18,252 nt are slightly larger than that of HeV.[22,23,33] These large genome sizes of >18 kb is a conserved feature of henipaviruses as even the recently discovered and more divergent CedV, GhV, and MojV all have genomes between 18,162 nt (CedV) to ~18,430 nt (GhV). Given the caveat that there is wide variation amongst individual species of the other paramyxovirus genera, the extra length of the henipavirus genome is primarily due to the longer 3’ untranslated regions (3’UTRs) at the end of several of its six genes, and a larger *P* gene relative to the other paramyxovirus genera. A comparison of genome length and gene organisation of representative members of the *Paramyxoviridae* is shown in Fig. 14.3A. The nomenclature of each of the six major genes common to all paramyxoviruses have been updated to reflect the latest ICTV convention.[35] An analysis of the 3’ UTR lengths between extant henipaviruses and representative species from each paramyxovirus genera that infects mammals is presented in Fig. 14.3B. The most significant differences are in the 3’UTRs of *N*, *P*, and *RBP* genes even given the wide variance seen within each genus. The significance of long 3’ UTRs, especially in HeV and NiV, is generally unknown. However, the N gene 3’UTR of NiV appears to play a role in the downregulation of N mRNAs via specific interactions with hnRNP D.[36]

<FIG14.3A><FIG14.3B>

The genome organization of henipaviruses resembles that in the genera *Respirovirus* and *Morbillivirus*. The first 12 nt of the 3′ and 5′ genomic terminal sequences of paramyxoviruses are highly conserved and complementary, containing promoter elements for replication and transcription (see Chapter 13). The first 3 nt of the henipavirus genome termini are 5′-ACC-3′—a sequence that is absolutely conserved in members of the family *Paramyxoviridae* and different from that found in the family *Pneumoviridae.*

<H2>Virus Proteins and Their Properties

Analysis of purified viruses by polyacrylamide gel electrophoresis reveals RdRP (L), P, RBP (G), F_0, N, F_1, M, and F_2 proteins[20,24] where F_0 is the uncleaved and F_1 and F_2 the cleaved products of the *F* gene (Fig. 14.4A). Interestingly, F_0 is more readily detected in HeV compared with other paramyxoviruses, including NiV,[24,37] which may suggest that HeV-F is less efficiently cleaved. Overall, the proteins of henipaviruses are typical of those of *Paramyxoviridae*, with the exception of the P protein, which is significantly larger than cognate proteins in this virus family.[26] The P protein is translated from messenger RNA (mRNA) that is co-linear with genomic RNA. For most henipaviruses, the *P* gene also encodes V and W proteins, produced from mRNA in which one and two nontemplated G residues, respectively, are inserted at the RNA editing site during transcription. The P, V, and W proteins, therefore, are identical for the first 405 amino acid residues. A C protein is encoded by the 5′ end of the gene in an overlapping reading frame and is produced by an internal translational initiation mechanism, which is common to other members of *Paramyxoviridae*, except for rubulaviruses (see Chapter 13). The *P*-derived V, W, and C proteins are present in HeV-infected and/or NiV-infected cells.[24,38] It is worth noting that Cedar virus lacks both the RNA editing and expression of the V protein.[28] Similar to other paramyxoviruses, P itself and its derived proteins (V, W and C in the case of most

Commented [BHL2]: **LFW:** I obtained (free) permission/license to use this figure from your review in *Microbes & Infection*, 2001. It shows all the NiV and HeV proteins on the same PAGE gel in the order listed. Fig.14.4**A** complements (now) Fig. 14.4**B** (HNV life cycle) very well. It illustrates your point beautifully about HeV-F_0 being more detectable, and it technically does not add a figure.

BTW, matrix wasn't mentioned at all, so I did it at the end of this section. Our instructions were to talk about the *unique* aspects of the HNV life cycle in this infectious agent section. I referred to Fig. 14.4B quite a bit at the end of this section because F cleavage and matrix is relatively unique.

henipaviruses), all antagonize innate immune responses via a diverse set of mechanisms.[39,40] The C protein also interacts with the host cell ESCRT machinery and the virus matrix (M) protein itself to facilitate virus budding (see Pathogenesis section for more details on function of these P-derived proteins).[41]

<FIG14.4A><FIG14.4B>

Paramyxovirus N, P, and RdRP proteins are necessary and sufficient for replication of viral RNA both *in vitro* and *in vivo* as discussed in chapter 13. This has been confirmed for henipaviruses by reverse genetics. Using a minigenome replicon containing leader and trailer sequences of the NiV genome with its entire coding region replaced with a reporter gene, it was shown that efficient genome replication was achieved only when all three proteins were expressed in the same cell.[34,42] NiV N, P, and RdRP proteins were also able to rescue a minigenome constructed from the leader and trailer sequences of the HeV genome, demonstrating the close genetic relationship between the viruses. Full-length recombinant NiV bearing the matrix (M), fusion (F) and receptor binding protein (RBP) genes from HeV, singly or in combination, can also be functionally rescued. These chimeric but isogenic rNiVs replicate well in primary human endothelial and neuronal cells, further underscoring the genetic relatedness of NiV and HeV that allows for heterotypic complementation between their major structural proteins.[43] These henipavirus structural proteins contribute to unique aspects of the henipavirus life cycle (Fig. 14.4B), and play different roles in the spread and virulence of henipaviruses (as discussed in the Pathogenesis section).

The RdRP protein of nonsegmented, negative-stranded RNA (NNR) viruses in the order *Mononegavirales* contains a highly conserved GDNQ motif, believed to be important for polymerase activity.[44] Henipaviruses were the first NNR viruses in which GDNQ was replaced by GDNE. It was speculated that this motif might be unique to paramyxoviruses with relatively large genomes[21,24]; however, the GDNE motif has since been found in the

RdRP protein of Mossman virus that has a genome length of 16,650 nt.[45] Conversely, other henipaviruses of both Asiatic (CedV) and African lineages (GhV) also have the GDNQ motif that is common to most other NNR viruses. High-resolution cryo-EM structures of VSV-RdRP (another NNR virus, family *Rhabdoviridae*) suggest that GDN is the truly conserved catalytic motif essential for the polymerase function of RdRP.[46]

The receptor binding proteins (RBPs) of the *Paramyxoviridae* display hemagglutination (H) and neuraminidase (N) activities in a predominantly genus-specific manner. Viruses in *Respirovirus, Avulavirus*, and *Rubulavirus* genera possess both activities, hence their RBPs were formerly termed HN proteins[47] whereas viruses in the genus *Morbillivirus* do not behave uniformly and only some possess hemagglutination activity, but nonetheless their RBPs were formerly termed H.[48] This is an unfortunate misnomer. Paramyxoviruses with RBPs bearing HN activity genuinely use sialic acid based receptors for entry whereas "H" activity of some morbilliviruses arise from the ability of their RBPs to bind CD46 expressed on some non-human primate (NHP) red blood cells.[49] In contrast, henipavirus RBPs have neither of these activities[24,50]; rather, they utilize at least ephrin-B2, and in many cases, also ephrin-B3 expressed on host cell surfaces as attachment and entry receptors [51-54,28,55]. The exception is MojV, which does not appear to use any known paramyxovirus receptors.[56]

Recent solution structures of various henipavirus RBPs alone and in complex with the ephrin-B2 and/or ephrin-B3 receptors have revealed the details of the virus–host cell binding process, distinguishing it from other paramyxoviruses's receptor binding strategies.[57-59, 49,55,56] (Fig. 14.5)

Commented [BHL3]: **Note to Co-authors:** I added this structural phylogeny figure which shows all published HNV-RBPs in relation to RBPs from each of the other PMV genera. It highlights that MojV is an outlier.

<FIG14.5>

Proteolytic processing of paramyxovirus F proteins is essential for the generation of a fusogenic form of the protein. For most paramyxoviruses that generate systemic infections, furin-like proteases in the secretory pathway cleave their F proteins at a multibasic cleavage site.[60] Surprisingly, henipavirus F proteins are cleaved without the involvement of furin and, although cleavage occurs at a single basic residue—lysine for HeV and arginine for NiV.[37]—activation of the NiV F protein does not require a basic amino acid at the cleavage site.[61] Instead, endosomal/lysosomal cysteine proteases such as cathepsins L and B are responsible for the cleavage of henipavirus F proteins.[62-65] To access cathepsins L/B, henipavirus F proteins are endocytosed upon initially reaching the cell surface in an uncleaved form, and subsequently have to traffic back to the cell surface once they are cleaved into a fusion-competent form (Fig. 14.4B). [55,57] Classical endocytic YXXØ motifs [66,67] in the cytoplasmic tails of henipavirus F proteins and specific residues in the transmembrane domain of at least HeV-F regulate this trafficking behaviour through the early/sorting (S490) and recycling endosomal (Y498) compartments.[68] Henipavirus F proteins also have the ability to bud by themselves forming F-only particles in the absence of M.[69,70]

The matrix protein (M) plays a major role in the efficient assembly and budding of infectious paramyxoviruses.[71,72] M interacts specifically with N, the cytoplasmic tail of F, as well as cognate lipid ligands along the vesicular trafficking pathway. These orchestrated interactions help to co-ordinate the envelopment of encapsidated viral genomes (RNPs) and the budding of infectious virions bearing a relatively high density of viral envelope glycoprotein spikes. General details on the role that matrix plays in paramyxovirus assembly and budding are given in the previous chapter 13.

Henipavirus M exhibits some relatively unique behaviours and functions. For example, NiV and HeV M have classical nuclear localization and export sequences (NLS, NES) that when mutated give the relevant nuclear exclusion and nuclear retention phenotypes, respectively.[73] Time-course monitoring of M trafficking during live NiV and HeV infection indicates that M first appears in the nucleus, localizes to subnuclear compartments, including the nucleolus, before exiting into the cytoplasm and trafficking to the plasma membrane to coordinate virus assembly and budding (Fig. 14.4B).[73,74] This nuclear transit is regulated by ubiquitination and is critical for M to acquire its budding functionality.[73,75] Henipavirus M is multi-mono and poly-ubiquitinated; a lysine to arginine mutation of a key residue in its bipartite NLS is sufficient to dysregulate ubiquitination of M and abrogate its budding function.[73,75] The latter is true not only in HeV and NiV, but also in divergent henipaviruses (GhV, CedV, MojV), albeit to varying degrees.[76] Matrix interactome studies have identified many nuclear interacting factors, including importins and exportins, consistent with the nuclear-cytoplasmic trafficking phenotype of M.[75,77].

<H2>Host Range

For most paramyxoviruses, host range is limited and interspecies transmission is rare. In contrast, henipaviruses display a broad species tropism. In addition to a large number of bat hosts at various geographic locations (see more detail in Epidemiology section), NiV has naturally infected pigs, humans, dogs, horses, and cats,[10,11,78-80] whereas HeV infects all four Australian flying fox species and has naturally infected humans, horses and dogs.[2,81-83] Confirmation of the wide host range of these henipaviruses and the identical cell tropism of HeV and NiV were obtained early using an *in vitro* cell fusion system that relies on vaccinia virus–mediated cell surface expression of G and F glycoproteins.[84-86] Bats, guinea pigs, hamsters, ferrets, squirrel monkeys, and African green monkeys are also susceptible to experimental NiV infection.[87-91] Laboratory studies have added cats, guinea pigs, hamsters, ferrets, dogs and African green monkeys to the list of HeV-susceptible species.[83,92-96] CedV, isolated from flying foxes (*Pteropus sp.*) in Cedar Grove, Australia, can establish transient but non-pathogenic infections in laboratory challenged guinea pigs and ferrets. GhV and MojV, known only from sequence information, were identified from bats[29] and rats,[30] respectively.

<H1>Pathogenesis and Pathology

<H2>Entry into the host

Epidemiological and experimental studies generally support an oronasal [8,97,98] or oropharygeal[99-101] route of entry. This occurs directly or indirectly via saliva, urine, or oronasal scerections that are contaminated with relatively high levels of HeV or NiV.[83,95,97,102-113] Fig. 14.6 summarizes what is known about how NiV is transmitted to humans either directly from their pteropid reservoir hosts—fruit bats or flying foxes—or indirectly via a secondary amplifying host such as pigs, horses and humans. Direct bat-to-human transmission has been associated with consuming contaminated fruit or liquid (e.g. date palm sap) [99-101]. Human-to-human transmission of NiV has been well-documented, especially in Bangladesh[107,114-116] and the most recent outbreak in Kerala (India).[17] However, all 7 known cases of HeV infections in humans have resulted from contact with sick horses already exhibiting symptoms of respiratory distress,[117] likely after being exposed to food or water contaminated with saliva and urine/droppings from HeV-infected flying foxes.[118] While patients that succumb to henipavirus infections eventually die from encephalitic complications that often involve brainstem neuronal dysfunction,[98,119] there is diversity of clinical presentations.[120] For example, NiV-MY infected patients in Malaysia present less often with respiratory involvement (~40%) than the vast majority of NiV-BD infected patients in Bangladesh and India, including the latest outbreak in Kerala (India). The handful of HeV infected humans have also presented with encephalitic illness with or without a respiratory component (see Clinical Features section). To better understand the pathogenesis and pathology associated with henipavirus disease in humans, many relevant animal models have been developed that either represent natural amplifying hosts (pigs and horses) or that reproduce well the respiratory and encephalitic disease seen in humans (hamsters, ferrets and African green monkeys (AGMs)). [121,122] Despite the broad host range mentioned in the prior section, other animal challenge models (guinea pigs, cats, dogs) either do not represent the full spectrum of disease seen in humans, or are relatively asymptomatic when challenged with NiV and/or HeV.[83,121]

<FIG14.6>

<H2>Site of primary replication, virus spread, and cell and tissue tropism

The clinical and pathological data from humans (see Clinical Features section), relevant animal models, and cell tropism studies (Table 14.3) indicate that the initial site of replication is within the respiratory system, likely involving cells lining the oro-/naso-pharyngeal epithelium as well as bronchiolar epithelial cells, type I pneumocytes, alveolar macrophages or pulmonary airway dendritic cells (DCs).[40,122-125] The distribution and time of appearance of lesions throughout the vasculature and in the brain and lung in NiV encephalitis suggest that secondary infection probably arises via hematogenous spread of the virus, with secondary replication occurring in vascular endothelium.[126] Inflammation of blood vessels (vasculitis) occurs in most organs but is particularly prominent in the brain, lung, heart, kidney and spleen.[9,126,127]Vasculitis is limited to small arteries, arterioles, and capillaries where NiV antigen is found in both endothelial cells and the smooth muscle of the tunica media. The pattern and time of appearance of vasculitis and viral antigen distribution are consistent with endothelial cell infection occurring before infection of the smooth muscle. The 5-day interval between maximal vasculitis in the brain and parenchymal infection in acute NiV encephalitis suggests that virus replication occurs first in endothelial cells, with infection of neurons occurring as a result of vascular damage and breakdown of the blood-brain barrier (BBB). In humans as well as in pigs and hamsters, viral antigen positive brain microvascular endothelial cells can be observed in areas of compromised BBB integrity.[126,128,129] The presence of inclusion bodies and viral antigen in neurons, along with widespread histologic and radiologic lesions, suggests that neurologic impairment in NiV (and HeV) encephalitis may be caused by both the effects of ischemia and infarction and viral infection of neurons.[12,97,126]

<TAB14.3>

In addition to the systemic vasculitis, NiV antigen is clearly present in the parenchyma of multiple organs mentioned above. The same pathology has been reported for a fatal case of acute HeV encephalitis.[130] Similar widespread infection and multiorgan vasculopathy has been demonstrated in relevant animal models with the highest viral antigen load consistently seen in the lung and brain (Fig. 14.7). [121,122] Interestingly, intranasal inoculation in pigs, hamsters, and ferrets suggests that NiV can also directly invade the central nervous system (CNS) through the olfactory epithelium (OE). NiV antigen positive neurons or olfactory nerves can be found extending through the cribiform plate or in the nasal turbinate into the olfactory bulb.[123,129,131] While primary human OE cells are susceptible to henipavirus infection[132], the olfactory route is unlikely to be the major mode of CNS invasion in humans (and the AGM model). Primates have a relatively smaller area of OE compared to hamsters, pigs, or ferrets where the majority of evidence regarding the olfactory route of entry has been gathered.[87,129,131] In addition, NiV antigen was not detected in the olfactory bulbs of at least nine infected patients from the autopsy series reported from the Malaysian outbreak.[126]

<FIG14.7>

The widespread cell and tissue tropism of NiV and HeV seen in humans and the various animal models is determined by the expression pattern of the henipavirus receptors, ephrin-B2 and ephrin-B3.[51-54] The ephrinB ligands and their cognate EphB receptors are both receptor tyrosine kinases that mediate bidirectional cell–cell signaling events upon engaging each other. They are critical modulators of cell remodeling events, especially within the nervous and vascular systems.[133,134] They are also highly conserved among vertebrates, which explains, at least in part, the unusually broad species tropism of henipaviruses. Ephrin-B2 is found in arteries, arterioles, and capillaries in multiple organs, and is more broadly distributed across multiple tissues and cell types (Fig. 14.8), including neurons, arterial smooth muscle and human bronchiolar epithelial cells [133,135-137] but is absent from venous components of the vasculature.[138,139] In contrast, ephrin-B3 is essentially restricted to the brain[134] where it is expressed higher than ephrin-B2 (Fig. 14.8). Its relative absence from organs such as lung, kidney, and spleen, as well as from arterial endothelium suggest that the systemic vasculitis, respiratory distress, and other end-organ pathologies seen in henipavirus infections is largely a consequence of ephrin-B2-mediated entry. Conversely, ephrin-B3 mediated entry may play a larger role in the spread of HeV and NiV within the CNS.

<FIG14.8>

Human lymphocytes (mainly T- and B- cells), and monocytes, despite being non-permissive for NiV infection[140] or HeV-F/RBP induced fusion[84], can bind and transfer NiV to permissive microvascular endothelial cells *in vitro*. [140] This trans-infection phenotype, thought to be mediated by heparan sulfates,[141] may be an efficient form of hematogenous spread in addition to the free form viruses that make up the plasma viremia seen in acute NiV and HeV infections in humans. This acute but transient viremia appears about 4-7 days post-onset of illness (POI) and in survivors, is usually undetectable within 2-4 weeks POI depending on severity of the disease course.[17,142-144] A similar course of plasma viremia can also be detected in AGMs challenged intratracheally and orally with NiV.[88] The next section on the Immune Response will discuss possible mechanisms by which viremia is cleared in survivors.

<H2>Immune Response

Viral antagonism of innate immune responses

HeV and NiV have a broad host range that is uncommon in paramyxoviruses. This ability to replicate, spread, and cause acutely lethal disease amongst multiple species suggest that these viruses have evolved effective means to antagonize or evade innate antiviral responses that might otherwise keep them in check until adaptive immune responses develop. In henipaviruses, as in other paramyxoviruses, [40] the major anti-interferon (IFN) activities are encoded by the *P* gene and *in vitro* studies indicate that *P* gene products inhibit both IFN induction and signalling (Fig. 14.9). [39] In IFN induction, various forms of viral double-stranded RNA (dsRNA) are detected by cytoplasmic RNA helicases[145-147] such as RIG-I and MDA-5, and by toll-like receptor-3 (TLR-3). [147,148] Both of these dsRNA signaling pathways, via a cascade of signaling events depicted in Fig. 14.9 (left) lead to activation and nuclear translocation of interferon regulatory factor (IRF)-3, or if present, IRF-7, and nuclear factor kappa B (NF-kB); transcription factors that induce the expression of IFN-α/β proteins. HeV and NiV inhibit dsRNA signaling via its V protein, which binds to MDA-5 and prevents downstream signaling[145,149,150] but does not abrogate dsRNA signaling through TLR-3.[151] Recent evidence suggest that NiV-V also binds RIG-I and its regulator (TRIM25), disrupting downstream signalling. [152] In an additional strategy unique to henipaviruses, the W protein, by virtue of a NLS located in the unique carboxy terminal, inhibits induction of IFN-α/β expression in the nucleus by targeting a process that is part of both helicase-dependent and TLR-3–dependent signaling pathways.[153] Unexpectedly, the NiV M protein, can also inhibit IFN-α/β induction by indirectly antagonizing the activity of IKKε, the kinase responsible for IRF-3 activation.[154]

<FIG14.9>

In the IFN signaling pathway (Fig. 14.9, right), IFN binds to the IFN-α/β receptor (IFNAR) on cells and initiates a signaling sequence that leads to activation of members of a family of proteins called *signal transducers and activators of transcription* (STAT).[155,156] Henipaviruses inhibit IFN signaling by sequestering STAT proteins in high molecular weight complexes and preventing their phospho-activation .[157,158] The anti-IFN signaling activity is a property of the V protein, as has been observed for other paramyxoviruses, but also of the W and P proteins,[151,157,158] [159]The P, V, and W proteins of henipaviruses have an N-terminal extension of 100 to 200 amino acids compared with cognate proteins in the subfamily,[20,23] and the STAT binding domain of NiV V maps to this region.[151,160,161] The V and P proteins bind STAT in the cytoplasm, whereas the W protein, which is imported into the nucleus via specific interactions of its NLS with importin α-3, [162] likely sequesters unactivated STAT1 in the nucleus.[151,153] Recently, NiV and HeV N proteins were also found to antagonize IFN-signaling by interfering with formation of activated phospho-(p)STAT1 complexes. This NiV-N mediated antagonism of pSTAT1 nuclear accumulation does not rely on direct binding to or inhibiting phosphorylation of STAT1 itself. [163] NiV C has also been demonstrated to interfere with IFN-signaling.[164,165] However, its mechanism and true function remains unclear. Antagonizing IFN-signaling is unlikely to be the main function of the C protein since recombinant C-deficient NiVs have an attenuated replication phenotype even in Vero cells, [41,159,166]which are genetically deficient in IFN production.

There is little doubt that inhibition of IFN-α/β production is one major contributor to the acute virulence of HeV and NiV *in vivo*. Daily administration of poly(I)-poly(C(12)U), a potent IFN-inducer, results in >80% survival in a lethal challenge hamster model for NiV infections. [167] Most of the studies described previously were carried out *in vitro* using single-gene transfection expression systems. Understanding the true function(s) of these proteins requires assessing their impact in the context of a live virus infection or the phenotype of viruses deficient in the cognate protein(s). For example, IFN signaling apparently remains functional during live HeV and NiV infection of human cell lines, whereas IFN production was inhibited.[168] However, henipaviruses also clearly antagonize both IFN production and signaling in bat cells derived from *Pteropus sp*. that serve as their natural hosts. [169]

The diverse phenotypes observed in the various animal models when challenged with recombinant viruses deficient in the various P-derived accessory proteins makes interpretation difficult. The clearest phenotype comes from rNiVs engineered to be V-deficient. rNiV-ΔV are extremely attenuated in both hamster and ferret challenge models.[166,170] Each model re-capitulates the respiratory and neurological disease seen in human infections when infected with wt NiV, albeit in different ways and in a dose-dependent fashion.[93,121,171] However, challenge with V-deficient rNiV under conditions where their wt counterparts effectuate 100% mortality always results in 100% survival, and almost never results in any overt respiratory or neurological signs. [166,170] On the other hand, C-deficient rNiVs give a variety of phenotypes that range from strong attenuation in the hamster model, [172] to 100% mortality in the ferret model, albeit with less severe histological lesions in the lung. [173] Similarly, W-deficient rNiVs did not show significant attenuation compared to their wild-type counterparts in both the hamster[166] and ferret models, although the disease symptomology appeared to be more neurologic in the latter. [170,173] Interestingly, a rNiV lacking both C and W still resulted in 60% mortality with reduced respiratory disease but markedly increased neurological disease. [173]

Adaptive immune responses.

In patients with encephalitis, anti-NiV antibodies were observed more frequently in the serum than the CSF. Immunoglobulin M (IgM) antibodies occurred more frequently than immunoglobulin G (IgG) antibodies in both locations. [9,12,126]The appearance of specific IgM antibodies in serum preceded their appearance in the CSF, a sequence consistent with viremia preceding central nervous system (CNS) infection. Anti-NiV antibodies were present in most patients with clinical NiV encephalitis; however, no difference was observed in clinical features, laboratory results, or mortality between seropositive and seronegative patients. [98,174] Seroconversion of IgG against HeV was seen in two human cases of HeV infection in 2008, with one progressing to fatal encephalitis. [8] From these cases, seroconversion in acute henipavirus disease may have diagnostic but no apparent prognostic value. Conversely, in the most recent NiV outbreak in Kerala involving 23 cases, careful monitoring of humoral and cellular immune responses in the 2 survivors revealed that there was a dramatic increase in activated (Ki67+) CD8+ T-cells with an acute effector phenotype (CD38+/HLA-DR+/Granzyme B+ /PD1+), suggesting that virus-specific naïve T-cells were actively proliferating in response to the virus. Interestingly, clearance of viremia coincided with the peak of proliferating Ki67+ CD8+ T-cells, and occurred before peak IgG titers. [142] Increases in activated CD8+ T-cells in lethal (NHP)[175] and non-lethal (Swine) animal models of NiV infection have also been observed; [176] in the latter, prevention of virus shedding appears correlated with such T-cell responses. However, most animal studies do not monitor adaptive immune responses with the requisite precision and sophistication in order to make definitive statements regarding the relative importance of T- or B- cell responses in protective immunity against acute henipavirus infections.[177]

The high CFR associated with the acute nature of henipavirus infections already suggests that the virus interferes with the timely generation of effective T- or B- cell responses during the *natural* history of the disease in the majority of infected individuals. Indeed, although NiV replicates poorly in DCs (Table 14.3), [140,178] *in vitro* NiV-infected DCs induces an inflammatory pro-apoptotic milieu (TNF-a, IL-1β, IL-8) that leads not only to defective T-cell priming, but apoptosis of cognate T-cells and DCs. [178] This in vitro phenomenon, if recapitulated *in vivo* by NiV-infected DCs that traffic to lymph nodes, may account for the lymphoid depletion and lymphoid necrotic lesions seen in humans[126], and various relevant animal models. [179,180] [170]

<H2>Release from host and transmission

Transmission is generally accepted to occur via the oronasal route[8,97,98]or liquid/food (palm sap/fruit),[99-101] with transmissibility of HeV (horse-human or human-to-human) [181,182] being much less efficient than that of NiV transmission (pig-to-human or human-to-human).[9,15,17,98,105,107,116,183,184] Infectious NiV can be recovered from urine and the tracheal and nasopharyngeal secretions of infected patients in the early phase of their illness in the original Malaysian outbreak. [3,97,98] Nonetheless, human-to-human transmission in the Malaysian outbreak was extremely rare[185], although it has been reported in the more recent outbreak in the Philippines. In contrast, person-to-person transmission in Bangladesh has been well-documented and typically occurs during the final stages of disease in the index patient when not only symptoms of respiratory involvement are most prominent, but also more frequent and closer contact with familial caretakers occurs. [115,183,184]

Replication in the respiratory epithelium and shedding in oronasal secretions is most likely responsible for virus release and transmission. Ferrets have been used to model the respiratory transmission of henipaviruses[102,180] as their respiratory symptoms (cough, serous nasal discharge, dyspnea) mimic the human disease.[87] Despite shedding relatively large amounts of virus in their oronasal secretions (4-5 log10 $TCID_{50}$ equivalents/ml at peak titers), passively cohoused naïve ferrets never got infected, regardless of whether the index ferrets were infected with HeV, NiV-MY, or NiV-BD.[102,180] However, following assisted or direct exposure to infectious oronasal fluids from the index ferrets, all the naïve ferrets developed acute infections, regardless of virus strain used. [102] Thus, the efficiency of NiV transmission appears low, and likely requires direct and repeated oro- or naso-mucosal exposure to infectious oronasal secretions. This is consistent with epidemiological investigations into the many recurrent outbreaks in Bangladesh where despite chains of person-to-person transmission, the estimated basic reproductive number R_0 of NiV-BD strains that have spilled over in Bangladesh have averaged 0.48.[183]

<H2>Virulence

Infection with HeV appears more severe than infection with NiV-MY and NiV-BD in the hamster[128,186] and AGM model.[187] The dose-dependent disease symptomology in the hamster model[128,188] makes it difficult to make generalize statements about any intrinsic pathogenic difference between these three genetically distinct henipaviruses. However, the intratracheal and intranasal AGM challenge model appears to recapitulate the clinical pathologies seen in human patients more robustly.[88,93] Interestingly, it is also in this model that NiV-BD is shown to be more pathogenic than NiV-MY,[187] which reflects the observed differences in pathogenicity between NiV-MY and NiV-BD.   Previous studies comparing NiV-MY and NiV-BD in hamsters[186] and in ferrets[102] gave conflicting and confusing results. But more careful comparisons of fully-sequenced NiV-MY and NiV-BD strains in the AGM model provides support that NiV-BD is more pathogenic than NiV-MY,[187] which is consistent with the higher mortality rates associated with NiV-Bang (see Table 14.1C). Under identical experimental conditions, NiV-BD is not only shown to be more pathogenic in terms of viral load in lungs, spleen, and blood, but also that the therapeutic window for post-exposure prophylaxis using human monoclonal antibody (m102.4) is significantly shorter in NiV-BD infected AGMs.[187] m102.4 is a potent neutralizing mAb previously shown to rescue NiV-MY and HeV infected AGMs even when administered up to 7 days post-infection.[189,190]

CedV lacks the editing site in the *P* gene and does not make the V protein,[28] a potent type I IFN antagonist required for henipavirus virulence *in vivo*.[39] Not surprisingly, CedV, which is naturally V-deficient, also does not induce any overt disease in ferrets and guinea pigs.[28] Reactive hyperplasia of oropharyngeal lymphoid tissues and detection of viral RNA in select bronchial associated lymphoid tissue (BALT) accompanied by increasing neutralizing Ab titers suggests that CedV likely replicates transiently in the upper and lower respiratory tracts of the challenged animals.[28] While recent evidence suggests that the CedV P protein itself is also a less efficient antagonizer of IFN-α signaling compared to HeV-P,[191] whether CedV is truly non-pathogenic in humans or horses remains to be seen. Given the fact that CedV does not appear to use ephrin-B3 as receptor, one might also predict CedV to be less encephalopathic even in more susceptible animals.

<H2>Persistence

A small percentage of NiV-infected patients (3-7%) experience late-onset or relapse encephalitis months to years after the initial NiV infection; the former describes patients with initially mild or asymptomatic NiV infections while the latter are survivors of acute NiV encephalitis.[98,119,192,193] One case of fatal late-onset HeV encephalitis has also been reported. [3,130] These cases suggest that NiV and HeV can persist in sanctuary sites like the brain especially since virus has never been isolated from oronasal secretions of relapsed encephalitis cases.[9,97,126,192-194] (see Clinical Features section for details). There is one report of NiV RNA being detected in the semen of a NiV survivor 4 weeks post-onset of illness, but this was gone 2 weeks later. Longitudinal studies on 2 HeV survivors shows no evidence of viral shedding by RT-qPCR, up to 6 years post-symptom onset.[144] It is unlikely that NiV and HeV in humans can persist in infectious forms. Thus, relapse henipavirus encephalitis might resemble measles virus associated subacute sclerosing panencephalitis, which can occur years after recovery from measles virus infection.[195,196]

<H1>Epidemiology

<H2>Age

The age of patients with encephalitis in the Malaysian outbreak ranged from 9 to 76 years, with almost 50% of cases occurring in those 40 to 44 years.[98,126,143,174,197] The male-to-female ratio was approximately 3:1, and more than 80% of the patients were Chinese, with statistics reflecting the increased risk to those working with infected pigs.[98,197] In Bangladesh, the age of patients ranged from 4 to 60 years, and males constituted 47% and 67% of the cases in the 2001 and 2003 outbreaks, respectively.[14,198] The outbreak in early 2011 in Bangladesh claimed at least 35 lives, including many children and infants, with ages ranging from 2 to 56 years.[117,199] Among the 18 suspected patients in the 2014 oubreak in the Phillipines, the age distribution is between 21-60 years old.[18] In the most recent NiV outbreak in Kerala, India, the age distribution ranged from a 17 year old male to a 75 year old female among a total of 23 human cases.[17]

<H2>Morbidity and Mortality

In Malaysia, between September 1998 and June 1999, 256 patients who developed acute NiV encephalitis were admitted to Malaysian hospitals and 105 died, a mortality rate of approximately 40%.[9,197] The rate of subclinical infection in households and farms where cases of NiV encephalitis occurred was calculated to be 8% and 11%, respectively.[192,197] In Singapore, where 11 patients were confirmed to have acute NiV encephalitis, a further 2 asymptomatic abattoir workers were serologically positive, representing a rate of subclinical infection of 15%.[200] Subsequently, 89 individuals were identified on the basis of positive serology as having experienced either an asymptomatic or mildly symptomatic NiV infection.[119] This increases the number of people infected with NiV to 345 and decreases the mortality rate to approximately 30%.[127] In Bangladesh, 98 of 135 patients died in eight outbreaks from 2001 to 2008, giving a combined case fatality rate of 73%.[107,184,198] In the Philippines outbreak, the case mortality is 53% out of all the suspected cases or 81% for those with encephalitic clinical signs.[18] The case mortality is 94% for the 2018 Kerala outbreak in India.[17] There have only been seven known human cases of HeV infection in

Australia in the past 16 years, four of which have been fatal (three acute and one case of relapsed encephalitis).[2,3,8]

<H2>Origin and Spread of Epidemics

Fruit bats (flying foxes) in the genus *Pteropus*, family *Pteropodidae*, suborder *Megachiroptera* are main reservoir hosts of HeV and NiV.[5,6,13,79,201] In Australia, HeV has been shown to occur in all four flying fox species, with the crude seroprevalence of 47%, indicating an endemic pattern of infection throughout Australia.[81] Serologic tests show that NiV or NiV-related virus is widely dispersed in bats from Malaysia to the west border of Africa (Fig. 14.10). NiV was first isolated from the urine of Island flying foxes and from the saliva on partially eaten fruit[13] and has since been isolated from Lyle's flying foxes (*Pteropus lylei*) in Cambodia[79] and *Pteropus medius* in Bangladesh.[202] The Indian flying fox (*Pteropus giganteus*) is the main pteropid species throughout Bangladesh and the Indian subcontinent with a high seroprevelence of henipavirus-specific antibody.[14,100,183,203] Additional serologic and limited nucleic acid evidence has suggested that related henipaviruses are circulating in other regions, including Thailand, Indonesia, Vietnam, China, Madigascar, and several countries and regions in Africa (Fig. 14.10 and Table 14.4-online).

<FIG14.10>

Neither HeV nor NiV appear to cause clinical disease in flying foxes infected naturally,[81,198,201,204] and experimental infection with doses of HeV, consistently shown to be lethal in horses, generates only sporadic vasculitis in the lung, spleen, meninges, kidney, and gastrointestinal tract and only in a proportion of infected bats.[95] Viral antigen is detected in the tunica media rather than endothelial cells, a fact that may spare the flying fox from the clinical effects associated with vasculitis.[205] In infected pregnant flying foxes, antigen was observed in similar locations and in the placenta.[206] The mode of transmission between flying foxes is unknown. Transplacental transmission has also been observed experimentally without apparent harm to the fetus.[206] Experimental infection of flying foxes with NiV produced a subclinical infection with a transient presence of virus within selected viscera along with periodic viral excretion in bat urine and seroconversion with neutralizing antibody present.[90]

The spillover and epidemic hosts of HeV and NiV were horses in Australia and pigs in Malaysia. All human infections with HeV in Australia and NiV in Malaysia have only occurred through transmission from these domestic animal hosts.[9,197] No evidence exists of direct transmission from pteropid bats to humans in Australia or Malaysia, despite many opportunities in Australia for transmission to bat carers.[192,207] In contrast, flying foxes apparently play a direct role in the transmission of NiV to humans in the many recent outbreaks of disease in Bangladesh, where epidemiologic evidence in support of a role for an intermediate host was lacking.[14,198] Three pathways of NiV transmission from bats to people have been identified based on epidemiologic investigations in Bangladesh.[107] Consumption of fresh date palm sap appears to be the predominant risk factor, and infrared camera studies have confirmed that *P. giganteus* bats frequently visit date palm sap trees and consume sap during collection.[208] In the 2005 NiV outbreak in Tangail District, Bangladesh, drinking raw date palm sap was the only activity significantly associated with illness (64% among cases vs. 18% among controls).[100] Another route of transmission for NiV from bats to people in Bangladesh could be via domestic animals. Contact with a sick cow in Meherpur, Bangladesh, in 2001 was strongly associated with NiV infection,[14] and contact with pigs and diseased goats have also been implicated in other occurrences of NiV in Bangladesh.[107] Although NiV has never been isolated in domestic livestock animals, serological evidence was reported for antibodies to NiV or NiV-related viruses in cattle, goats and pigs in Bangladesh.[209] Transmission via direct contact with NiV-infected bat secretions also appears possible from evidence in the Goalando outbreak in 2004, where individuals who climbed trees were more likely to develop NiV infection than controls.[210]

The mode of transmission from bats to spillover hosts in Australia and Malaysia remains to be determined. Three principal hypotheses exist. One is that masticated pellets of virus-contaminated, residual fruit pulp spat out by flying foxes are ingested by horses or pigs.[201] The second is that urine from infected animals contaminates pastures or pigsties. The third is that infected fetal tissues or fluids contaminate pastures or sties and are ingested. The latter is based largely on the fact that the HeV outbreaks have occurred during the birthing period of some species of flying fox and is supported by the isolation of virus from a pregnant flying fox and its fetus.[6]

HeV has been transmitted from horse to man on seven occasions from 1994 to 2009, twice during the initial outbreak in Brisbane,[1,2] twice during necropsy of horses that died in the field,[1,4] twice during either daily nasal cavity lavage and participating in a necropsy,[8] and once from performing an endoscopy on an infected horse.[8,211] HeV is rarely found in the bronchi or bronchioles of infected horses, which suggests that aerosol transmission to either man or horses is less likely[212] and horse-to-horse transmission of HeV has not been demonstrated.[95] The presence of HeV in equine saliva, however, suggests that close contact with infected horses, such as might occur during manual feeding of the animals, may facilitate horse-to-human transmission.[1] The presence of virus in a wide range of tissues and in the nasal discharge commonly found at the terminal stage of infection offers a range of sources for virus transmission during necropsy.[2,213] As shown in Fig. 14.6A, high level of viral antigen can be detected in the nasal cavity of HeV-infected horses.

In the Malaysian NiV outbreak, contact with pigs or fresh pig products was required for transmission of the virus to humans, with greater likelihood of transfer to those in direct contact with sick or dying pigs on farms or in abattoirs.[9,197] The presence of NiV in the respiratory epithelium of naturally and experimentally infected pigs (Fig. 14.6B) indicated that virus probably spread to humans and within the pig population by aerosol or by direct contact with oropharyngeal or nasal secretions.[10,111,129] The presence of the virus in a wide range of organs indicates that humans may also have been infected during processes such as slaughtering or farrowing. In Bangladesh, pigs were excluded as potential sources of NiV on epidemiolog grounds, and human-to-human transmission was observed.[116,184] The virus may have been transmitted to human index cases directly through contact with fruit bat secretions in contaminated fruit or date palm sap before circulation in the human population.[13,14,107,198] Nosocomial transmission has been detected in some of the Bangladesh and India outbreaks.[15,107,214] In the Philippines NiV outbreak, Of the 17 case-patients, a total of 7 (41%) had participated in horse slaughtering and horse meat consumption; 3 (18%) had only consumed horse meat and but had no history of slaughtering or meat preparation; 5 (29%) case-patients had been exposed to other human casepatients but not to any horses. So it is possible that both horse-to-human and human-to-human transmission might have occurred.[18] For the Kerala NiV outbreak, while it remains undefined how the index case was exposed to NiV, it was evident that human-to-human transmission was the main driver for the spread of the virus in both family/community and hospital settings.[17]

<H2>Genetic Diversity

Genome sequencing revealed that HeV isolated from equine and human sources during the outbreak in Brisbane appears identical and differs little from HeV isolated from flying foxes 2 years later.[2,6] Sequencing of five additional horse isolates from five different locations from the 2006 to 2008 HeV occurrences has also demonstrated a very high genetic similarity.[215] Similar observations were made in Malaysia, where it was demonstrated that NiV isolated from pigs at the height of the outbreak and at its geographic focus were essentially identical to human isolates made at that time and isolates obtained from flying foxes several years later.[13,22,23,216]

In Bangladesh, four human isolates obtained in 2004 demonstrate significant genetic heterogeneity, which might suggest multiple spillovers of NiV from flying foxes into the human population.[33] The NiV sequences detected from human patients in India from 2001 to 2018 were more related to NiV from Bangladesh than NiV from Malaysia.[15,17,214] A recently whole genome sequencing study of ten NiV isolates obtained from bats in 2013 in Bangladesh at two different geographic locations revealed that the isolated viruses were very similar in sequences. This suggests that multiple strains were not co-circulating in the bat population at the time.[202] Furthermore, none of the bat NiV isolate sequences was identical with any previously detected human NiV isolate sequences, suggesting that NiV spillover into humans is a rare event and more intensive surveillance is required to appreciate the full genetic diversity of NiV in bats in an area with frequent spillover events into human populations.[202] NiV isolated from the flying fox *Pteropus lylei* in Cambodia[79] represents an evolutionary lineage that is separated from the Malaysia or Bangladesh/India cluster . Partial *N* gene sequences detected in *Pteropus lylei* in Thailand indicate the circulation of at least two lineages of NiV—one related to the Bangladesh NiV and the other more related to the Malaysian NiV.[109] On the other hand, NiV sequences detected in *Pteropus hypomelanus* in southern Thailand are clearly more related to the Malaysian NiV.[217] As the RNA-dependent RNA polymerase (RdRP), also known as the L protein, is the most highly conserved protein for paramyxoviruses, it has been used as a more reliable indicator of evolutionary relationship among different paramyxoviruses. Shown in Fig. 14.11 is phylogenetic tree based on RdRP proteins of prototype species of each genera in the family *Paramyxoviridae* with major members of the genus *Henipavirus*, selected to represent major species origin, geographic location and time of isolation.

<FIG14.11>

The presence of henipavirus-reactive (but not neutralizing) antibodies and viral RNA in bats from other regions of the world (Fig. 14.10 and Table 14.4-online) predicts that a much greater genetic diversity of henipaviruses exists in different bat populations. This also suggests the existence of henipaviruses with different transmissibility and pathogenicity in non-bat hosts.

<H1>Clinical Features

<H2>Incubation Period

Based on the time interval between the last exposure to pigs and onset of disease, the incubation period for NiV ranged from 2 to 45 days; however, for 90% of patients, it was 2 weeks or less.[98,143,174] An estimate of 2 to 3 weeks was made based on the time interval between importation of pigs from NiV-affected areas of Malaysia and development of human disease at a Singaporean abattoir.[200] A mean incubation period of 9.4 days was calculated for four patients who had a fixed period of exposure.[174] The NiV outbreak in Kerala, India, 2018, revealed a median incubation period was 9.5 days among 23 cases, 22 of which were nosocomial.[17] In the occurrence of HeV in Australia in 2008, a detailed examination of exposure histories from two infected patients (one fatal) suggested a likely incubation period of 9 to 16 days, with exposure occurring some 3 days before the onset of symptoms of HeV infection in the horse.[8]

<H2>Acute Clinical Features

The first two patients infected with HeV presented with fever, myalgia, headaches, lethargy, and vertigo. One patient recovered; however, the other developed pneumonitis, respiratory failure, renal failure, and arterial thrombosis, and died of cardiac arrest 7 days after admission to the hospital. Findings at autopsy were consistent with a viral infection; both lungs were congested, hemorrhagic, and filled with serous fluid, and the histology revealed focal necrotizing alveolitis with many giant cells, some syncytial formation, and viral inclusions.[1] Although the predominant clinical features of NiV encephalitis derived from CNS involvement in the initial Malaysia outbreak, a proportion (40%) of patients displayed pulmonary involvement, which presented as an atypical pneumonia with fever, cough, and headache.[12,98,126,174] In the Kerala, India NiV outbreak, 19 cases (83%) presented with acute respiratory distress syndrome (ARDS) and shortness of breath, and 20 cases (87%) had respiratory symptoms.[17] The clinical presentation of NiV infections in Bangladesh also shows severe respiratory disease involvement.[218] A chest X-ray of an acute NiV-infected human case showing diffuse bilateral opacities of the lung fields consistent with ARDS (Fig. 14.12A) and a similar presentation was reported in the first fatal human HeV case.[1] Chest X-ray images of NiV and HeV-infected AGMs have also demonstrated severe respiratory disease (Fig. 14.12B). Case definitions for suspected NiV infection in Bangladesh[218] and India[15,17] generally included individuals residing in an outbreak area who presented with fever, headache, vomiting or altered mental status or with history of cough or shortness of breath and fever.

<FIG14.12>

With NiV in Malaysia, most patients presented with acute encephalitis characterized by fever, headache, drowsiness, dizziness, myalgia, and vomiting, and more than 50% had a reduced level of consciousness.[9,12,98,174] The major clinical signs included drowsiness, areflexia, segmental myoclonus, tachycardia, hypertension, pinpoint pupils, and an abnormal doll's eye reflex. Such clinical features as these suggested involvement of the brain stem and upper cervical spinal cord, and were observed more frequently in patients with a reduced level of consciousness.[98,219]In Kerala, India, 2018, NiV-infected patients presented with altered sensorium (74%), along with myalgia, headache, vomiting, and seizures.[17] Patients in the NiV-Malaysia outbreak who retained normal levels of consciousness throughout their illness recovered fully; however, only 15% with reduced levels of consciousness survived. Figure 14.13 shows magnetic resonance imaging (MRI) images of human cases of acute NiV encephalitis (India, 2018 and Malaysia, 1999). Such neurologic manifestations are consistent with vasculitis-induced thrombosis in the brain and the direct infection of neurons.[9,11,12,126] The multiple discrete lesions 1 to 5 mm in diameter in the cerebral white matter detected by MRI may be the site of such microinfarctions and are distinct from lesions caused by other viruses.[9,12,130,192,220] A third case of HeV infection presented first with meningitis and a 12-day history of sore throat, headache, drowsiness, vomiting, and neck stiffness. After an apparent full recovery, this patient developed fatal encephalitis 13 months later and was admitted to the hospital with a generalized tonic-clonic seizure after 2 weeks of irritable mood and low back pain. Recurrent focal motor seizures occurred over the next 7 days, as did secondarily generalized seizures and low-grade fever, followed by dense right hemiplegia, signs of brain stem involvement, and depressed consciousness requiring intubation. The patient remained comatose and died 25 days after admission.[3] Two patients in 2008[8] presented with initial influenza-like illness, although soon after apparent clinical improvement and an absence of fever, encephalitis developed in both. MRI revealed widespread cortical, subcortical, and deep white matter involvement, similar to the previous late-onset case of HeV encephalitis[3] and to NiV encephalitis cases (Fig. 14.13B).[98,192,221]

<FIG14.13>

<H2>Outcome of Infection

Most patients who survived acute NiV encephalitis in Malaysia made a full recovery; however, approximately 20% had residual neurologic deficits.[98,174,222] Neurologic sequelae included cognitive difficulties, tetraparesis, cerebellar signs, nerve palsies, and clinical depression. A few patients remained in a vegetative state. In patients with encephalitis who recovered, most brain lesions revealed by MRI disappeared or became smaller over a period of 12 to 18 months, although some remained unchanged during this period.[222] Approximately 7.5% of patients who recovered from acute encephalitis and 3.4% of those who experienced nonencephalitic or asymptomatic infection developed late neurologic disease (Fig. 14.13).[98,119,192,193] Relapse encephalitis and late-onset encephalitis presented several months to 4 years after the initial infection, with the longest reported case 11 years after an initial acute infection.[223] Relapsed cases had elevated IgG, but not IgM, and no vasculitis, and unlike the situation in acute encephalitis, virus was not isolated from throat and nasal secretions.[9,97,126,192-194]

The clinical features associated with relapse and late-onset encephalitis resembled those found with acute NiV encephalitis, although decreased incidence was seen of fever, coma, segmental myoclonus, and meningism and an increased occurrence of seizures and focal cortical signs compared with the acute manifestation of the disease.[119] The clinical, radiologic, and pathologic features of relapse NiV encephalitis resembled those of the patient who became infected with HeV, suffered mild, transient aseptic meningitis, and recovered but died of a fatal meningoencephalitis 13 months later.[3,130] Most patients with relapse and late-onset encephalitis had only one neurologic episode, although some patients experienced two episodes separated by a mean of 7.6 months (6 weeks to 1 year).[119] The mortality rate associated with relapse and late-onset encephalitis at 18% was lower than that associated with acute encephalitis, at 30% to 40%. However, 61% of patients with relapse and late onset had further neurologic sequelae compared with 22% after acute encephalitis. Among NiV survivors in Bangladesh, some 30% have moderate to severe persistent neurologic dysfunction for years following acute infection.[192]

The demographics, clinical features, serology, and MRI of patients with relapsed and late-onset encephalitis were similar, suggesting that the two diseases have identical pathogenesis[119] and that the initial infection in late-onset encephalitis patients may not have been sufficiently severe to cause neurologic symptoms. MRI abnormalities similar to those observed in patients with acute encephalitis were also seen in 16% of asymptomatic patients, although the lesions were fewer in number. [219] The involvement of the cortex in relapse and late-onset encephalitis suggests a different pathologic mechanism compared with acute encephalitis. Relapse and late-onset encephalitis are considered to be caused by the recrudescence and rapid replication of virus that had persisted following acute or asymptomatic NiV infection.[119] NiV, however, was not isolated from CSF and brain tissue of patients with relapse and late-onset encephalitis.[119]

<FIG14.14>

The generalized understanding of the infection process along with the clinical features of human NiV and HeV infection is diagrammed in Fig. 14.14. Transmission is likely via the oronasal route as described the above section on Pathogenesis. The initial site of replication in humans is likely within the respiratory system and disease onset is characterized by fever, myalgia, shortness of breath, cough, which may progress to ARDS. Possible invasion into the CNS via the olfactory bulb has also been suggested and has been demonstrated in animal models. [224]Virus replication and hematogenous systemic spread occurs (cell-free or cell-associated viremia) and established infection is characterized by a widespread vasculitis with endothelial and smooth muscle cell tropism resulting in multinucleated syncytial cells.[126] CNS infection and encephalitis results in altered sensorium, headache, vomiting, and seizures along with fever and other systemic symptoms. Infection of other organs in addition to lung and brain in human cases occurs and has been reported in kidney, heart, spleen and lymphoid

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tissues,[126] and similar widespread infection has been demonstrated in multiple animal models.[121]

<H1>Diagnosis

Virus isolation, EM, immuno-EM, immunohistochemistry (IHC), serology, and polymerase chain reaction (PCR) played key roles in the initial discovery of HeV[2] and NiV,[11] and they remain essential elements in a repertoire of procedures for the rapid and specific diagnosis of henipavirus infections in humans and animals.

During investigation of a suspected disease outbreak, attempts to grow henipaviruses may be initiated in a BSL3 laboratory. However, if a cytopathic effect (CPE) is observed and the growth of henipavirus is confirmed by PCR or immune staining, infected cultures should be handled under BSL4 conditions and subsequent work with live virus restricted to BSL4. Both HeV and NiV replicate in various cell lines—a feature that contributed to the efficiency with which they were isolated during the initial disease outbreak investigations.[2,11] Vero cells are commonly used, generating titers of virus as high as 10^8 infectious virions per milliliter.[143,225] In fatal cases, attempts should be made to isolate virus from brain, lung, kidney, and spleen.[225] For tissue specimens containing a high virus load, direct examination by immuno-EM and IHC can be very useful in providing early diagnosis. Various antibody reagents have been developed for this purpose, including polyclonal antisera, monospecific antibodies raised against recombinant antigens,[20] and monoclonal antibodies (mAb) raised against whole virions or vaccinia virus–expressed viral proteins.[226-229] Using HeV- or NiV-specific mAb, it is possible to differentiate between the two viruses.[228,229] Quantitative real-time PCR (TaqMan assay) has been the method of choice to detect viral materials in infected tissues because of its speed, specificity, and sensitivity. The first-generation henipavirus TaqMan assays are either HeV specific[230] or NiV specific.[231] Since then, several consensus henipavirus real-time PCR assays have been developed that target different conserved regions of the viral genome.[232] It should be cautioned that the current PCR tests may not work with new henipaviruses yet to be discovered, especially those from African bats, owing to expected greater genetic divergence than those detected in Australia and Asia.

For henipaviruses, serologic tests are important both during outbreak investigation and for disease surveillance. The virus neutralization test (VNT) is accepted as the reference standard.[225] Few laboratories, however, can conduct neutralization tests because of the requirement to handle live virus at BSL4. For surveillance and diagnostic purposes, three types of tests that do not require BSL4 containment have been developed:

1. *Enzyme-linked immunosorbent assay (ELISA):* Several ELISA-based tests have been reported for the detection of henipavirus antibodies.[225,233,234] For diagnosis of human infections, two different ELISA tests have been applied: an IgM capture ELISA for early diagnosis of infection and an indirect ELISA for detection of IgG antibodies.[225]
2. *Liquid protein array multiplex test:* A Luminex-based test based on recombinant soluble G proteins of HeV and NiV was developed that is capable of mimicking VNT with great sensitivity and differentiating between antibody responses of HeV versus NiV infection.[235]
3. *Pseudotype virus:* Different pseudotype systems carrying the henipavirus F and G proteins have been developed as a surrogate VNT for detecting henipavirus-specific antibodies.[18,236-238] Incorporation of reporter genes in these systems resulted in greater sensitivity and reproducibility.

<H1>Prevention, and Control

<H2>Drugs and Small molecules

Ribavirin, which inhibits replication of HeV *in vitro*,[239] was used during the NiV outbreak in Malaysia in an open-label study in which 140 patients with encephalitis were given the drug, and 54 patients who presented before ribavirin became available or who refused treatment acted as controls.[240] Mortality in the treated group was 32% compared with 54% in the control group, representing a 35% reduction ($P = 0.011$). Duration of ventilation and total hospital stay were both significantly shorter in the ribavirin group ($P = 0.0002$ and <0.0001, respectively). In the absence of other therapies, ribavirin may be an option for treatment of henipavirus infections. However, two HeV-infected patients in 2008[8] were given a high-dose intravenous regimen of ribavirin, although basal concentrations appeared inadequate given the results of *in vitro* susceptibility testing of HeV, and the efficacy of ribavirin as therapy or prophylaxis in people remains at best uncertain. Chloroquine, an antimalarial drug, was first demonstrated to block the critical proteolytic processing needed for HeV F maturation and function.[241] Not surprisingly, the drug was later shown to inhibit NiV and HeV infection in cell culture experiments.[242] Chloroquine was administered along with ribavirin to one HeV-infected individual in 2009[243] with no apparent clinical benefit. Remdesivir (GS-5734) is a nucleotide analog prodrug that showed therapeutic efficacy in a nonhuman primate model of Ebola virus infection.[244] GS-5734 has been administered under compassionate use to Ebola virus infected patients and is currently in Phase 2 clinical development for treatment of Ebola virus disease. The antiviral activities of GS-5734 has been demonstrated to be broad showing *in vitro* activity against viruses in the *Coronaviridae, Filoviridae* and the *Paramyxoviridae* including NiV and HeV.[245]

In vivo, ribavirin only delayed but did not prevent deaths caused by NiV and had no effect on HeV infection in a hamster model.[167,246] Ribavirin treatment also only delayed disease onset by 1 to 2 days in African green monkeys challenged with HeV with no significant benefit for disease progression or outcome.[93] Chloroquine administration, either alone or in combination with ribavirin, had no therapeutic benefit in ferrets challenged with NiV or hamsters challenged with either NiV or HeV.[246,247] Poly(I)-poly(C12U), which induces IFN-α and -β production and can completely block NiV replication *in vitro*, displayed some protective activity in the hamster model of lethal NiV infection.[167]

<H2>Peptide Fusion Inhibitors

The first potential henipavirus-specific therapeutic was shown to be a heptad peptide-based fusion inhibitor,[85] analogous to HIV-1-specific peptide, enfuvirtide (Fuzeon) approved by the Food and Drug Administration (FDA) in March 2003. The henipavirus F_1 glycoprotein resembles other fusion glycoproteins in having α-helical heptad repeat (HR) domains proximal to both the fusion peptide at the amino (N) terminus and the transmembrane domain near the carboxy (C) terminus of the protein. The HR domains are involved in the formation of a trimer-of-hairpins structure during the membrane fusion and virus infection process. Addition of exogenous peptide from either HR domain blocks formation of the trimer-of-hairpins and abrogates membrane fusion and virus infection.[84,85,248] These observations were followed up with testing cholesterol tagged HR-derived peptides in the hamster model of NiV infection providing the first evidence of *in vivo* effectiveness of a fusion inhibiting peptide against NiV.[249]

<H2>Vaccines

A wide variety of immunization strategies have been developed for NiV and HeV prevention and include live-recombinant virus platforms, protein subunit, virus-like particles and DNA vaccines, however many of these approaches have only been examined for HeV- or NiV-specific neutralizing antibody induction.[250] A more limited number of vaccine candidates have been examined for both immune response and efficacy using a variety of animal challenge models (Table 14.5). Attenuated vaccinia virus strain NYVAC recombinants encoding either the NiV F or G glycoproteins used to immunize hamsters provided complete protection from NiV-mediated disease following challenge and demonstrated that an immune response to the viral envelope glycoproteins were an important immunogen in protection.[251] A second poxvirus-based approach was developed as a potential livestock vaccine using recombinant canarypox virus with the NiV F and G genes inserted into ALVAC vectors (Table 14.5). ALVAC vectors expressing either NiV F or NiV G were tested individually and in combination by vaccination of piglets, and animals were challenged intranasally with NiV. Piglets were protected from NiV-mediated illness by either ALVAC vector alone or in combination and immunized animals shed only low levels of nucleic acid detectable virus and no recoverable virus was evident.[252] Three other viral vector-based NiV vaccines have been examined in animal challenge studies (Table 14.5). An adeno-associated virus (AAV) platform using the NiV G glycoprotein was tested in the hamster model using NiV challenge, showing complete protection against NiV, but only low level cross-protection (3 of 6 animals) against a HeV challenge.[253] A recombinant measles virus vector encoding the NiV G glycoprotein was examined using two different vectors; the HL (rMV-HL-G) and Edmonston (rMV-Ed-G) measles virus strains[254] (Table 14.5). Here, complete protection from NiV disease was achievable in the hamster challenge model following vaccination with either rMV-HL-G or rMV-Ed-G, and the rMV-Ed-G was tested in the nonhuman primate model demonstrating 2 of 2 AGMs were protected against NiV challenge. Several groups have tested the vesicular stomatitis virus (VSV) based platform using the NiV F or G glycoproteins (Table 14.5). In the ferret model, a single immunization with VSV encoding either NiV F or NiV G could afford complete protection from NiV challenge.[255] Similarly, NiV G or F glycoproteins in VSV vectors were successful in protection from NiV challenge in the hamster model,[256,257] and a single immunization of NiV G encoding VSV was protective in the AGM model.[258,259]

<TAB14.5>

The most extensively tested vaccine against NiV and HeV has been a recombinant protein subunit strategy because of the inherent safety of such an approach. Soluble, secreted, oligomeric forms of the G glycoprotein (sG) from both NiV and HeV were developed and tested as possible vaccine candidates and the HeV-sG was show capable of eliciting a more potent cross-reactive polyclonal antibody response and also a better cross-protective response[260,261] (Table 14.5). HeV-sG is a soluble and secreted version of the molecule with a genetically deleted transmembrane and cytoplasmic tail that is produced in mammalian cell culture systems and is properly N-linked glycosylated[262] (Fig. 14.15). HeV-sG has been shown to retain many native characteristics including oligomerization and ability to bind ephrin receptors,[52,260] and is capable of eliciting potent cross-reactive (HeV and NiV) neutralizing antibody responses in a variety of animals including mice, rabbits, cats, ferrets, monkeys and horses.[250] The HeV-sG vaccine in the cat model provided complete protection against a lethal NiV challenge[261] and suggested a single subunit vaccine (HeV-sG) could be effective against both HeV and NiV. Further studies in the cat model revealed that antibody titers as low as 1:32 could protect against NiV-Malaysia challenge[263] (Table 14.5). Studies in the ferret model with low vaccine doses of HeV-sG formulated in CpG and Allhydrogel™ could completely protect against high dose HeV challenge.[96] Immunization and challenge studies in ferrets with the NiV-Bangladesh strain also demonstrated complete protection.[264] In addition, good durable immunity was shown in other ferrets challenged 434 days post-vaccination.[264] However, preliminary studies with HeV-sG as a vaccine in the pig model was less effective against HeV and un-protective against NiV, and both humoral and cellular immune responses were required for protection of swine against henipaviruses in that challenge model.[176]

<FIG14.15>

The HeV-sG vaccine has also been extensively evaluated in nonhuman primates (AGMs). Monkeys immunized with HeV-sG formulated in Allhydrogel^{TM} and CpG were completely protected against intratracheal challenge with NiV-Malaysia, some with pre-challenge neutralizing titers as low as 1:28, and no evidence of clinical disease, virus replication, or pathology was observed in any vaccinated monkeys.[265] Similarly, HeV-sG vaccination and protection from a HeV challenge in AGMs has also been shown, including HeV-sG formulated in Allhydrogel^{TM} alone.[266] The inherent safety and effectiveness of the HeV-sG vaccine led to its development as an equine vaccine to prevent not only HeV infection of horses but also as the means to reduce the risk of HeV transmission to people (Figure 14.16A). HeV-sG was licensed by Zoetis™ Inc., (formerly Pfizer Animal Health) and developed as an equine vaccine for use in Australia. Horse HeV-sG vaccination and HeV challenge studies were conducted at the BSL-4 facilities of the Animal Health Laboratories (AAHL) in Geelong[267] (Table 14.5). HeV-sG was formulated in an approved equine adjuvant (Zoetis, Inc.) and two initial efficacy studies in horses tested 50 µg and 100 µg doses of the same HeV-sG used in published animal studies was used to immunize horses. Additional studies used a 100 µg dose of HeV-sG produced in CHO cells (Zoetis, Inc.). Immunizations were two doses given intramuscularly 3-weeks apart. All horses in these efficacy studies were challenged oronasally with 2×10^6 $TCID_{50}$ of HeV. Seven horses were challenged at 28 days, and 3 horses were challenged 194 days, after the second immunization. All vaccinated horses remained clinically healthy following challenge, demonstrating protection with pre-challenge HeV neutralizing titers as low as 1:16.[267] There was no gross or histologic evidence of HeV infection in any of the vaccinated horses at study completion, and all tissues examined were negative for HeV antigen by immunohistochemistry, with no viral genome detected in any tissue. In 9 of 10 vaccinated horses, HeV nucleic acid was not detected in daily nasal, oral, or rectal swab specimens or from blood, urine, or feces samples collected before euthanasia, and no recoverable virus was present.[267]

<FIG14.16>

Equivac® HeV was launched in November 2012 on a Minor Use Permit by the regulatory authority, the Australian Pesticides and Veterinary Medicines Authority (APVMA), and is the first commercially developed and deployed vaccine against a BSL-4 agent and is currently the only licensed antiviral approach for henipavirus infection. All vaccinated horses receive a microchip and database is maintained, and in August, 2015 Equivac® HeV received full registration by the APVMA. To date, >640,000 doses of Equivac® HeV vaccine has been administered to >153,000 unique horses (Fig. 14.16B). Since vaccine release, laboratory confirmed HeV infections in horses (n=21) have only occurred in unvaccinated horses. The HeV-sG recombinant subunit vaccine for NiV and HeV is now in clinical development with support from the Coalition for Epidemic Preparedness Innovations (CEPI)[268] and Phase I clinical trials set to commence in 2019. Recent vaccination and challenge studies in nonhuman primates have shown that a single immunizing dose of HeV-sG in Allhydrogel™ can protect against HeV and NiV-Bangladesh challenge (Geisbert and Broder, unpublished) and together with the results of recombinant VSV-based NiV vaccines, these findings highlight their potential usefulness in an emergency-use or outbreak scenario.

<H2>Passive immunization

Initial passive immunization studies using either NiV G and F-specific polyclonal antiserums, or mouse monoclonal antibodies (mAbs) specific for the NiV or HeV G or F glycoproteins were shown to be protective in the hamster model.[92,251,269] HeV- and NiV-neutralizing human mAbs reactive to the G glycoproteins of both HeV and NiV have been developed using recombinant antibody technology.[270] One mAb, m102.4, possessed strong cross-reactive neutralizing activity against HeV and NiV and was later produced in an IgG1 format in a CHO-K1 cell line.[271] The m102.4 mAb epitope maps to the receptor binding site of G and engages G in a similar fashion as the ephrin receptors[272] (Figure 14.5). The m102.4 mAb can neutralize all HeV and NiV isolates tested, including HeV-1994, HeV-Redlands, NiV-Malaysia and NiV-Bangladesh.[87,273] NiV challenge experiments in the ferret demonstrated complete protection was possible by a single dose of m102.4 mAb administered by intravenous infusion 10 hrs following a lethal virus challenge.[87] The therapeutic efficacy of mAb m102.4 has also been examined in nonhuman primates against both NiV and HeV challenge as a post-exposure therapeutic reflective of a potential real life virus exposure scenario[189,190] (Table 14.5). In one study, AGMs were challenged intratracheally with HeV and following virus administration they were infused twice with m102.4 (~15 mg/kg) beginning at 10 hrs, 24 hrs or 72 hrs post-infection followed by a the second infusion ~48 hrs after the first. In this study, all subjects became infected following challenge, and all animals that received m102.4 survived whereas all controls succumbed to severe systemic disease 8 days following virus challenge. Animals in a 72 hrs treatment group did exhibit neurological signs but all recovered by day 16, but there was no evidence of HeV-specific pathology in any of the m102.4-treated animals, and no infectious HeV could be recovered from any tissues from any m102.4-treated subjects. A later study evaluated the efficacy of m102.4 against NiV-Malaysia challenge in AGMs, at several time points after virus exposure by intratracheal challenge including as late as the onset of clinical illness.[190] Here, animals were infused twice with m102.4 (15 mg/kg) beginning at either 1, 3, or 5 days following NiV-Malaysia challenge and again 48 hrs after the first dose. All animals became infected following virus exposure, and again all subjects that received m102.4 therapy survived the infection, whereas the untreated control subjects succumbed to disease between days 8 and 10 following virus challenge. The m102.4 mAb was also shown to be effective the nonhuman primate model against NiV-Bangladesh infection, although its therapeutic window was much shorter as compared to NiV-Malaysia infected subjects.[187] Taken together, these studies have shown that m102.4 mAb therapy can prevent HeV or NiV lethal disease in exposed subjects is the only effective post-exposure therapeutic tested *in vivo* in nonhuman primates. The mAb m102.4 has since been used by emergency protocol in 13 people in Australia and one in the U.S. because of significant risk of HeV or NiV infection.[250] The m102.4 antibody has completed a Phase I clinical trial in Australia, and has recently been supplied to Government of India and included in an international Nipah treatment protocol sponsored by the Indian Council of Medical Research (ICMR) with support from the NIAID, NIH, USA.

<H1>Perspective and Geographic Considerations

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The high virulence of the henipaviruses and the requirement for BSL-4 facilities have hampered investigations into the biology and pathogenesis of these novel paramyxoviruses. Recent investigations into the structure and function of henipavirus proteins expressed from cloned genes have provided insight into the functions of many henipavirus proteins in infected cells. It remains to be determined if all of the functional characteristics of the henipavirus proteins determined *in vitro* accurately reflect the role that they play in the cells of both terrestrial and chiropteran hosts.

Although significant strides and an expanded understanding of the henipaviruses have been made during the past two decades, many questions relating to the ecology and biology of henipaviruses remain unanswered. It is clear that Pteropus species of fruit bats are the major reservoir host of these viruses, but new studies have suggested that an even wider group of chiropteran species may host the known henipaviruses and certainly yet to be identified new henipavirus species. With the wide geographic range of Pteropus species as overlapping populations, extending from islands in the South Pacific through Australia, and southern Asia to Pakistan, and with additional species on islands off the eastern coast of Africa, together with the cross-reactive serologic evidence of henipavirus presence,(61),(81) it would seem that many other related viruses remain to be identified.(43),(56) The emergence of these and related viruses is probably associated with the destruction of the flying fox native habitats, driving the animals to seek food from orchards and ornamental trees in urban and periurban areas. Thus, with continued deforestation, undoubtedly further outbreaks of HeV, NiV, and novel related members of the genus will occur. The mechanisms by which henipaviruses are transmitted between fruit bats and maintained within their colonies, as well as the pathways leading to the infection of spillover hosts, remain to be elucidated.

<H1>References

1. Selvey LA, Wells RM, McCormack JG, et al. Infection of humans and horses by a newly described morbillivirus. *Med J Aust.* 1995;162(12):642-645.
2. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science.* 1995;268(5207):94-97.
3. O'Sullivan JD, Allworth AM, Paterson DL, et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet.* 1997;349(9045):93-95.
4. Rogers RJ, Douglas IC, Baldock FC, et al. Investigation of a second focus of equine morbillivirus infection in coastal Queensland. *Aust Vet J.* 1996;74(3):243-244.
5. Young PL, Halpin K, Selleck PW, et al. Serologic evidence for the presence in Pteropus bats of a paramyxovirus related to equine morbillivirus. *Emerg Infect Dis.* 1996;2(3):239-240.
6. Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol.* 2000;81(Pt 8):1927-1932.

7. Summary of Hendra virus incidents in horses. *Business Queensland* [Web Page]. 2018; https://www.business.qld.gov.au/industries/service-industries-professionals/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary. Accessed 20 Sep 2018.

8. Playford EG, McCall B, Smith G, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis.* 2010;16(2):219-223.

9. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. *Lancet.* 1999;354(9186):1257-1259.

10. Mohd Nor MN, Gan CH, Ong BL. Nipah virus infection of pigs in peninsular Malaysia. *Rev Sci Tech.* 2000;19(1):160-165.

11. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science.* 2000;288(5470):1432-1435.

12. Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. *Lancet.* 1999;354(9186):1253-1256.

13. Chua KB, Koh CL, Hooi PS, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect.* 2002;4(2):145-151.

14. Hsu VP, Hossain MJ, Parashar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis.* 2004;10(12):2082-2087.

15. Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis.* 2006;12(2):235-240.

16. Harit AK, Ichhpujani RL, Gupta S, et al. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. *Indian J Med Res.* 2006;123(4):553-560.

17. Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis.* 2018.

18. Ching PK, de los Reyes VC, Sucaldito MN, et al. Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis.* 2015;21(2):328-331.

19. Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. *Nat Commun.* 2014;5:5342.

20. Wang LF, Michalski WP, Yu M, et al. A novel P/V/C gene in a new member of the Paramyxoviridae family, which causes lethal infection in humans, horses, and other animals. *J Virol.* 1998;72(2):1482-1490.

21. Wang LF, Yu M, Hansson E, et al. The exceptionally large genome of Hendra virus: support for creation of a new genus within the family Paramyxoviridae. *J Virol.* 2000;74(21):9972-9979.

22. Harcourt BH, Tamin A, Halpin K, et al. Molecular characterization of the polymerase gene and genomic termini of Nipah virus. *Virology.* 2001;287(1):192-201.

23. Harcourt BH, Tamin A, Ksiazek TG, et al. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. *Virology.* 2000;271(2):334-349.

24. Wang L, Harcourt BH, Yu M, et al. Molecular biology of Hendra and Nipah viruses. *Microbes Infect.* 2001;3(4):279-287.

25. Lamb RA, Collins PL, Kolakofsky D, Parks GD. Family Paramyxoviridae. In: Fauquet CM, Mayo J, Maniloff J, Desselberger U, Ball LA, eds. *Virus Taxonomy: 8th Report of the International Committee on Taxonomy of Viruses.* San Diego: Elsevier Academic Press; 2005:655-668.

26. Amarasinghe GK, Arechiga Ceballos NG, Banyard AC, et al. Taxonomy of the order Mononegavirales: update 2018. *Arch Virol.* 2018;163(8):2283-2294.

27. Maes P, Amarasinghe GK, Ayllon MA, et al. Taxonomy of the order Mononegavirales: second update 2018. *Arch Virol.* 2019.

28. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog.* 2012;8(8):e1002836.

29. Drexler JF, Corman VM, Muller MA, et al. Bats host major mammalian paramyxoviruses. *Nat Commun.* 2012;3:796.

30. Wu Z, Yang L, Yang F, et al. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis.* 2014;20(6):1064-1066.

31. Goldsmith CS, Whistler T, Rollin PE, et al. Elucidation of Nipah virus morphogenesis and replication using ultrastructural and molecular approaches. *Virus Res.* 2003;92(1):89-98.

32. Hyatt AD, Zaki SR, Goldsmith CS, Wise TG, Hengstberger SG. Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. *Microbes Infect.* 2001;3(4):297-306.

33. Harcourt BH, Lowe L, Tamin A, et al. Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis.* 2005;11(10):1594-1597.

34. Halpin K, Bankamp B, Harcourt BH, Bellini WJ, Rota PA. Nipah virus conforms to the rule of six in a minigenome replication assay. *J Gen Virol.* 2004;85(Pt 3):701-707.

35. Rima BK, Balkema-Buschmann A, Dundon WG, et al. Family Paramyxoviridae Classification and features. *J Gen Virol.* 2019;in press.

36. Hino K, Sato H, Sugai A, Kato M, Yoneda M, Kai C. Downregulation of Nipah virus N mRNA occurs through interaction between its 3' untranslated region and hnRNP D. *J Virol.* 2013;87(12):6582-6588.

37. Michalski WP, Crameri G, Wang L, Shiell BJ, Eaton B. The cleavage activation and sites of glycosylation in the fusion protein of Hendra virus. *Virus Res.* 2000;69(2):83-93.

38. Lo MK, Harcourt BH, Mungall BA, et al. Determination of the henipavirus phosphoprotein gene mRNA editing frequencies and detection of the C, V and W proteins of Nipah virus in virus-infected cells. *J Gen Virol.* 2009;90(Pt 2):398-404.

39. Basler CF. Nipah and hendra virus interactions with the innate immune system. *Curr Top Microbiol Immunol.* 2012;359:123-152.

40. Parks GD, Alexander-Miller MA. Paramyxovirus activation and inhibition of innate immune responses. *J Mol Biol.* 2013;425(24):4872-4892.

41. Park A, Yun T, Vigant F, et al. Nipah Virus C Protein Recruits Tsg101 to Promote the Efficient Release of Virus in an ESCRT-Dependent Pathway. *PLoS Pathog.* 2016;12(5):e1005659.

42. Freiberg A, Dolores LK, Enterlein S, Flick R. Establishment and characterization of plasmid-driven minigenome rescue systems for Nipah virus: RNA polymerase I- and T7-catalyzed generation of functional paramyxoviral RNA. *Virology.* 2008;370(1):33-44.

43. Yun T, Park A, Hill TE, et al. Efficient reverse genetics reveals genetic determinants of budding and fusogenic differences between Nipah and Hendra viruses and enables real-time monitoring of viral spread in small animal models of henipavirus infection. *J Virol.* 2015;89(2):1242-1253.

44. Poch O, Blumberg BM, Bougueleret L, Tordo N. Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. *J Gen Virol.* 1990;71 (Pt 5):1153-1162.

45. Miller PJ, Boyle DB, Eaton BT, Wang LF. Full-length genome sequence of Mossman virus, a novel paramyxovirus isolated from rodents in Australia. *Virology.* 2003;317(2):330-344.

46. Liang B, Li Z, Jenni S, et al. Structure of the L Protein of Vesicular Stomatitis Virus from Electron Cryomicroscopy. *Cell.* 2015;162(2):314-327.

47. Lamb RA, Parks GD. *Paramyxoviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, eds. *Fields Virology.* Vol 6th Edition. Philadelphia: Lippincott Williams & Wilkins; 2013:957-995.

48. Langedijk JP, Daus FJ, van Oirschot JT. Sequence and structure alignment of Paramyxoviridae attachment proteins and discovery of enzymatic activity for a morbillivirus hemagglutinin. *J Virol.* 1997;71(8):6155-6167.

49. Lee B, Ataman ZA. Modes of paramyxovirus fusion: a Henipavirus perspective. *Trends Microbiol.* 2011;19(8):389-399.

50. Yu M, Hansson E, Langedijk JP, Eaton BT, Wang LF. The attachment protein of Hendra virus has high structural similarity but limited primary sequence homology compared with viruses in the genus Paramyxovirus. *Virology.* 1998;251(2):227-233.

51. Bishop KA, Stantchev TS, Hickey AC, et al. Identification of Hendra virus G glycoprotein residues that are critical for receptor binding. *J Virol.* 2007;81(11):5893-5901.

52. Bonaparte MI, Dimitrov AS, Bossart KN, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A.* 2005;102(30):10652-10657.

53. Negrete OA, Levroney EL, Aguilar HC, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature.* 2005;436(7049):401-405.

54. Negrete OA, Wolf MC, Aguilar HC, et al. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog.* 2006;2(2):e7.

55. Lee B, Pernet O, Ahmed AA, Zeltina A, Beaty SM, Bowden TA. Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. *Proc Natl Acad Sci U S A.* 2015;112(17):E2156-2165.

56. Rissanen I, Ahmed AA, Azarm K, et al. Idiosyncratic Mojiang virus attachment glycoprotein directs a host-cell entry pathway distinct from genetically related henipaviruses. *Nat Commun.* 2017;8:16060.

57. Bowden TA, Aricescu AR, Gilbert RJ, Grimes JM, Jones EY, Stuart DI. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. *Nat Struct Mol Biol.* 2008;15(6):567-572.

58. Bowden TA, Crispin M, Harvey DJ, Jones EY, Stuart DI. Dimeric architecture of the Hendra virus attachment glycoprotein: evidence for a conserved mode of assembly. *J Virol.* 2010;84(12):6208-6217.

59. Xu K, Rajashankar KR, Chan YP, Himanen JP, Broder CC, Nikolov DB. Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. *Proc Natl Acad Sci U S A.* 2008;105(29):9953-9958.

60. Bose S, Jardetzky TS, Lamb RA. Timing is everything: Fine-tuned molecular machines orchestrate paramyxovirus entry. *Virology.* 2015;479-480:518-531.

61. Moll M, Diederich S, Klenk HD, Czub M, Maisner A. Ubiquitous activation of the Nipah virus fusion protein does not require a basic amino acid at the cleavage site. *J Virol.* 2004;78(18):9705-9712.

62. Pager CT, Dutch RE. Cathepsin L is involved in proteolytic processing of the Hendra virus fusion protein. *J Virol.* 2005;79(20):12714-12720.

63. Diederich S, Sauerhering L, Weis M, et al. Activation of the Nipah virus fusion protein in MDCK cells is mediated by cathepsin B within the endosome-recycling compartment. *J Virol.* 2012;86(7):3736-3745.

64. Kruger N, Hoffmann M, Weis M, et al. Surface glycoproteins of an African henipavirus induce syncytium formation in a cell line derived from an African fruit bat, Hypsignathus monstrosus. *J Virol.* 2013;87(24):13889-13891.

65. Pager CT, Craft WW, Jr., Patch J, Dutch RE. A mature and fusogenic form of the Nipah virus fusion protein requires proteolytic processing by cathepsin L. *Virology.* 2006;346(2):251-257.

66. Meulendyke KA, Wurth MA, McCann RO, Dutch RE. Endocytosis plays a critical role in proteolytic processing of the Hendra virus fusion protein. *J Virol.* 2005;79(20):12643-12649.

67. Vogt C, Eickmann M, Diederich S, Moll M, Maisner A. Endocytosis of the Nipah virus glycoproteins. *J Virol.* 2005;79(6):3865-3872.

68. Popa A, Carter JR, Smith SE, Hellman L, Fried MG, Dutch RE. Residues in the hendra virus fusion protein transmembrane domain are critical for endocytic recycling. *J Virol.* 2012;86(6):3014-3026.

69. Cifuentes-Munoz N, Sun W, Ray G, et al. Mutations in the Transmembrane Domain and Cytoplasmic Tail of Hendra Virus Fusion Protein Disrupt Virus-Like-Particle Assembly. *J Virol.* 2017;91(14).

70. Johnston GP, Contreras EM, Dabundo J, et al. Cytoplasmic Motifs in the Nipah Virus Fusion Protein Modulate Virus Particle Assembly and Egress. *J Virol.* 2017;91(10).

71. El Najjar F, Schmitt AP, Dutch RE. Paramyxovirus glycoprotein incorporation, assembly and budding: a three way dance for infectious particle production. *Viruses.* 2014;6(8):3019-3054.

72. Harrison MS, Sakaguchi T, Schmitt AP. Paramyxovirus assembly and budding: building particles that transmit infections. *Int J Biochem Cell Biol.* 2010;42(9):1416-1429.

73. Wang YE, Park A, Lake M, et al. Ubiquitin-regulated nuclear-cytoplasmic trafficking of the Nipah virus matrix protein is important for viral budding. *PLoS Pathog.* 2010;6(11):e1001186.

74. Monaghan P, Green D, Pallister J, et al. Detailed morphological characterisation of Hendra virus infection of different cell types using super-resolution and conventional imaging. *Virol J.* 2014;11:200.

75. Pentecost M, Vashisht AA, Lester T, et al. Evidence for ubiquitin-regulated nuclear and subnuclear trafficking among Paramyxovirinae matrix proteins. *PLoS Pathog.* 2015;11(3):e1004739.

76. McLinton EC, Wagstaff KM, Lee A, et al. Nuclear localization and secretion competence are conserved among henipavirus matrix proteins. *J Gen Virol.* 2017;98(4):563-576.

77. Bauer A, Neumann S, Karger A, et al. ANP32B is a nuclear target of henipavirus M proteins. *PLoS One.* 2014;9(5):e97233.

78. Hooper P, Zaki S, Daniels P, Middleton D. Comparative pathology of the diseases caused by Hendra and Nipah viruses. *Microbes Infect.* 2001;3(4):315-322.

79. Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. *Emerg Infect Dis.* 2005;11(7):1042-1047.

80. Mills JN, Alim AN, Bunning ML, et al. Nipah virus infection in dogs, Malaysia, 1999. *Emerg Infect Dis.* 2009;15(6):950-952.

81. Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J. The natural history of Hendra and Nipah viruses. *Microbes Infect.* 2001;3(4):307-314.

82. Kirkland PD, Gabor M, Poe I, et al. Hendra Virus Infection in Dog, Australia, 2013. *Emerg Infect Dis.* 2015;21(12):2182-2185.

83. Middleton DJ, Riddell S, Klein R, et al. Experimental Hendra virus infection of dogs: virus replication, shedding and potential for transmission. *Aust Vet J.* 2017;95(1-2):10-18.

84. Bossart KN, Wang LF, Eaton BT, Broder CC. Functional expression and membrane fusion tropism of the envelope glycoproteins of Hendra virus. *Virology.* 2001;290(1):121-135.

85. Bossart KN, Wang LF, Flora MN, et al. Membrane fusion tropism and heterotypic functional activities of the Nipah virus and Hendra virus envelope glycoproteins. *J Virol.* 2002;76(22):11186-11198.

86. Tamin A, Harcourt BH, Ksiazek TG, Rollin PE, Bellini WJ, Rota PA. Functional properties of the fusion and attachment glycoproteins of Nipah virus. *Virology.* 2002;296(1):190-200.

87. Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute nipah virus infection. *PLoS Pathog.* 2009;5(10):e1000642.

88. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One.* 2010;5(5):e10690.

89. Marianneau P, Guillaume V, Wong T, et al. Experimental infection of squirrel monkeys with nipah virus. *Emerg Infect Dis.* 2010;16(3):507-510.

90. Middleton DJ, Morrissy CJ, van der Heide BM, et al. Experimental Nipah virus infection in pteropid bats (Pteropus poliocephalus). *J Comp Pathol.* 2007;136(4):266-272.

91. Wong KT, Grosjean I, Brisson C, et al. A golden hamster model for human acute Nipah virus infection. *Am J Pathol.* 2003;163(5):2127-2137.

92. Guillaume V, Wong KT, Looi RY, et al. Acute Hendra virus infection: Analysis of the pathogenesis and passive antibody protection in the hamster model. *Virology.* 2009;387(2):459-465.

93. Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. *J Virol.* 2010;84(19):9831-9839.

94. Williamson MM, Hooper PT, Selleck PW, Westbury HA, Slocombe RF. A guinea-pig model of Hendra virus encephalitis. *J Comp Pathol.* 2001;124(4):273-279.

95. Williamson MM, Hooper PT, Selleck PW, et al. Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. *Aust Vet J.* 1998;76(12):813-818.

96. Pallister J, Middleton D, Wang LF, et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine.* 2011;29(34):5623-5630.

97. Chua KB, Lam SK, Goh KJ, et al. The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. *J Infect.* 2001;42(1):40-43.

98. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med.* 2000;342(17):1229-1235.

99. Islam MS, Sazzad HM, Satter SM, et al. Nipah Virus Transmission from Bats to Humans Associated with Drinking Traditional Liquor Made from Date Palm Sap, Bangladesh, 2011-2014. *Emerg Infect Dis.* 2016;22(4):664-670.

100. Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. *Emerg Infect Dis.* 2006;12(12):1888-1894.

101. Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector Borne Zoonotic Dis.* 2012;12(1):65-72.

102. Clayton BA, Middleton D, Arkinstall R, Frazer L, Wang LF, Marsh GA. The Nature of Exposure Drives Transmission of Nipah Viruses from Malaysia and Bangladesh in Ferrets. *PLoS Negl Trop Dis.* 2016;10(6):e0004775.

103. Edson D, Field H, McMichael L, et al. Routes of Hendra Virus Excretion in Naturally-Infected Flying-Foxes: Implications for Viral Transmission and Spillover Risk. *PLoS One.* 2015;10(10):e0140670.

104. Middleton DJ, Weingartl HM. Henipaviruses in their natural animal hosts. *Curr Top Microbiol Immunol.* 2012;359:105-121.

105. Homaira N, Rahman M, Hossain MJ, et al. Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One.* 2010;5(10):e13570.

106. Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. *Malays J Pathol.* 2010;32(2):69-73.

107. Luby SP, Gurley ES, Hossain MJ. Transmission of human infection with Nipah virus. *Clin Infect Dis.* 2009;49(11):1743-1748.

108. Fogarty R, Halpin K, Hyatt AD, Daszak P, Mungall BA. Henipavirus susceptibility to environmental variables. *Virus Res.* 2008;132(1-2):140-144.

109. Wacharapluesadee S, Hemachudha T. Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats. *J Virol Methods.* 2007;141(1):97-101.

110. Looi LM, Chua KB. Lessons from the Nipah virus outbreak in Malaysia. *Malays J Pathol.* 2007;29(2):63-67.

111. Middleton DJ, Westbury HA, Morrissy CJ, et al. Experimental Nipah virus infection in pigs and cats. *J Comp Pathol.* 2002;126(2-3):124-136.

112. Chakraborty A, Sazzad HM, Hossain MJ, et al. Evolving epidemiology of Nipah virus infection in Bangladesh: evidence from outbreaks during 2010-2011. *Epidemiol Infect.* 2016;144(2):371-380.

113. Hegde ST, Sazzad HM, Hossain MJ, et al. Investigating Rare Risk Factors for Nipah Virus in Bangladesh: 2001-2012. *Ecohealth.* 2016;13(4):720-728.

114. Blum LS, Khan R, Nahar N, Breiman RF. In-depth assessment of an outbreak of Nipah encephalitis with person-to-person transmission in Bangladesh: implications for prevention and control strategies. *Am J Trop Med Hyg.* 2009;80(1):96-102.

115. Sazzad HM, Hossain MJ, Gurley ES, et al. Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. *Emerg Infect Dis.* 2013;19(2):210-217.

116. Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. *Emerg Infect Dis.* 2007;13(7):1031-1037.

117. Luby SP, Gurley ES. Epidemiology of henipavirus disease in humans. *Curr Top Microbiol Immunol.* 2012;359:25-40.

118. Field HE. Hendra virus ecology and transmission. *Curr Opin Virol.* 2016;16:120-125.

119. Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. *Ann Neurol.* 2002;51(6):703-708.

120. Wong KT, Tan CT. Clinical and pathological manifestations of human henipavirus infection. *Curr Top Microbiol Immunol.* 2012;359:95-104.

121. Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. *Curr Top Microbiol Immunol.* 2012;359:153-177.

122. de Wit E, Munster VJ. Animal models of disease shed light on Nipah virus pathogenesis and transmission. *J Pathol.* 2015;235(2):196-205.

123. Baseler L, Scott DP, Saturday G, et al. Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. *PLoS Negl Trop Dis.* 2016;10(11):e0005120.

124. Hammoud DA, Lentz MR, Lara A, et al. Aerosol exposure to intermediate size Nipah virus particles induces neurological disease in African green monkeys. *PLoS Negl Trop Dis.* 2018;12(11):e0006978.

125. Lambrecht BN, Hammad H. Lung dendritic cells in respiratory viral infection and asthma: from protection to immunopathology. *Annu Rev Immunol.* 2012;30:243-270.

126. Wong KT, Shieh WJ, Kumar S, et al. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol.* 2002;161(6):2153-2167.

127. Wong KT, Shieh WJ, Zaki SR, Tan CT. Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer Semin Immunopathol.* 2002;24(2):215-228.

128. Rockx B, Brining D, Kramer J, et al. Clinical outcome of henipavirus infection in hamsters is determined by the route and dose of infection. *J Virol.* 2011;85(15):7658-7671.

129. Weingartl H, Czub S, Copps J, et al. Invasion of the central nervous system in a porcine host by nipah virus. *J Virol.* 2005;79(12):7528-7534.

130. Wong KT, Robertson T, Ong BB, et al. Human Hendra virus infection causes acute and relapsing encephalitis. *Neuropathol Appl Neurobiol.* 2009;35(3):296-305.

131. Munster VJ, Prescott JB, Bushmaker T, et al. Rapid Nipah virus entry into the central nervous system of hamsters via the olfactory route. *Sci Rep.* 2012;2:736.

132. Borisevich V, Ozdener MH, Malik B, Rockx B. Hendra and Nipah Virus Infection in Cultured Human Olfactory Epithelial Cells. *mSphere.* 2017;2(3).

133. Miao H, Wang B. Eph/ephrin signaling in epithelial development and homeostasis. *Int J Biochem Cell Biol.* 2009;41(4):762-770.

134. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell.* 2008;133(1):38-52.

135. Barquilla A, Pasquale EB. Eph receptors and ephrins: therapeutic opportunities. *Annu Rev Pharmacol Toxicol.* 2015;55:465-487.

136. Bennett KM, Afanador MD, Lal CV, et al. Ephrin-B2 reverse signaling increases alpha5beta1 integrin-mediated fibronectin deposition and reduces distal lung compliance. *Am J Respir Cell Mol Biol.* 2013;49(4):680-687.

137. Genotype-Tissue Expression (GTEx) Portal: dbGaP Accession phs000424.v7.p2. The Broad Institute of MIT and Harvard; 2019. https://gtexportal.org/home/. Accessed 01/31/2019.

138. Gale NW, Baluk P, Pan L, et al. Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. *Dev Biol.* 2001;230(2):151-160.

139. Shin D, Garcia-Cardena G, Hayashi S, et al. Expression of ephrinB2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. *Dev Biol.* 2001;230(2):139-150.

140. Mathieu C, Pohl C, Szecsi J, et al. Nipah virus uses leukocytes for efficient dissemination within a host. *J Virol.* 2011;85(15):7863-7871.

141. Mathieu C, Dhondt KP, Chalons M, et al. Heparan sulfate-dependent enhancement of henipavirus infection. *MBio.* 2015;6(2):e02427.

142. Arunkumar G, Devadiga S, McElroy AK, et al. Adaptive immune responses in humans during Nipah virus acute and convalescent phases of infection. *Clin Infect Dis.* 2019.

143. Chua KB. Nipah virus outbreak in Malaysia. *J Clin Virol.* 2003;26(3):265-275.

144. Taylor C, Playford EG, McBride WJ, McMahon J, Warrilow D. No evidence of prolonged Hendra virus shedding by 2 patients, Australia. *Emerg Infect Dis.* 2012;18(12):2025-2027.

145. Andrejeva J, Childs KS, Young DF, et al. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc Natl Acad Sci U S A.* 2004;101(49):17264-17269.

146. Levy DE, Marie IJ. RIGging an antiviral defense--it's in the CARDs. *Nat Immunol.* 2004;5(7):699-701.

147. Jensen S, Thomsen AR. Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion. *J Virol.* 2012;86(6):2900-2910.

148. Matsumoto M, Oshiumi H, Seya T. Antiviral responses induced by the TLR3 pathway. *Rev Med Virol.* 2011;21(2):67-77.

149. Parisien JP, Bamming D, Komuro A, et al. A shared interface mediates paramyxovirus interference with antiviral RNA helicases MDA5 and LGP2. *J Virol.* 2009;83(14):7252-7260.

150. Childs KS, Andrejeva J, Randall RE, Goodbourn S. Mechanism of mda-5 Inhibition by paramyxovirus V proteins. *J Virol.* 2009;83(3):1465-1473.

151. Shaw ML, Garcia-Sastre A, Palese P, Basler CF. Nipah virus V and W proteins have a common STAT1-binding domain yet inhibit STAT1 activation from the cytoplasmic and nuclear compartments, respectively. *J Virol.* 2004;78(11):5633-5641.

152. Sanchez-Aparicio MT, Feinman LJ, Garcia-Sastre A, Shaw ML. Paramyxovirus V Proteins Interact with the RIG-I/TRIM25 Regulatory Complex and Inhibit RIG-I Signaling. *J Virol.* 2018;92(6).

153. Shaw ML, Cardenas WB, Zamarin D, Palese P, Basler CF. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. *J Virol.* 2005;79(10):6078-6088.

154. Bharaj P, Wang YE, Dawes BE, et al. The Matrix Protein of Nipah Virus Targets the E3-Ubiquitin Ligase TRIM6 to Inhibit the IKKepsilon Kinase-Mediated Type-I IFN Antiviral Response. *PLoS Pathog.* 2016;12(9):e1005880.

155. Aaronson DS, Horvath CM. A road map for those who don't know JAK-STAT. *Science.* 2002;296(5573):1653-1655.

156. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol.* 2005;5(5):375-386.

157. Rodriguez JJ, Wang LF, Horvath CM. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. *J Virol.* 2003;77(21):11842-11845.

158. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol.* 2002;76(22):11476-11483.

159. Ciancanelli MJ, Volchkova VA, Shaw ML, Volchkov VE, Basler CF. Nipah virus sequesters inactive STAT1 in the nucleus via a P gene-encoded mechanism. *J Virol.* 2009;83(16):7828-7841.

160. Rodriguez JJ, Cruz CD, Horvath CM. Identification of the nuclear export signal and STAT-binding domains of the Nipah virus V protein reveals mechanisms underlying interferon evasion. *J Virol.* 2004;78(10):5358-5367.

161. Ludlow LE, Lo MK, Rodriguez JJ, Rota PA, Horvath CM. Henipavirus V protein association with Polo-like kinase reveals functional overlap with STAT1 binding and interferon evasion. *J Virol.* 2008;82(13):6259-6271.

162. Smith KM, Tsimbalyuk S, Edwards MR, et al. Structural basis for importin alpha 3 specificity of W proteins in Hendra and Nipah viruses. *Nat Commun.* 2018;9(1):3703.

163. Sugai A, Sato H, Takayama I, Yoneda M, Kai C. Nipah and Hendra Virus Nucleoproteins Inhibit Nuclear Accumulation of Signal Transducer and Activator of Transcription 1 (STAT1) and STAT2 by Interfering with Their Complex Formation. *J Virol.* 2017;91(21).

164. Park MS, Shaw ML, Munoz-Jordan J, et al. Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus V, W, and C proteins. *J Virol.* 2003;77(2):1501-1511.

165. Lo MK, Peeples ME, Bellini WJ, Nichol ST, Rota PA, Spiropoulou CF. Distinct and overlapping roles of Nipah virus P gene products in modulating the human endothelial cell antiviral response. *PLoS One.* 2012;7(10):e47790.

166. Yoneda M, Guillaume V, Sato H, et al. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One.* 2010;5(9):e12709.

167. Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. *Antimicrob Agents Chemother.* 2006;50(5):1768-1772.

168. Virtue ER, Marsh GA, Wang LF. Interferon signaling remains functional during henipavirus infection of human cell lines. *J Virol.* 2011;85(8):4031-4034.

169. Virtue ER, Marsh GA, Baker ML, Wang LF. Interferon production and signaling pathways are antagonized during henipavirus infection of fruit bat cell lines. *PLoS One.* 2011;6(7):e22488.

170. Satterfield BA, Cross RW, Fenton KA, et al. The immunomodulating V and W proteins of Nipah virus determine disease course. *Nat Commun.* 2015;6:7483.

171. Rockx B. Recent developments in experimental animal models of Henipavirus infection. *Pathog Dis.* 2014;71(2):199-206.

172. Mathieu C, Guillaume V, Volchkova VA, et al. Nonstructural Nipah virus C protein regulates both the early host proinflammatory response and viral virulence. *J Virol.* 2012;86(19):10766-10775.

173. Satterfield BA, Cross RW, Fenton KA, et al. Nipah Virus C and W Proteins Contribute to Respiratory Disease in Ferrets. *J Virol.* 2016;90(14):6326-6343.

174. Chong HT, Kunjapan SR, Thayaparan T, et al. Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. *Can J Neurol Sci.* 2002;29(1):83-87.

175. Cong Y, Lentz MR, Lara A, et al. Loss in lung volume and changes in the immune response demonstrate disease progression in African green monkeys infected by small-particle aerosol and intratracheal exposure to Nipah virus. *PLoS Negl Trop Dis.* 2017;11(4):e0005532.

176. Pickering BS, Hardham JM, Smith G, et al. Protection against henipaviruses in swine requires both, cell-mediated and humoral immune response. *Vaccine.* 2016;34(40):4777-4786.

177. Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. *Vaccine.* 2016;34(26):2971-2975.

178. Gupta M, Lo MK, Spiropoulou CF. Activation and cell death in human dendritic cells infected with Nipah virus. *Virology.* 2013;441(1):49-56.

179. Berhane Y, Weingartl HM, Lopez J, et al. Bacterial infections in pigs experimentally infected with Nipah virus. *Transbound Emerg Dis.* 2008;55(3-4):165-174.

180. Leon AJ, Borisevich V, Boroumand N, et al. Host gene expression profiles in ferrets infected with genetically distinct henipavirus strains. *PLoS Negl Trop Dis.* 2018;12(3):e0006343.

181. Barclay AJ, Paton DJ. Hendra (equine morbillivirus). *Vet J.* 2000;160(3):169-176.

182. McCormack JG, Allworth AM, Selvey LA, Selleck PW. Transmissibility from horses to humans of a novel paramyxovirus, equine morbillivirus (EMV). *J Infect.* 1999;38(1):22-23.

183. Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001-2007. *Emerg Infect Dis.* 2009;15(8):1229-1235.

184. Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect.* 2010;138(11):1630-1636.

185. Mounts AW, Kaur H, Parashar UD, et al. A cohort study of health care workers to assess nosocomial transmissibility of Nipah virus, Malaysia, 1999. *J Infect Dis.* 2001;183(5):810-813.

186. DeBuysscher BL, de Wit E, Munster VJ, Scott D, Feldmann H, Prescott J. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. *PLoS Negl Trop Dis.* 2013;7(1):e2024.

187. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep.* 2016;6:30916.

188. Rockx B, Winegar R, Freiberg AN. Recent progress in henipavirus research: molecular biology, genetic diversity, animal models. *Antiviral Res.* 2012;95(2):135-149.

189. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med.* 2011;3(105):105ra103.

190. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med.* 2014;6(242):242ra282.

191. Lieu KG, Marsh GA, Wang LF, Netter HJ. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral Res.* 2015;124:69-76.

192. Sarji SA, Abdullah BJ, Goh KJ, Tan CT, Wong KT. MR imaging features of Nipah encephalitis. *AJR Am J Roentgenol.* 2000;175(2):437-442.

193. Wong SC, Ooi MH, Wong MN, Tio PH, Solomon T, Cardosa MJ. Late presentation of Nipah virus encephalitis and kinetics of the humoral immune response. *J Neurol Neurosurg Psychiatry.* 2001;71(4):552-554.

194. Chua KB, Lam SK, Tan CT, et al. High mortality in Nipah encephalitis is associated with presence of virus in cerebrospinal fluid. *Ann Neurol.* 2000;48(5):802-805.

195. Griffin DE, Lin WW, Nelson AN. Understanding the causes and consequences of measles virus persistence. *F1000Res.* 2018;7:237.

196. Watanabe S, Shirogane Y, Sato Y, Hashiguchi T, Yanagi Y. New Insights into Measles Virus Brain Infections. *Trends Microbiol.* 2019;27(2):164-175.

197. Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. *J Infect Dis.* 2000;181(5):1755-1759.

198. Field HE, Mackenzie JS, Daszak P. Henipaviruses: emerging paramyxoviruses associated with fruit bats. *Curr Top Microbiol Immunol.* 2007;315:133-159.

199. Luby SP. The pandemic potential of Nipah virus. *Antiviral Res.* 2013;100(1):38-43.

200. Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. *J Infect Dis.* 2000;181(5):1760-1763.

201. Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerg Infect Dis.* 2001;7(3):439-441.

202. Anderson DE, Islam A, Crameri G, et al. Isolation and Full-Genome Characterization of Nipah Viruses from Bats, Bangladesh. *Emerg Infect Dis.* 2019;25(1):166-170.

203. Epstein JH, Prakash V, Smith CS, et al. Henipavirus infection in fruit bats (Pteropus giganteus), India. *Emerg Infect Dis.* 2008;14(8):1309-1311.

204. Rahman SA, Hassan SS, Olival KJ, et al. Characterization of Nipah virus from naturally infected Pteropus vampyrus bats, Malaysia. *Emerg Infect Dis.* 2010;16(12):1990-1993.

205. Eaton BT, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol.* 2006;4(1):23-35.

206. Williamson MM, Hooper PT, Selleck PW, Westbury HA, Slocombe RF. Experimental hendra virus infectionin pregnant guinea-pigs and fruit Bats (Pteropus poliocephalus). *J Comp Pathol.* 2000;122(2-3):201-207.

207. Selvey L, Taylor R, Arklay A, Gerrard J. Screening of bat carers for antibodies to equine morbillivirus. *Commun Dis Intell* 1996;20:477-478.

208. Khan MS, Hossain J, Gurley ES, Nahar N, Sultana R, Luby SP. Use of infrared camera to understand bats' access to date palm sap: implications for preventing Nipah virus transmission. *Ecohealth.* 2010;7(4):517-525.

209. Chowdhury S, Khan SU, Crameri G, et al. Serological evidence of henipavirus exposure in cattle, goats and pigs in Bangladesh. *PLoS Negl Trop Dis.* 2014;8(11):e3302.

210. Montgomery JM, Hossain MJ, Gurley E, et al. Risk factors for Nipah virus encephalitis in Bangladesh. *Emerg Infect Dis.* 2008;14(10):1526-1532.

211. Mahalingam S, Herrero LJ, Playford EG, et al. Hendra virus: an emerging paramyxovirus in Australia. *Lancet Infect Dis.* 2012;12(10):799-807.

212. Hooper PT, Westbury HA, Russell GM. The lesions of experimental equine morbillivirus disease in cats and guinea pigs. *Vet Pathol.* 1997;34(4):323-329.

213. Murray K, Rogers R, Selvey L, et al. A novel morbillivirus pneumonia of horses and its transmission to humans. *Emerg Infect Dis.* 1995;1(1):31-33.

214. Arankalle VA, Bandyopadhyay BT, Ramdasi AY, et al. Genomic characterization of Nipah virus, West Bengal, India. *Emerg Infect Dis.* 2011;17(5):907-909.

215. Marsh GA, Todd S, Foord A, et al. Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. *Emerg Infect Dis.* 2010;16(11):1767-1769.

216. Chan YP, Chua KB, Koh CL, Lim ME, Lam SK. Complete nucleotide sequences of Nipah virus isolates from Malaysia. *J Gen Virol.* 2001;82(Pt 9):2151-2155.

217. Wacharapluesadee S, Samseeneam P, Phermpool M, et al. Molecular characterization of Nipah virus from Pteropus hypomelanus in Southern Thailand. *Virol J.* 2016;13:53.

218. Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. *Clin Infect Dis.* 2008;46(7):977-984.

219. Tan K-S, Sarji SA, Tan C-T, et al. Patients with asymptomatic Nipah virus infection may have abnormal cerebral MR imaging. *Neurol J Southeast Asia.* 2000;5:69-73.

220. Lim CC, Lee KE, Lee WL, et al. Nipah virus encephalitis: serial MR study of an emerging disease. *Radiology.* 2002;222(1):219-226.

221. Lee KE, Umapathi T, Tan CB, et al. The neurological manifestations of Nipah virus encephalitis, a novel paramyxovirus. *Ann Neurol.* 1999;46(3):428-432.

222. Lim CC, Lee WL, Leo YS, et al. Late clinical and magnetic resonance imaging follow up of Nipah virus infection. *J Neurol Neurosurg Psychiatry.* 2003;74(1):131-133.

223. Abdullah S, Chang LY, Rahmat K, Goh KJ, Tan CT. Late-onset Nipah virus encephalitis 11 years after the initial outbreak: A case report. *Neurol Asia.* 2012;17(1):71-74.

224. Broder CC, Wong KT. Henipaviruses. In: Reiss CS, ed. *Neurotropic Viral Infections.* Vol 1:Neurotropic RNA Viruses. 2 ed. Switzerland: Springer International Publishing; 2016:45-83.

225. Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. *Curr Top Microbiol Immunol.* 2012;359:179-196.

226. Imada T, Abdul Rahman MA, Kashiwazaki Y, Tanimura N, Syed Hassan S, Jamaluddin A. Production and characterization of monoclonal antibodies against formalin-inactivated Nipah virus isolated from the lungs of a pig. *J Vet Med Sci.* 2004;66(1):81-83.

227. Tanimura N, Imada T, Kashiwazaki Y, Shahirudin S, Sharifah SH, Aziz AJ. Monoclonal antibody-based immunohistochemical diagnosis of Malaysian Nipah virus infection in pigs. *J Comp Pathol.* 2004;131(2-3):199-206.

228. Tanimura N, Imada T, Kashiwazaki Y, et al. Reactivity of anti-Nipah virus monoclonal antibodies to formalin-fixed, paraffin-embedded lung tissues from experimental Nipah and Hendra virus infections. *J Vet Med Sci.* 2004;66(10):1263-1266.

229. White JR, Boyd V, Crameri GS, et al. Location of, immunogenicity of and relationships between neutralization epitopes on the attachment protein (G) of Hendra virus. *J Gen Virol.* 2005;86(Pt 10):2839-2848.

230. Smith IL, Halpin K, Warrilow D, Smith GA. Development of a fluorogenic RT-PCR assay (TaqMan) for the detection of Hendra virus. *J Virol Methods.* 2001;98(1):33-40.

231. Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). *J Virol Methods.* 2004;120(2):229-237.

232. Feldman KS, Foord A, Heine HG, et al. Design and evaluation of consensus PCR assays for henipaviruses. *J Virol Methods.* 2009;161(1):52-57.

233. Eshaghi M, Tan WS, Mohidin TB, Yusoff K. Nipah virus glycoprotein: production in baculovirus and application in diagnosis. *Virus Res.* 2004;106(1):71-76.

234. Kashiwazaki Y, Na YN, Tanimura N, Imada T. A solid-phase blocking ELISA for detection of antibodies to Nipah virus. *J Virol Methods.* 2004;121(2):259-261.

235. Bossart KN, McEachern JA, Hickey AC, et al. Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. *J Virol Methods.* 2007;142(1-2):29-40.

236. Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. *J Virol Methods.* 2009;160(1-2):7-13.

237. Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. *J Virol Methods.* 2012;179(1):226-232.

238. Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. *J Virol Methods.* 2009;160(1-2):1-6.

239. Wright PJ, Crameri G, Eaton BT. RNA synthesis during infection by Hendra virus: an examination by quantitative real-time PCR of RNA accumulation, the effect of ribavirin and the attenuation of transcription. *Arch Virol.* 2005;150(3):521-532.

240. Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. *Ann Neurol.* 2001;49(6):810-813.

241. Pager CT, Wurth MA, Dutch RE. Subcellular localization and calcium and pH requirements for proteolytic processing of the Hendra virus fusion protein. *J Virol.* 2004;78(17):9154-9163.

242. Porotto M, Orefice G, Yokoyama CC, et al. Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy. *J Virol.* 2009;83(10):5148-5155.

243. Anonymous. *Hendra virus, human, equine - Australia (05): Queensland.* International Society for Infectious Diseases; 10-Sep 2009. 20090910.3189.

244. Warren TK, Jordan R, Lo MK, et al. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature.* 2016;531(7594):381-385.

245. Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. *Sci Rep.* 2017;7:43395.

246. Freiberg AN, Worthy MN, Lee B, Holbrook MR. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. *J Gen Virol.* 2010;91(Pt 3):765-772.

247. Pallister J, Middleton D, Crameri G, et al. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. *J Virol.* 2009;83(22):11979-11982.

248. Eaton BT, Broder CC, Wang LF. Hendra and Nipah viruses: pathogenesis and therapeutics. *Curr Mol Med.* 2005;5(8):805-816.

249. Porotto M, Rockx B, Yokoyama CC, et al. Inhibition of Nipah virus infection in vivo: targeting an early stage of paramyxovirus fusion activation during viral entry. *PLoS Pathog.* 2010;6(10):e1001168.

250. Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. *Vaccine.* 2016;34(30):3525-3534.

251. Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. *J Virol.* 2004;78(2):834-840.

252. Weingartl HM, Berhane Y, Caswell JL, et al. Recombinant nipah virus vaccines protect pigs against challenge. *J Virol.* 2006;80(16):7929-7938.

253. Ploquin A, Szecsi J, Mathieu C, et al. Protection against henipavirus infection by use of recombinant adeno-associated virus-vector vaccines. *J Infect Dis.* 2013;207(3):469-478.

254. Yoneda M, Georges-Courbot MC, Ikeda F, et al. Recombinant measles virus vaccine expressing the Nipah virus glycoprotein protects against lethal Nipah virus challenge. *PLoS One.* 2013;8(3):e58414.

255. Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J.* 2013;10:353.

256. DeBuysscher BL, Scott D, Marzi A, Prescott J, Feldmann H. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. *Vaccine.* 2014;32(22):2637-2644.

257. Lo MK, Bird BH, Chattopadhyay A, et al. Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters. *Antiviral Res.* 2014;101:26-29.

258. Prescott J, DeBuysscher BL, Feldmann F, et al. Single-dose live-attenuated vesicular stomatitis virus-based vaccine protects African green monkeys from Nipah virus disease. *Vaccine.* 2015;33(24):2823-2829.

259. DeBuysscher BL, Scott D, Thomas T, Feldmann H, Prescott J. Peri-exposure protection against Nipah virus disease using a single-dose recombinant vesicular stomatitis virus-based vaccine. *NPJ Vaccines.* 2016;1.

260. Bossart KN, Crameri G, Dimitrov AS, et al. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol.* 2005;79(11):6690-6702.

261. Mungall BA, Middleton D, Crameri G, et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol.* 2006;80(24):12293-12302.

262. Colgrave ML, Snelling HJ, Shiell BJ, et al. Site occupancy and glycan compositional analysis of two soluble recombinant forms of the attachment glycoprotein of Hendra virus. *Glycobiology.* 2012;22(4):572-584.

263. McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine.* 2008;26(31):3842-3852.

264. Pallister JA, Klein R, Arkinstall R, et al. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J.* 2013;10:237.

265. Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci Transl Med.* 2012;4(146):146ra107.

266. Mire CE, Geisbert JB, Agans KN, et al. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol.* 2014;88(9):4624-4631.

267. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis.* 2014;20(3):372-379.

268. Plotkin SA. Vaccines for epidemic infections and the role of CEPI. *Hum Vaccin Immunother.* 2017;13(12):2755-2762.

269. Guillaume V, Contamin H, Loth P, et al. Antibody prophylaxis and therapy against Nipah virus infection in hamsters. *J Virol.* 2006;80(4):1972-1978.

270. Zhu Z, Dimitrov AS, Bossart KN, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. *J Virol.* 2006;80(2):891-899.

271. Zhu Z, Bossart KN, Bishop KA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J Infect Dis.* 2008;197(6):846-853.

272. Xu K, Rockx B, Xie Y, et al. Crystal structure of the Hendra virus attachment G glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog.* 2013;9(10):e1003684.

273. Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. *Curr Opin Virol.* 2012;2(2):176-187.

Figure legends

Figure 14.1. Syncytia induced in vero cells 24 hours after infection by Hendra virus (HeV) (A) and Nipah virus (NiV) (B). Methanol-fixed infected cells were labeled with rabbit monospecific antiserum to the HeV P protein and fluorescein-conjugated goat antirabbit immunoglobulin G. P protein is detected in extensive perinuclear ribonucleoprotein complexes (*small arrow*) and in discrete regularly shaped arrays (*large arrow*) distributed throughout the cytoplasm and believed to be sites of virus egress from the cell. Nuclei are indicated by *chevrons.*

Figure 14.2. A: Electron micrograph of Nipah virus (NiV)-infected Vero cells showing tubule-like structures, both in the cytoplasm and in a maturing virus particle (*arrows*). **B:** Electron micrograph of negatively stained Hendra virus (HeV) displaying the double fringe at the virus envelope (*small arrow*) and the herringbone nucleocapsids (*large arrow*). (Courtesy of Dr. Alex Hyatt, CSIRO Australian Animal Health Laboratory.)

Figure 14.3. Genome size and organization of Hendra virus in comparison with the type species in each of the other six genera in the family *Paramyxoviridae*. A: Genome length (in nucleotides) are given in brackets after each virus. Color coding: blue, proteins for the inner most nucleocapsid responsible for replication and transcription; green, envelope proteins. **B:** 3'UTR of certain genes in henipaviruses (N, P and G) are longer than their counterparts from representative members of paramyxovirus genera that infect mammals. The 3'UTR length (number of nucleotides) of each gene from representative members of the indicated genera is compared separately in box-and-whisker plots (N, P, M, F, RBP, and RdRP). P values are calculated based on one-tailed t-test (*Henipavirus* vs all other genera); five virus species from *Henipavirus* (HeV, NC_001906; NiV, NC_002728; CedV, NC_025351.1; GhV, NC_025256.1; MojV,

NC_025352.1), and three each from *Morbillivirus,* (MeV, NC_001498.1; CDV, NC_001921.1; FeMV, NC_039196.1), *Rubulavirus* (MuV, NC_002200.1; HPIV-2, NC_003443.1; PIV-5, NC_006430.1) and *Respirovirus* (SeV, NC_001552; HPIV-1, NC_003461; HPIV-3, NC_001796.2). Courtesy of Satoshi Ikegame and Christian Stevens, Icahn School of Medicine at Mount Sinai, New York.

Figure 14.4. The major proteins of Hendra virus and Nipah virus and their roles in the henipavirus life cycle. A: Viruses were purified and analyzed as described in Wang LF et al.[1] Major virus proteins and the molecular weights of standard marker proteins are shown on the right and left, respectively. Reproduced with permission from Wang LF et al, Molecular biology of Hendra and Nipah viruses. *Microbes Infect* 2001; 3:279-87**. B:** Henipavirus replication cycle is depicted. The virus receptor binding protein (RBP, deep blue lollipops) attaches the virus to the cell via its cognate receptor, typically ephrinB2/B3 **(1)**, and triggers virus-cell membrane fusion mediated by the viral fusion protein (F, filled orange trapezoids) **(2)**. Fusion delivers the nucleoprotein (N) encapsidated negative-sense virus RNA genome [vRNA(−), cyan] to the cytosol **(3).** The vRNA(-) serves as template for viral transcripts made by the transcriptase complex comprised of the phosphoprotein (P) and the large RNA-dependent RNA-polymerase (RdRP) (P-RdRP, transparent hexagons) **(4).** Viral transcripts are made following a 3′ to 5′ attenuation gradient. The vRNA(-) is also the template for making full-length antigenomic cRNA(+) **(5),** which in turn is a template for vRNA(−) synthesis during virus genome replication **(6).** Henipavirus genome replication mediated by the N-P-RdRP replicase complex is similar to the details described for the *Paramyxoviridae* in chapter 13. However, some aspects of henipavirus assembly are relatively unique. The matrix protein (M, green octagons) needs to transit through the nucleus **(7)** in a ubiquitin-regulated manner **(8),** in order to traffic properly to

the plasma membrane. This nuclear sojourn involves shuttling through subnuclear compartments such as nucleoli **(9).** Proper trafficking is required for M to orchestrate the assembly of the viral RNPs with the viral envelope glycoproteins into budding infectious virions bearing the vRNA(-) **(10).** Matrix also has non-structural functions such as antagonizing type-I IFN responses **(11);** see Fig. 14.8 and text for details. Additionally, in order for the henipavirus F protein to be cleaved and become fusion-competent, it needs to access cathepsins L/B in the endosomal compartment. To do this, F first reaches the plasma membrane via the secreory pathway in an uncleaved form (open orange trapezoids) **(12).** Subsequent endocytosis and recycling back to the plasma membrane **(12a)** is required to form infectious virions where cleaved fusion-competent F (filled orange trapezoids) **(12b)** is complexed with the RBP in its proper oligomeric state. Where this occurs along the vesicular trafficking pathway and/or the plasma membrane is unresolved **(13).** F protein cleavage is seldom complete and uncleaved F can be detected in budded virions **(14)** (Adapted from Aguilar HC and Lee B. Emerging paramyxoviruses: molecular mechanisms and antiviral strategies. *Expert Rev Mol Med* 2011;13:e6)

Figure 14.5. A structure-based phylogeny of paramyxoviral receptor binding proteins. Structure-based phylogenetic analysis on extant structures of all henipavirus RBPs and representative RBPs from at least one virus in other paramyxovirus genera for which structural data is available. RBPs are shown besides the tree and are rendered as surface representation (grey). All receptor-binding proteins, with the exception of the MojV-G, are shown in complex with their respective receptor molecules (yellow), with the receptor-binding interface colored red. Analysis was performed and produced as described in Rissanen et al.[2] Paramyxoviral RBPs generally cluster according to their respective genera and cellular receptor usage with the

exception MojV-RBP, which is almost equidistant to all structurally studied genera. Courtesy of Dr. Thomas Bowden, University of Oxford, U.K.

Figure 14.6. Nipah virus transmission. Major pathways of human Nipah virus infection are summarized for Malaysia (blue arrows), Bangladesh (red arrows), the Philippines (green arrows), and the latest outbreak in Kerala, India (orange arrows). (Adapted from Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. *Curr Opin Virol* 2017; 22:97-104)

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Figure 14.7. Immunohistochemistry detection of Nipah virus (NiV) and Hendra virus (HeV) antigen in henipavirus-infected tissues. A-D: Hendra virus infected tissues. **A:** Staining of HeV antigen (N protein-specific rabbit antibody) within the wall of superficial arteriole in the sub-mucosa of the nasal cavity in a HeV-infected horse. **B:** Immmunolabeling (N protein-specific rabbit antibody) of the endothelium of medium caliber vessels and alveolar septae in lung of a HeV-infected African green monkey (AGM) (10x). **C:** Immunolabeling of HeV antigen (N protein-specific rabbit antibody) within neurons in the brainstem of a HeV-infected AGM (40x). **D:** Immunolabeling of HeV antigens (mouse anti-HeV polyclonal antibody) in neurons of acute HeV-infected human case (40x). **E:** Positive immunolabelling (using anti-Nipah virus rabbit serum) within the lung of a NiV-infected pig. Involvement of bronchial epithelium and airway debris is noted. **F:** Immunolabeling (N protein-specific antibody) of the endothelium of medium caliber vessels and alveolar septae in lung of a NiV-infected AGM (20x). **G:** Immunolabeling of NiV antigen (N protein-specific antibody) within neurons in the brainstem of a NiV-infected AGM (40x). **H:** Immunolabeling of NiV antigens (hyperimmune anti-NiV mouse ascitic fluid) in the cytoplasm and nuclei of neurons, and neuronal processes in the brain of acute NiV-infected human case (158x). A and E, courtesy of Dr. Deborah Middleton, CSIRO Australian Animal Health Laboratory. B, C, F and G, courtesy of Dr. Karla Fenton, University of

Texas Medical Branch at Galveston, Texas. D, courtesy of Dr. Kum Thong Wong, University of Malaya; H, from Wong et al.[3]

Figure 14.8. Ephrin-B2 and ephrin-B3 RNA-seq expression data across representative human non-disease tissues. Shown here are ephrin-B2 (upper) and ephrin-B3 (lower) expression data obtained from the Genotype-Tissue Expression (GTEx) Portal. The GTEx project is a public resource where up to 53 non-disease tissue sites obtained from nearly 1,000 individuals are subjected to RNA-seq by next-generation sequencing amongst other assays. Details on datasets can be found on https://gtexportal.org. Data from all available brain regions are shown. The non-brain tissues are chosen for illustrative purposes based on their relevance to henipavirus pathogenesis. Expression data in transcripts per million are plotted as box plots with the medians and 25th and 27th percentiles shown; points are displayed as outliers if they are above or below 1.5 times the interquartile range. 1a Artery – Aorta; 1b Artery – Coronary; 2a Brain – Amygdala; 2b Brain - Anterior cingulate cortex (BA24); 2c Brain - Caudate (basal ganglia); 2d Brain - Cerebellar Hemisphere; 2e Brain – Cerebellum; 2f Brain – Cortex; 2g Brain - Frontal Cortex (BA9); 2h Brain – Hippocampus; 2i Brain – Hypothalamus; 2j Brain - Nucleus accumbens (basal ganglia); 2k Brain - Putamen (basal ganglia); 2l Brain - Spinal cord (cervical c-1); 2m Brain - Substantia nigra; 3 Breast - Mammary Tissue; 4 Colon – Transverse; 5 Esophagus - Gastroesophageal Junction; Esophagus – Mucosa; 6 Heart - Left Ventricle; 7 Kidney – Cortex; 8 Liver; 9 Lung; 10 Minor Salivary Gland; 11 Spleen; 12 Whole Blood.

Figure 14.9. Innate immune evasion mechanisms of the Nipah virus N, P, V, W and M proteins. Left: The V and W proteins (green diamonds) block IFN-α/β expression by different mechanisms. Depicted are simplified versions of the RIG-I-like receptor (RLR) signaling (blue

arrows) and TLR3 signaling (black arrows) pathways. Both lead to activation of interferon regulatory factors 3 and 7 (IRF-3 and IRF-7) (dashed arrow). RIG-I, MDA5 and TLR3 can sense RNAs produced during viral infection. Activation of RIG-I or MDA5 leads to their interaction with the mitochondria-associated adapter protein MAVS (1). This leads to activation of the kinases IKKε and/or TBK-1 (2), which phosphorylate and activate IRF-3 and, when present, IRF-7 (3). For clarity, MAVS activation of NF-κB is not shown. Activated IRF-3 and IRF-7 translocate into and accumulate in the nucleus; both contribute to IFN-α/β gene transcription (4). TLR3 signals through adaptor protein TRIF to activate IKKε/TBK-1 (5). The cytoplasmic NiV and HeV V proteins interact with and inhibit MDA5, thereby inhibiting the induction of IFN-α/β expression early on in the pathway (a); however NiV V does not appear to inhibit TLR3 signaling. The nucleo-cytoplasmic shuttling NiV M protein (green hexagon), while it is in the cytoplasm, antagonizes induction of type-I IFNs by blocking IKKε activation (b). This prevents downstream phosphorylation and nuclear translocation of IRF3, which also leads to a failure of IFN-α/β induction. The nuclear W protein (green trapezoid) effectively blocks RLR and TLR3 activation of the IFN-β promoter by preventing the accumulation of phosphorylated IRF-3 (c). This function requires the nuclear localization of W. **Right:** The Nipah virus N, P, V and W proteins each block IFN signaling pathways at distinct steps (red arrows), ultimately leading to reduced expression of interferon- stimulated (antiviral) genes (ISGs). The P, V and W proteins can each inhibit the phosphorylation and activation of STAT1 and STAT2 in response to IFN-α/β (6) and block the phosphorylation and activation of STAT1 in response to IFN-γ. The inhibition of the IFN-α/β signaling pathway is depicted. The cytoplasmic P and V proteins interact with and inhibit STAT1 (d). The V protein also interacts with STAT2, in a STAT1-dependent manner. P and V prevent nuclear accumulation of pSTAT1 /pSTAT2 following IFN-α/β addition to cells and cause the STAT proteins to accumulate in a high molecular weight complex. The NiV and HeV N proteins (green oval) were recently reported to disrupt phospho-STAT1 complex formation (7), though not phosphorylation of STAT1 itself (e). Nonetheless, this N-mediated disruption of STAT complexes reduces nuclear accumulation of the activated STAT complexes required to stimulate ISG expression (8). The nuclear W protein relocalizes non-phosphorylated STAT1 from its typically cytoplasmic localization to the nucleus (f). STAT1 relocalized to the nucleus by W remains unphosphorylated and does not activate transcription of ISGs in response to IFN-α/β addition. (Adapted from Basler CF. Nipah and Hendra virus interactions with the innnate immune system. *Curr Top Microbiol Immunol* 2012; 359: 123-52)

Figure 14.10. Brain MR scans of acute Nipah (A) and relapsing Nipah encephalitis (B) showing hyperintense discrete and more confluent lesions, respectively, corresponding to discrete pathological lesions (C) and confluent lesions (D). H&E stains (C, D). Magnification, objective x4 (Reproduced with permission from Wong KT and Tan CT. Clnical and Pathological manifestations of human henipavirus infection. *Curr Top Microbiol Immunol* 2012; 359: 95-104).

Figure 14.11. Distribution of frugivorous bats belonging to various genera in the *Pteropodidae* family where evidence of henipavirus presence have been detected. The presence of henipavirus is graded into four levels with known outbreak (red), virus isolation (pink), molecular detection (blue), and antibody detection (green).

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Figure 14.12: Phylogenetic tree based on L-proteins of selected henipaviruses and type species of the other six genera in the family *Paramyxoviridae.* Tree was constructed by using Neighbor-Joining approach with 1,000 bootstrap replicates. Numbers at the nodes represent

bootstrap values. Scale bar indicates amino acid substitutions per site. Sequences are labelled with the following ordination when all relative information is available for any given genome sequence: Virus/Country/Host/Year/Strain (GenBank accession number). For example, NiV/BD/Human/2010/Faridpur(JN808864) represents a Nipah virus isolated in Bangladesh from humans in 2010 with Faridpur as its isolate name and JN808864 as its Genbank accession number. (Two accession numbers still missing from figure, pending Genbank release)

Figure 14.13. Chest radiographs of a Nipah virus (NiV) infected patient and Nipah virus (NiV) and Hendra virus (HeV) infected African green monkeys. A: Chest X-ray of patient infected with Nipah virus (Nipah-Bangladesh clade) from Kerala, India, 2018, at 6 days following onset of symptoms showing diffuse bilateral opacities covering the majority of the lung fields, consistent with acute respiratory distress syndrome (ARDS). **B:** Top, comparison of pre-challenge to Nipah virus (Nipah-Malaysia clade) infected African green monkey at 11 days post-challenge showing congestion and pneumonia with infiltrates on the lung fields. Bottom, comparison of pre-challenge to Hendra virus infected African green monkey at 8 days post-challenge showing diffuse interstitial infiltrates and pulmonary consolidation and severe respiratory distress. Panel A, courtesy of Prof. Govindakarnavar Arunkumar, Manipal Centre for Virus Research, Manipal Academy of Higher Education (deemed to be University), Karnataka State, India and Dr. Suresh Kumar. E.K, Malabar Institute of Medical Sciences, Kerala State, India. Panel B, top, from Geisbert et al.[4], bottom, from Rockx et al.[5] (reproduced with permission)

Figure 14.14. Magnetic resonance imaging scans of patients with Nipah virus (NiV) or Hendra virus (HeV) encephalitis. A: Left, patient with NiV encephalitis (Nipah-Bangladesh clade) from Kerala, India, 2018, at 6 days following onset of symptoms showing cortical and

subcortical hyperintense foci (arrow heads). Middle, patient with acute NiV encephalitis (Nipah-Malaysia clade) from Malaysia, 1999, showing multiple discrete hyperintense lesions in the grey and white matter. Right, patient with relapsed NiV encephalitis from Malaysia, 1999, showing confluent lesions involving primarily the cortical grey matter. **B:** Left, acute HeV encephalitis in a patient on day 18 of illness, showing cortical and subcortical hyperintense foci. Right, relapsed HeV encephalitis in a patient on day 14 following symptoms onset showing widespread multifocal neocortical involvement. Panel A, left (India), courtesy of Prof. Govindakarnavar Arunkumar, Manipal Centre for Virus Research, Manipal Academy of Higher Education (deemed to be University), Karnataka State, India and Dr. Suresh Kumar. E.K, Malabar Institute of Medical Sciences, Kerala State, India. A, middle and right (Malaysia) from Goh, et al.[6] B, left, from Playford et al.[7]; right, from O'Sullivan et al.[8] (reproduced with permission)

Figure 14.15. Generalized understanding of infection and clinical features of human Nipah virus (NiV) and Hendra virus (HeV) infection. **A:** Transmission is generally accepted to occur via the oronasal route presumably by virus contaminated respiratory secretions or droplets from infected animals or people, or liquid/food (palm sap/fruit); **B:** The initial site of replication in humans is ill-defined but is thought to occur within the respiratory system and disease onset is characterized by fever, myalgia, shortness of breath, cough, which may progress to an acute respiratory distress syndrome (ARDS); **C:** Possible invasion into the central nervous system (CNS) via the olfactory bulb has also been suggested and has been demonstrated in animal models; **D:** Virus replication and hematogenous systemic spread occurs (cell-free or cell-associated viremia) and established infection is characterized by a widespread vasculitis with endothelial and smooth muscle cell tropism resulting in multinucleated syncytial cells; **E:** CNS infection and encephalitis results in altered sensorium, headache, vomiting, and seizures along

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with fever and other systemic symptoms; **F:** Infection of other organs in addition to lung and brain in human cases occurs and has been reported in kidney, heart, spleen and lymphoid tissues, and similar widespread infection has been demonstrated in multiple animal models.

Figure 14.16. Model of the Hendra virus soluble G glycoprotein subunit vaccine (HeV-sG) and its complex with the Hendra and Nipah virus neutralizing human monoclonal antibody m102.4. A: The HeV-sG glycoprotein subunit vaccine is composed of the entire ectodomain (amino acids 76-604) of the HeV G glycoprotein. HeV-sG is shown as dimer with one monomer colored green and the other cyan. The secondary structure elements of the two globular head domains are derived from the crystal structure of the HeV G head domain, the stalk regions of each G monomer (residues 77-136) are modeled, and N-linked glycosylation sites shown as gray spheres. The ephrin-binding face of the cyan globular head is facing forward with an overlay of the interacting ephrin-B2 G-H loop residues in yellow. **B:** The HeV-sG dimer is modeled in complex with two m102.4 Fab antibody fragments. The two HeV-sG monomers are colored green and cyan as in panel A and rotated slightly to the right, and the two Fab molecules are shown with their heavy chains colored in magenta and light chain – in yellow, each binding one globular head domains of G. Modified from Broder et al.[9]

Figure 14.17. One Health Vaccine strategy. A: The known modes of henipavirus transmission in different countries. In Australia, all seven human cases were infected via contacting infected horses. A One Health vaccine strategy was developed for immunization of horses with the dual purposes of saving horses from lethal Hendra virus infection and prevention of transmission from bats to humans via horses. **B:** Location densities of Equivac® HeV vaccine administered to horses around Australia, courtesy of Dr. Richard L'Estrange, Zoetis Inc.

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1. Wang LF, Michalski WP, Yu M, et al. A novel P/V/C gene in a new member of the Paramyxoviridae family, which causes lethal infection in humans, horses, and other animals. *J Virol.* 1998;72(2):1482-1490.
2. Rissanen I, Ahmed AA, Azarm K, et al. Idiosyncratic Mojiang virus attachment glycoprotein directs a host-cell entry pathway distinct from genetically related henipaviruses. *Nat Commun.* 2017;8:16060.
3. Wong KT, Shieh WJ, Kumar S, et al. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol.* 2002;161(6):2153-2167.
4. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. *PLoS One.* 2010;5(5):e10690.
5. Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. *J Virol.* 2010;84(19):9831-9839.
6. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med.* 2000;342(17):1229-1235.
7. Playford EG, McCall B, Smith G, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. *Emerg Infect Dis.* 2010;16(2):219-223.
8. O'Sullivan JD, Allworth AM, Paterson DL, et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet.* 1997;349(9045):93-95.
9. Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. *Antiviral Res.* 2013;100(1):8-13.

TABLE 14.1A. Summary of Hendra Virus Outbreaks in Horses in Australia

Year	Month	State[1]	Location	Total Cases[2]
1994	Aug	QLD	Mackay	2
1994	Sep	QLD	Hendra	20
1999	Jan	QLD	Cairns	1
2004	Oct	QLD	Cairns	1
	Dec	QLD	Townsville	1
2006	Jun	QLD	Peachester	1
	Oct	NSW	Murwillumbah	1
2007	Jun	QLD	Peachester	1
	Jul	QLD	Cairns	1
2008	Jun	QLD	Redlands	8
	Jul	QLD	Propserpine	4
2009	Jul	QLD	Cawarral	4
	Sep	QLD	Bowen	2
2010	May	QLD	Tewantin	1
2011	Jun	QLD	Beaudesert	1
		QLD	Boonah	3
		QLD	Logan	1
		NSW	Wollongbar	2
	Jul	QLD	Park Ridge	1
		NSW	Macksville	1
		QLD	Kuranda	1
		NSW	Lismore	1
		QLD	Hervey Bay	1
		QLD	Boondall	1
		QLD	Chinchilla	1
		NSW	Mulumbimby	1
	Aug	NSW	South Ballina	1
		NSW	South Balinna	2
		NSW	Mullumbinby	1
		QLD	Gold Coast Hinterland	1
		NSW	North Ballina	1
	Oct	QLD	Beachmere	3
2012	Jan	QLD	Townsville	1
	May	QLD	Rockhampton	1
		QLD	Ingham	1
	Jun	QLD	Mackay	1
	Jul	QLD	Rockhampton	3
		QLD	Cairns	1
	Sep	QLD	Port Douglas	1
	Oct	QLD	Ingham	1
	Jan	QLD	Mackay	1
	Feb	QLD	Atherton Tabelands	1
	Jun	NSW	Macksville	1

2013		QLD	Brisbane Valley	1
	Jul	QLD	Gold Coast Hinterland	1
		NSW	Macksville	1
		NSW	Kempsey	2
2014	Mar	QLD	Bundaberg	1
	Jun	QLD	Beenleigh	1
		NSW	Murvillumbah	1
	Jul	QLD	Gladstone	1
2015	Jun	NSW	Murwillumbah	1
	Jul	QLD	Atherton Tableland	1
	Spt	NSW	Lismore	1
2016	Dec	NSW	Casino	1
2017	May	QLD	Gold Cost Hinterland	1
	Jun	NSW	Lismore	1
	Aug	NSW	Murwillumbah	1
		NSW	Lismore	1
2018	Sep	NSW	Tween Heads	1
			Total	***103***

[1] QLD = Queensland; NSW = New South Wales
[2] All cases were deceased

TABLE 14.1B. Summary of Hendra Virus Outbreaks in Humans

Year	**Month**	**Country**	**Location**	**No. cases**	**No. deaths**	**Case Fatality Rate**
1994	Aug	Australia	Mackay	1	1	100%
1994	Sep		Hendra	2	1	50%
2004	Oct		Cairns	1	1	100%
2008	Jul		Redlands	2	1	60%
2009	Aug		Cararral	1	0	0%
			Total	*7*	***4***	***57%***

Table 14.1C. Summary of Nipah Virus Outbreaks in Humans

Year	**Month**	**Country**	**Location**	**No. cases**	**No. deaths**	**NiV Clade (M/B)**		**Case Fatality Rate**
1998	Spt-Apr 1999	Malaysia	Started from Nipah near Ipoh	265	105	M		40%
1999	Mar	Singapore	Singapore	11	1	M		9%
2001	Feb	India	Siliguri	66	45		B	68%
2001	Apr-May	Bangladesh	Meherpur	13	9		B	69%
2003	Jan		Naogaon	12	8		B	67%
2004	Jan		Raibari	31	23		B	74%
	Apr		Faridpur	36	27		B	75%
2005	Jan-Mar		Tangail	12	11		B	92%

2007	Jan-Feb		Thakurgaon	7	3		B	43%
	Mar		Kushtia	8	5		B	63%
	Apr		Pabna, Natore, Naogaon	3	1		B	33%
	Apr	India	Nadia	5	5		B	100%
2008	Feb	Bangladesh	Manikgonj	4	4		B	100%
	Apr		Rajbari	7	5		B	71%
2009	Jan		Gaibandha, Rangpur, Nilphamari	3	0		B	0%
			Rajbari	1	1		B	100%
2010	Feb-Mar		Faridpur, Rajbari, Gopalgonj, Kurigram	17	15		B	88%
2011	Jan-Feb		Lalmohirhat, Dinajpur, Comilla, Nilphamari, Faridpur, Rajbari	44	40		B	91%
2012	Jan		Joypurhat	12	10		B	83%
2013	Jan-Apr		Pabna, Natore, Naogaon, Gaibandha, Manikganj	24	21			88%
2014	Jan-Feb		13 Districts	18	9		B	50%
2014	Mar-May	The Philippines	Senator Ninoy Aquino	17	9	M		53%
2015	Jan-Feb	Bangladesh	Nilphamari, Ponchoghor, Faridpur, Magura, Naugaon, Rajbari	9	6		B	67%
2018	May	India	Kerala	23	21		B	94%
				293	***115***	***M***		***39%***
				355	***269***		***B***	***76%***
			Total	***648***	***384***	***M***	***B***	***59%***

TABLE 14.2. Summary of Henipaviruses Isolated from Different Species and Geographic Locations

		Isolation details	

Virus	**Isolate name and number**	**Year**	**Country**	**Host species/tissue**	**Reference**
Hendra	Horse-1 VR-1	1994	Australia	Horse/spleen, lung Human/lung, liver, kidney, spleen	1
	Bat-1-1 Bat-1-2 Bat-2	1996	Australia	Grey-headed flying fox (*Pteropus poliocephalus*)/uterine fluid Grey-headed flying fox (*P. poliocephalus*)/fetus Black flying fox (*Pteropus alecto*)/fetal lung	2
	Murwillumbah Clifton Beach Peachester Redlands Proserpine	2006 2007 2008	Australia	Horse/lung Horse/lung Horse/blood Horse/lung Horse/lung	3
Nipah	PKL EKK WWS	1999	Malaysia	Human/cerebral spinal fluid Human/cerebral spinal fluid Human/cerebral spinal fluid	4,5
	UMMC1 UMMC2	1999	Malaysia	Human/cerebral spinal fluid Human/throat secretion	6
	UM-0128 VRI-0626 VRI-1413 VRI-2794	1999	Malaysia	Human Pig/lung Pig/lung Pig/lung	7
	B13/6-18 B13/6-43 JA13/6-4	2000	Malaysia	Bat/pooled urine Bat/pooled urine Bat/partially eaten jambu air fruit	8
	Rajbari-1 Rajbari-2 Faridpur Rajshahi	2004	Bangladesh	Human/oropharyngeal Human/cerebral spinal fluid Human/urine Human/urine	9
	CSUR381 CSUR382	2004	Cambodia	Flying fox (*Pteropus lylei*)/urine Flying fox (*Pteropus lylei*)/urine	10
	Raypur-31 Raypur-36 Raypur-38 Raypur-40 Raypur-41 Raypur-42 Raypur-43 Raypur-46	2013	Bangladesh	Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine	11

	Raypur-47 Sylhet-47			Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine Flying fox (*Pteropus medius*)/urine	

Table 14.3. Henipavirus Cell Tropism and Receptor Expression

Cell line/ Primary cells	**Species/Cell Type**	**Virus**	**EFNB2[a]**	**EFNB3[a]**	**Infection Permissive[b]**	**Reference[c]**
			HUMAN			
HeLa-CCL2	Human Cervical Cancer	NiV HeV	+	-	+	12 13
HeLa-USU	Human Cervical Cancer	NiV HeV	-	-	-	12
HeLa-USU-EFNB2	HeLa-USU stably expressing EFNB2	HeV CedV	+ +	- -	+ +	14 15
HeLa-USU-EFNB3	HeLa-USU stably expressing EFNB3	HeV CedV	- -	+ +	+ -	14 15
Vero (various subclones)	African green monke kidney epithelial	NiV HeV CedV GhV	++	+	+	1 4 12 16 14 17
293T	Human embryonic kidney fibroblast		+	+	+	18 19 20 21
A549	Human lung adenocarcinoma	NiV HeV	+	+	+	22 23 24

PCI 13	Human head and neck carcinoma	NiV HeV	+	+	+	12 25
U373-MG	Human astroglioma	NiV	+	++	+	12 25 26
U87-MG	Human glioblastoma	NiV GhV	+	+	+	27 28 17 29
HBMEC (Primary)	Human brain microvascular endothelial	NiV	+	-	+	30 16
HUVEC (Primary)	Human umbilical cord endothelial	NiV HeV	+	+	+	31 32
HuNSC Neurons/ Astrocytes (Primary)	Human neural stem cells derived neurons and astrovytes	NiV, HeV	+	+	+	33 32
NHBE (Primary)	(Normal) Human bronchial epithelial	NiV, HeV	+	+	+	34 35
SAEC (Primary)	Human small airway epithleilial	NiV HeV	+	+	+	34 35
Hu PBL (Primary)	Human peripheral blood lymphocytes	NiV	(+/-)[d]	(+/-)[d]	-	26
HuMono/ Mac/DCs (Primary)	Human monocytes, macrophages, dendritic cells	NiV	Mono/ Mac (+/-)[d] DC (+/-)[d]	Mono/ Mac (+/-)[d] DC (-)	Mono (-) Mac (-) DC (+/-)	26,36
HuOE (Primary)	Human olfactory epithelial	NiV HeV	(?)	+	+	37 38

Cell line/ Primary cells	**Species/Cell Type**	**Virus**	**EFNB2[a]**	**EFNB3[a]**	**Infection Permissive[b]**	**References[c]**
NON-HUMAN						
BHK-21	Baby hamster kidney	NiV	+	(?)	+	13 22

	fibroblast					
CHO	Chinese Hamster Ovary	NiV HeV	-	-	-	39 40 13
CHO-B2	CHO stably expressing ephrin-B2	NiV HeV	+	-	+	39 40
CHO-B3	CHO stably expressing ephrin-B3	NiV HeV	-	+	+	39 40
CRFK	Cat (Feline) kidney epithelial	NiV HeV	+	+	+	20
PBMEC (Primary)	Porcine brain microvascular endothelial	NiV	+	+	+	16 41
PAEC (Primary)	Pig Aorta Endothelial	NiV	-	-	-	16
PK13	Pig kidney fibroblast	NiV	-	-	-	39 42
Rat cortical neurons	Rat cortical neurons (Primary)	NiV	+	+	+	39 19
4/4RM4	Rat pleural mesothelial	NiV	+	ND	+	13 43
L2	Rat lung epithelial	NiV	+	ND	+	13 44
208f	Rat embryonic fibroblast	NiV	+	ND	-	13 44
P815	Mouse mast cell	NiV	+	ND	-	13 44
MyEnd	Mouse myocardial endothelial	NiV	(?)	(?)	-	16
PaLuT02	Immortalized *P. alecto* (fruit bat) lung cells	NiV HeV	+	+	+	45 46
PaKi	Primary *P. alecto* (fruit bat) kidney cells	HeV	+	+	+	45 46

PaBrH04	Immortalized *P. alecto* (fruit bat) brain cells	NiV HeV	+	+	+	[47] [46]
MMEC	Mouse microvascular endothelium	NiV HeV	(?)	(?)	(+/-)[d]	[22]

[a] Expression inferred from (1) transcriptional data (microarray, next-generation sequencing, RT-PCR, etc.) provided in the cited references, or (2) indepedently verified through the Gene Expression Omnibus (GEO) Database (https://www.ncbi.nlm.nih.gov/geo/), or (3) competition with soluble ephrinB ligands or interference with cognate henipavirus receptor binding proteins. [b] For clarity and uniformity, only infection-permissive (not fusion-permissive) data are indicated; infection includes use of live virus and/or pseudotyped virus. [c] References cited support expression, infection data, or cell-type origin where relevant, and are representative, not comprehensive. [d] Indicates >10-100-fold below positive control when quantitative data is avialable. ND, not determined; (?), not specifically mentioned.

TABLE 14.4 Detection of Nipah or Nipah-related Virus Infection in Bats (Geographic surveillance studies)

Bat Species	**Method and country of detection**			**Reference**
	Serology	**PCR**	**Isolation**	
Suborder: Yinpterochiroptera				
Cynopterus brachyotis	Malaysia			[48]
Cynopterus sphinx	Vietnam			[49]
Eidolon dupreanum	Madgascar			[50]
Eidolon helvum	Ghana, Zambia, Malawi, Tanzania, Uganda, Rio Muni, Bioko, Príncipe, Saõ Tomé, Annobón, Cameroon	Ghana, Gabon, Democratic Republic of Congo, Central African Republic,		[51], [52], [53], [54], [55]
Eonycteris spelaea	Malaysia			[48]
Epomophorus gambianus	Ghana	Democratic Republic of Congo, Gabon, Republic of Congo		[51], [53]
Hipposideros armiger	China			[56]
Hipposideros	Thailand	Thailand		[57]

larvatus				
Hipposideros pomona	China			[56]
Hypsignathus monstrosus	Ghana	Republic of Congo, Central African Republic,		[51], [53]
Myonycteris torquata		Democratic Republic of Congo, Gabon, Republic of Congo		[53]
Rhinolophus affinis	China			[56]
Rhinolophus sinicus	China			[56]
Rousettus leschenaulti	China, Vietnam			[56]; [49]
Rousettus aegyptiacus		Gabon		[53]
Pteropus giganteus	Bangladesh, India			[58]
Pteropus hypomelanus	Malaysia, Thailand	Thailand	Malaysia	[48]; [59]; [60]
Pteropus lylei	Cambodia, Thailand	Thailand	Cambodia	[10,61,62]
Pteropus medius		Bangladesh, India	Bangladesh	[63]; [11]; [64]
Pteropus rufus	Madgascar			[50]
Pteropus vampyrus	Malaysia, Thailand			[48]; [57]
Suborder: Yangochiroptera				
Carolia perspicillta		Costa Rica		[53]
Miniopterus spp.	China			[56]
Myotis daubentoni	China			[56]
Myotis ricketti	China			[56]
Pteronotus parnellii		Costa Rica		[53]
Scotophilus kuhlii	Malaysia			[48]

TABLE 14.5 | Summary of Advanced Henipavirus Vaccination Development Initiatives Evaluated in Animal Challenge Models

Platform	Viral antigen target or immunogen	Animal challenge model

Active vaccination		
Recombinant vaccinia virus	Nipah F and/or G glycoprotein	Hamster[a] (NiV) Pig[b] (NiV)
Recombinant canarypox virus	Nipah F and/or G glycoprotein	Ferret[c] (NiV), Hamster[d] (NiV), nonhuman primate[e] (NiV)
Recombinant VSV	Nipah F and/or G glycoprotein	Hamster[f] (NiV, HeV) Hamster[g], Nonhuman primate (NiV, HeV)
Recombinant AAV		
Recombinant measles virus	Nipah G glycoprotein Nipah G glycoprotein	Cat[h] (NiV), Ferret[i] (HeV, NiV), nonhuman primate[j] (HeV, NiV)
Recombinant subunit	Hendra soluble G glycoprotein	Horse[k] (HeV)
Passive immunization		
Human monoclonal antibody m102.4	Hendra / Nipah G glycoprotein	Ferret[l] (NiV) Nonhuman primate[m] (HeV, NiV)

[a] Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vaccinia viruses were protected against disease following intraperitoneal challenge with 10^3 PFU of NiV.[65]

[b] Pigs immunized with NiV F and/or G glycoprotein encoding recombinant canarypox viruses were protected against intranasal challenge with $2.5x10^5$ PFU of NiV.[66].

[c] Ferrets immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intranasal challenge with $5x10^3$ PFU of NiV.[67]

[d] Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intraperitoneal challenge with 10^5 $TCID_{50}$ of NiV;[68] or $6.8x10^4$ $TCID_{50}$ of NiV.[69]

[e] African green monkeys immunized with a NiV G encoding recombinant VSV vector were protected against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV.[70,71]

[f] Hamsters immunized with a NiV G encoding recombinant adeno-associated virus (AAV) vector were protected against lethal intraperitoneal with 10^4 PFU of NiV.[72]

[g] Hamsters and African green monkeys immunized with a NiV G encoding recombinant measles virus vector were protected against lethal intraperitoneal challenge with 10^3 $TCID_{50}$ of NiV (hamsters) or 10^5 $TCID_{50}$ of NiV (AGMs).[73]

[h] Hendra virus soluble G glycoprotein (HeV-sG) used to immunize cats protects against lethal subcutaneous (500 $TCID_{50}$)[74] or oronasal ($5x10^4$ $TCID_{50}$) NiV challenge.[75]

[i] HeV-sG used to immunize ferrets protects against lethal oronasal challenge with $5x10^3$ $TCID_{50}$ of HeV[76] or $5x10^3$ $TCID_{50}$ of NiV challenge.[77]

[j] HeV-sG used to immunize African green monkeys protects against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV[78] or $5x10^5$ PFU of HeV.[79]

[k] HeV-sG used to immunize horses protects against lethal oronasal challenge with $2x10^6$ $TCID_{50}$ of HeV[80].

[l] A NiV and HeV cross-reactive G glycoprotein specific neutralizing human mAb (m102.4) protects ferrets against lethal oronasal challenge with $5x10^3$ $TCID_{50}$ of NiV[81] or $5x10^3$ $TCID_{50}$ of HeV (J. Pallister and C. Broder, unpublished) by post-exposure infusion.

[m] Human mAb m102.4 protects African green monkeys by post-exposure infusion following lethal intratracheal challenge with $4x10^5$ $TCID_{50}$ of HeV[82] or lethal intratracheal challenge with $5x10^5$ PFU of NiV.[83,84]

REFERENCES

1. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science.* 1995;268(5207):94-97.
2. Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol.* 2000;81(Pt 8):1927-1932.
3. Marsh GA, Todd S, Foord A, et al. Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. *Emerg Infect Dis.* 2010;16(11):1767-1769.
4. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science.* 2000;288(5470):1432-1435.
5. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia [see comments]. *Lancet.* 1999;354(9186):1257-1259.
6. Chan YP, Chua KB, Koh CL, Lim ME, Lam SK. Complete nucleotide sequences of Nipah virus isolates from Malaysia. *J Gen Virol.* 2001;82(Pt 9):2151-2155.
7. AbuBakar S, Chang LY, Ali AR, Sharifah SH, Yusoff K, Zamrod Z. Isolation and molecular identification of Nipah virus from pigs. *Emerg Infect Dis.* 2004;10(12):2228-2230.
8. Chua KB, Lek Koh C, Hooi PS, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes Infect.* 2002;4(2):145-151.
9. Harcourt BH, Lowe L, Tamin A, et al. Genetic characterization of Nipah virus, Bangladesh, 2004. *Emerg Infect Dis.* 2005;11(10):1594-1597.
10. Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. *Emerg Infect Dis.* 2005;11(7):1042-1047.
11. Anderson DE, Islam A, Crameri G, et al. Isolation and Full-Genome Characterization of Nipah Viruses from Bats, Bangladesh. *Emerg Infect Dis.* 2019;25(1):166-170.
12. Bonaparte MI, Dimitrov AS, Bossart KN, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A.* 2005;102(30):10652-10657.
13. Yoneda M, Guillaume V, Ikeda F, et al. Establishment of a Nipah virus rescue system. *Proc Natl Acad Sci U S A.* 2006;103(44):16508-16513.
14. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog.* 2012;8(8):e1002836.
15. Laing ED, Amaya M, Navaratnarajah CK, et al. Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J.* 2018;15(1):56.
16. Erbar S, Diederich S, Maisner A. Selective receptor expression restricts Nipah virus infection of endothelial cells. *Virol J.* 2008;5:142.
17. Pernet O, Wang YE, Lee B. Henipavirus receptor usage and tropism. *Curr Top Microbiol Immunol.* 2012;359:59-78.
18. Yoneda M. Nipah and Hendra virus infectious diseases. *Nihon Rinsho.* 2016;74(12):1973-1978.
19. Talekar A, Pessi A, Porotto M. Infection of primary neurons mediated by nipah virus envelope proteins: role of host target cells in antiviral action. *J Virol.* 2011;85(16):8422-

8426.
20. Sawatsky B, Grolla A, Kuzenko N, Weingartl H, Czub M. Inhibition of henipavirus infection by Nipah virus attachment glycoprotein occurs without cell-surface downregulation of ephrin-B2 or ephrin-B3. *J Gen Virol.* 2007;88(Pt 2):582-591.
21. Martinez-Gil L, Vera-Velasco NM, Mingarro I. Exploring the Human-Nipah Virus Protein-Protein Interactome. *J Virol.* 2017;91(23).
22. Aljofan M, Porotto M, Moscona A, Mungall BA. Development and validation of a chemiluminescent immunodetection assay amenable to high throughput screening of antiviral drugs for Nipah and Hendra virus. *J Virol Methods.* 2008;149(1):12-19.
23. Bauer A, Neumann S, Karger A, et al. ANP32B is a nuclear target of henipavirus M proteins. *PLoS One.* 2014;9(5):e97233.
24. Dietzel E, Kolesnikova L, Sawatsky B, et al. Nipah Virus Matrix Protein Influences Fusogenicity and Is Essential for Particle Infectivity and Stability. *J Virol.* 2015;90(5):2514-2522.
25. Bossart KN, Crameri G, Dimitrov AS, et al. Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol.* 2005;79(11):6690-6702.
26. Mathieu C, Pohl C, Szecsi J, et al. Nipah virus uses leukocytes for efficient dissemination within a host. *J Virol.* 2011;85(15):7863-7871.
27. Bender RR, Muth A, Schneider IC, et al. Receptor-Targeted Nipah Virus Glycoproteins Improve Cell-Type Selective Gene Delivery and Reveal a Preference for Membrane-Proximal Cell Attachment. *PLoS Pathog.* 2016;12(6):e1005641.
28. Lee B, Pernet O, Ahmed AA, Zeltina A, Beaty SM, Bowden TA. Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. *Proc Natl Acad Sci U S A.* 2015;112(17):E2156-2165.
29. Khetawat D, Broder CC. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol J.* 2010;7:312.
30. Wong KT, Shieh WJ, Kumar S, et al. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol.* 2002;161(6):2153-2167.
31. Mathieu C, Guillaume V, Sabine A, et al. Lethal Nipah virus infection induces rapid overexpression of CXCL10. *PLoS One.* 2012;7(2):e32157.
32. Yun T, Park A, Hill TE, et al. Efficient reverse genetics reveals genetic determinants of budding and fusogenic differences between Nipah and Hendra viruses and enables real-time monitoring of viral spread in small animal models of henipavirus infection. *J Virol.* 2015;89(2):1242-1253.
33. Palomares K, Vigant F, Van Handel B, et al. Nipah virus envelope-pseudotyped lentiviruses efficiently target ephrinB2-positive stem cell populations in vitro and bypass the liver sink when administered in vivo. *J Virol.* 2013;87(4):2094-2108.
34. Escaffre O, Borisevich V, Carmical JR, et al. Henipavirus pathogenesis in human respiratory epithelial cells. *J Virol.* 2013;87(6):3284-3294.
35. Escaffre O, Borisevich V, Vergara LA, Wen JW, Long D, Rockx B. Characterization of Nipah virus infection in a model of human airway epithelial cells cultured at an air-liquid interface. *J Gen Virol.* 2016;97(5):1077-1086.
36. Gupta M, Lo MK, Spiropoulou CF. Activation and cell death in human dendritic cells infected with Nipah virus. *Virology.* 2013;441(1):49-56.
37. Borisevich V, Ozdener MH, Malik B, Rockx B. Hendra and Nipah Virus Infection in

Cultured Human Olfactory Epithelial Cells. *mSphere.* 2017;2(3).

38. Tanos T, Saibene AM, Pipolo C, Battaglia P, Felisati G, Rubio A. Isolation of putative stem cells present in human adult olfactory mucosa. *PLoS One.* 2017;12(7):e0181151.
39. Negrete OA, Levroney EL, Aguilar HC, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature.* 2005;436(7049):401-405.
40. Negrete OA, Wolf MC, Aguilar HC, et al. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog.* 2006;2(2):e7.
41. Erbar S, Maisner A. Nipah virus infection and glycoprotein targeting in endothelial cells. *Virol J.* 2010;7:305.
42. Chua KB, Wong EM, Cropp BC, Hyatt AD. Role of electron microscopy in Nipah virus outbreak investigation and control. *Med J Malaysia.* 2007;62(2):139-142.
43. Hudson AL, Weir C, Moon E, et al. Establishing a panel of chemo-resistant mesothelioma models for investigating chemo-resistance and identifying new treatments for mesothelioma. *Sci Rep.* 2014;4:6152.
44. Yoneda M, Fujita K, Sato H, Kai C. Reverse genetics of Nipah virus to probe viral pathogenicity. *Methods Mol Biol.* 2009;515:329-337.
45. Virtue ER, Marsh GA, Baker ML, Wang LF. Interferon production and signaling pathways are antagonized during henipavirus infection of fruit bat cell lines. *PLoS One.* 2011;6(7):e22488.
46. Bossart KN, Tachedjian M, McEachern JA, et al. Functional studies of host-specific ephrin-B ligands as Henipavirus receptors. *Virology.* 2008;372(2):357-371.
47. Crameri G, Todd S, Grimley S, et al. Establishment, immortalisation and characterisation of pteropid bat cell lines. *PLoS One.* 2009;4(12):e8266.
48. Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerg Infect Dis.* 2001;7(3):439-441.
49. Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of nipah virus infection in bats, Vietnam. *Emerg Infect Dis.* 2012;18(3):536-537.
50. Iehle C, Razafitrimo G, Razainirina J, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. *Emerg Infect Dis.* 2007;13(1):159-161.
51. Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. *PLoS One.* 2008;3(7):e2739.
52. Drexler JF, Corman VM, Gloza-Rausch F, et al. Henipavirus RNA in African bats. *PLoS One.* 2009;4(7):e6367.
53. Drexler JF, Corman VM, Muller MA, et al. Bats host major mammalian paramyxoviruses. *Nat Commun.* 2012;3:796.
54. Peel AJ, Sargan DR, Baker KS, et al. Continent-wide panmixia of an African fruit bat facilitates transmission of potentially zoonotic viruses. *Nat Commun.* 2013;4:2770.
55. Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. *Nat Commun.* 2014;5:5342.
56. Li Y, Wang J, Hickey AC, et al. Antibodies to Nipah or Nipah-like viruses in bats, China. *Emerg Infect Dis.* 2008;14(12):1974-1976.
57. Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. *Emerg Infect Dis.* 2005;11(12):1949-1951.
58. Epstein JH, Prakash V, Smith CS, et al. Henipavirus infection in fruit bats (Pteropus giganteus), India. *Emerg Infect Dis.* 2008;14(8):1309-1311.
59. Wacharapluesadee S, Samseeneam P, Phermpool M, et al. Molecular characterization of

Nipah virus from Pteropus hypomelanus in Southern Thailand. *Virol J.* 2016;13:53.

60. Chua KB. A novel approach for collecting samples from fruit bats for isolation of infectious agents. *Microbes Infect.* 2003;5(6):487-490.
61. Olson JG, Rupprecht C, Rollin PE, et al. Antibodies to Nipah-like virus in bats (Pteropus lylei), Cambodia. *Emerg Infect Dis.* 2002;8(9):987-988.
62. Wacharapluesadee S, Boongird K, Wanghongsa S, et al. A longitudinal study of the prevalence of Nipah virus in Pteropus lylei bats in Thailand: evidence for seasonal preference in disease transmission. *Vector Borne Zoonotic Dis.* 2010;10(2):183-190.
63. Epstein JH, Anthony SJ, Islam A, et al. Nipah virus ecology and infection dynamics in its bat reservoir, *Pteropus medius*, in Bangladesh. *International Journal of Infectious Diseases.* 2016;53:20-21.
64. Mourya DT, Yadav P, Sudeep AB, et al. A spatial association between a Nipah virus outbreak in Kozhikode, Kerala, India and Nipah virus infection in Pteropus bats. *Clin Infect Dis.* 2018.
65. Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. *J Virol.* 2004;78(2):834-840.
66. Weingartl HM, Berhane Y, Caswell JL, et al. Recombinant nipah virus vaccines protect pigs against challenge. *J Virol.* 2006;80(16):7929-7938.
67. Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J.* 2013;10:353.
68. Lo MK, Bird BH, Chattopadhyay A, et al. Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters. *Antiviral Res.* 2014;101:26-29.
69. DeBuysscher BL, Scott D, Marzi A, Prescott J, Feldmann H. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. *Vaccine.* 2014;32(22):2637-2644.
70. Prescott J, DeBuysscher BL, Feldmann F, et al. Single-dose live-attenuated vesicular stomatitis virus-based vaccine protects African green monkeys from Nipah virus disease. *Vaccine.* 2015;33(24):2823-2829.
71. DeBuysscher BL, Scott D, Thomas T, Feldmann H, Prescott J. Peri-exposure protection against Nipah virus disease using a single-dose recombinant vesicular stomatitis virus-based vaccine. *NPJ Vaccines.* 2016;1.
72. Ploquin A, Szecsi J, Mathieu C, et al. Protection against henipavirus infection by use of recombinant adeno-associated virus-vector vaccines. *J Infect Dis.* 2013;207(3):469-478.
73. Yoneda M, Georges-Courbot MC, Ikeda F, et al. Recombinant measles virus vaccine expressing the Nipah virus glycoprotein protects against lethal Nipah virus challenge. *PLoS One.* 2013;8(3):e58414.
74. Mungall BA, Middleton D, Crameri G, et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol.* 2006;80(24):12293-12302.
75. McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine.* 2008;26(31):3842-3852.
76. Pallister J, Middleton D, Wang LF, et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine.* 2011;29(34):5623-5630.
77. Pallister JA, Klein R, Arkinstall R, et al. Vaccination of ferrets with a recombinant G

glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J.* 2013;10:237.

78. Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci Transl Med.* 2012;4(146):146ra107.
79. Mire CE, Geisbert JB, Agans KN, et al. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol.* 2014;88(9):4624-4631.
80. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis.* 2014;20(3):372-379.
81. Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute nipah virus infection. *PLoS Pathog.* 2009;5(10):e1000642.
82. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med.* 2011;3(105):105ra103.
83. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med.* 2014;6(242):242ra282.
84. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep.* 2016;6:30916.

From: Lee, Benhur on behalf of Lee, Benhur <benhur.lee@mssm.edu>
To: Broder, Christopher
Cc: LinFa Wang Linfa
Subject: The Final DRAFT & everything else
Date: Thursday, March 7, 2019 10:13:27 AM
Attachments: Master List.Figures & Tables & PageCount.xlsx
CH14-HNV-Complete.190307.v9.0.docx
Figure Legends_Final.v5.Fields.docx
HNV_Fields Ref.enlp.zip
Henipavirus Tables 14.1-14.5-V10-190226.docx

HI Folks,

This is the final draft for now. I think it's still way too long by my counting, but at least everyone can see how everything hangs together.

The only edits and comments you see are new ones I added, mostly to LinFa's section, but I also took a couple sentences from Chris's section to put into my pathogenesis part to avoid duplication. I clearly indicated where.

I removed two old figures and replace with two new ones.

Attached is the (1) Master Excel Spread sheet to keep everything straight, (2) the text manuscript file, (3) Figure Legend text file, (4) Master endnote file., (5) Final Table Text file

I will send you a Googlelink to the ppt file with all the figures. The tiff files are individually exported, can share them in a dropbox if you want.

Anyway, I'm happy to take out anything in my section - it's just too long, and I already cut out so much. Let me know what you think is important.

Thanks. (LinFa, maybe you can tell Sean we'll be uploading soon. I'm afraid to talk to him!) Gottta go and get some sleep now.

Benhur

> On Mar 6, 2019, at 11:00 PM, Broder, Christopher <christopher.broder@usuhs.edu> wrote:
>
> hey! Congrats Benhur! So happy for you!
>
> I just landed in NRT on way home to MD.
>
> HAHA I know what you mean about being afraid to get back online to email!
> and NO need to
> apologize! are you kidding. Life is way more important than this!
>
> I hope you have fun things planned the rest of the week and weekend! and not the damn chapter!

all very best wishes!
Chris

On Wed, Mar 6, 2019 at 7:56 PM Lee, Benhur <benhur.lee@mssm.edu> wrote:

> Hi Guys,
>
> The reason why I wanted things earlier on to put together is because . . . I just got (b) (6) [redacted]. But my schedule is not your problem.
>
> I am also here at a GRC in Ventura (on Bioterrorism but speaking on CRISPR delivery, NOT NiV). Basically have been continuously working till 5 am or so every day since last week (except for flying here and on my (b) (6) [redacted] day).
>
> This chapter is such a bear to put together. Reading the instructions, they really want the subsections constructed as in the outline, so the whole book has the same organization. I had re-do my original sections almost completely, and have to keep referring to both your sections (that's why I need your sections to fully complete mine) to make everything consistent and avoid duplication.
>
> I've added substantial new matter for LinFa's into section on viral proteins properties, etc. To keep up with advances, I made an entirely original figure on B2/B3 expression across tissues using the original NGS data from the GTEx Portal. Then converting every figure into a high resolution tiff file for those that i don't have already is a nightmare.
>
> I'm going to send what I have even if the length is too long . . . to all of you by early am (Mar 7 EST). I may be upload it as well. We all have to revise the manuscript anyway, after review, and also after the substantial nomenclature changes are adopted later this month by ICTV.
>
> (b) (6) [redacted]. all week and we are taking off for the weekend, so if I still have to work on this chapter this weekend, this will be (b) (6) [redacted]. I can already tell the uploading process is a bear.
>
> So, I apologize. It's so much work than I thought! You see what I mean when i send everything. Thank you for your patience.
>
> Cheers,
>
> Benhur
>
> On Mar 6, 2019, at 1:42 AM, Wang Linfa <linfa.wang@duke-nus.edu.sg> wrote:

> A very nice updated figure!
>
> *Linfa (Lin-Fa) Wang, PhD FTSE*
> Professor & Director
> Programme in Emerging Infectious Diseases
> Duke-NUS Medical School
> 8 College Road, Singapore 169875
> Tel: +65 65168397
>
> ---
>
> **From:** Christopher Broder <christopher.broder@usuhs.edu>
> **Date:** Wednesday, 6 March 2019 at 5:35 PM
> **To:** Wang Linfa <linfa.wang@duke-nus.edu.sg>
> **Cc:** Benhur Lee <benhur.lee@mssm.edu>
> **Subject:** Re: GenBank AC#
>
> this was replacement HeV vaccine map.
>
> Must have been lost in the emails.
>
> cb
>
> On Sat, Mar 2, 2019 at 10:45 PM Wang Linfa <linfa.wang@duke-nus.edu.sg> wrote:
>
>> Dear Benhur,
>>
>> We have now got the GenBank AC# for the new NiV seuences:
>>
>> NiV/BD/Bat/2013/Sylhet-47(MK575070)
>> NiV/BD/Bat/2013/Raypur-31(MK575060)
>>
>> I will let you to update in the figure.
>>
>> Thanks
>>
>> LF
>>
>> ***Linfa (Lin-Fa) WANG, PhD FTSE***
>> **Professor & Director**
>> **Programme in Emerging Infectious Disease**
>> **Duke-NUS Medical School,**
>> **8 College Road, Singapore 169857**

Tel: +65 6516 8397

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Chapter 14

<TITLE>~~12~~14 Henipaviruses

Benhur Lee

Christopher C. Broder

Lin-Fa Wang

- History
- Infectious Agent
    - Classification
    - Propagation in Cell Culture and Cytopathic Effect
    - Virus Morphology
    - Genome Length and Organization
    - Genetic Diversity
    - Virus Proteins and Their Properties
    - Host Range
- Pathogenesis and Pathology
    - Cell and Tissue Tropism
    - Immune Response
    - Inhibition of the Interferon Response
    - Infections of Animals
    - Laboratory Animal Models
- Epidemiology
    - Age
    - Morbidity and Mortality
    - Origin and Spread of Epidemics
- Clinical Features
    - Incubation Period
    - Acute Clinical Features
    - Outcome of Infection
- Diagnosis
    - Laboratory Diagnosis
- Prevention and Control
    - Treatment
    - Vaccines and Passive Immunotherapy
    - Peptide Inhibitors
- Perspective and Geographic Considerations

<H1>History

Hendra virus (HeV), the first known member of the genus *Henipavirus* in the family *Paramyxoviridae*, came to light in September 1994 as the causative agent of a sudden outbreak of acute respiratory disease in thoroughbred horses at a stable in Brisbane, Australia. A total of 21 horses and 2 humans (a horse trainer and a stable hand) became infected. The horse trainer and 14 horses died, and 7 horses with mild or subclinical infection were euthanized.(130) A virus was isolated, initially called *Equine Morbillivirus* but later re-named *Hendra virus* after the Brisbane suburb where the outbreak occurred. A second person died from HeV infection 13 months after the Brisbane outbreak, a farmer from Mackay, nearly 1000 km north of Brisbane. Unlike the first case, however, the man succumbed to encephalitis caused by HeV infection.(107) A forensic retrospective investigation found that the farmer had suffered a mild meningitic illness 14 months earlier after assisting at the necropsy of two horses that had died of severe respiratory distress—later found to have been caused by HeV(125)—and that he became infected at that time. After initial serologic evidence suggested that fruit bats (flying foxes) of the genus *Pteropus* in the suborder Yinpterochiroptera were the reservoir hosts,(165) HeV was isolated from two species of flying fox.(56) In total, there have been 60 recognized occurrences of HeV in Australia between 1994–2018, with at least one occurrence per year since 2006 (Table 14.1A). (5)·(10) Every occurrence of HeV has involved horses as the initial infected host, causing lethal respiratory disease and encephalitis, along with a total of seven human cases arising from exposure to infected horses, among which four have been fatal and the most recent in 2009 (Table 14.1B).(6)·(10)·(115)

Commented [L1]: Can we cite webpage? https://www.business.qld.gov.au/industries/service-industries-professionals/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary

BHL REPLY: I have added the web-page into my Endnote database and cited it. Green highlighted Ref 1

Commented [BHL2]: Does this mean you still want to keep **Ref 5 and 10**, Please make the decision and add/delete ref accordingly. **PLEASE USE the Endnote File I sent you. All these (refs) from the original document Publisher sent (Knipe XXXXx) CANNOT be formatted – I DON'T have the time to go through every single one and do it myself.**

Commented [BHL3]: **TABLE 14.1A and 14.1B** should be moved to online materials**. TABLE 14.1C** (below) should be kept in the print version.

Nipah virus (NiV), the second known member of the genus *Henipavirus*, emerged as the cause of an outbreak of disease in pigs and humans in Peninsular Malaysia in 1998 through 1999. The epidemic started in Perak State as clusters of cases of encephalitis among pig farmers. It was initially believed to be caused by Japanese encephalitis virus; however, various features of the outbreak, including a high proportion of cases in direct contact with pigs and illness and deaths in pigs, differed from those expected with Japanese encephalitis.(30) Indeed, respiratory illness and encephalitis in pigs preceded human cases in the same district.(97) The epidemic spread south to the intensive pig-farming areas of Negeri Sembilan in December 1998 and subsequently peaked between February and April 1999. More than 1 million pigs were destroyed to halt the spread of the epidemic, and by late May, 265 human cases of acute encephalitis with 105 deaths were recorded.(29)·(30) A cluster of 11 cases with 1 death occurred among abattoir workers in Singapore.(114) In early March 1999, a virus was isolated from the cerebrospinal fluid (CSF) of a patient with encephalitis and identified as the etiologic agent.(29)·(30) Named *Nipah virus* after the village from which the patient had come, it was shown to be closely related to HeV. NiV was subsequently isolated from the urine of Malaysian flying foxes.(33) A highly related NiV emerged in Bangladesh in 2001,(68) (7) and outbreaks of NiV-related encephalitis have occurred in people from that country almost every year since, along with three reports of NiV encephalitis in India (23), albeit in Bangladesh adjacent regions. However, the most recent outbreak of NiV occurred in 2018 in Kerala (India), more than 2,500 km south-west of NiV-endemic Bangladesh counties.

This outbreak claim~~ing~~ed 21 lives out of 23 infected human cases. [2](60) In 2014, a NiV outbreak occurred in the Province of Sultan Kudarat, the Phillipines, which resulted in the death of 9 humans from 11 which showed an acute encephalitis syndrome.[3] The human case fatality rate of these NiV outbreaks averages around 60% (Table 14.1C).(68),(85) So far human NiV oubreaks have been documented in a total of five countries, Malaysia, Singapore, Bangladesh, India and the Philippines.

<TAB14.1~~A,B,~~C>

<H1>Infectious Agent

<H2>Classification ~~refs~~

When HeV was first isolated in 1994, partial sequencing of the matrix gene (M) revealed that it most closely resembled members of the genus *Morbillivirus* in the subfamily *Paramyxovirinae.*(104) Subsequent characterization of the full-length genome, however, revealed that many of the genetic features of HeV were unique among paramyxoviruses and that the virus did not fit within any of the three genera existing at that time, *Morbillivirus, Respirovirus*, and *Rubulavirus.*(147),(148) When NiV was isolated in 1999, it was initially described as Hendra-like on the basis of its strong reactivity with anti-HeV antibodies. Later it was shown that sera raised against HeV were able to neutralize NiV and vice versa, and that both viruses shared a high degree of similarity in genome organization and protein size and sequence.(29),(58),(59),(146) In 2002, the genus *Henipavirus* was created to accommodate these novel paramyxoviruses, and HeV was designated the type species.(75) Since 1994, there have been 38 isolations of HeV or NiV from humans, bats, horses, and pigs over a wide geographic area and spanning a period of approximately 20 years (Table 14.2). The susceptibility of humans, the virulence of the viruses, and absence of therapeutics and vaccines led to classification of HeV and NiV as biosafety level 4 (BSL4) pathogens. In the latest report from the paramyxovirus study group under the order Mononegavirales, the genus *Henipavirus* has been expanded to include three new species,[4,5] they are *Cedar henipavirus* (Cedar virus, CedV) isolated from bats in Australia, [6] *Ghanaian bat henipavirus* (Ghana virus, GhV) detected in bats from Ghana,[7] and *Mojiang henipavirus* (Mòjiāng, MojV) detected in rats in China.[8] *Henipavirus* is now classified as one of the seven ICTV-approved genera in the family *Paramyxoviridae*.

<TAB14.2>

<H2>Propagation in Cell Culture and Cytopathic Effect

The ultrastructural characteristics of henipavirus-infected cells resemble those found in cells infected by other members of the *Paramyxoviridae*. Shared features include generation of large syncytia and the presence of viral nucleocapsids in cytoplasmic inclusion bodies and underlying electron-dense areas of the plasma membrane.(49),(69) In Vero cells, NiV-induced syncytia are significantly larger than those generated by HeV and nuclei and nucleocapsids are frequently located at the cell periphery, compared with HeV-induced syncytia, where they tend to be more centrally located or distributed randomly throughout the cytoplasm (Fig.

Commented [L4]: Arunkumar et al (2018) JID. DOI: 10.1093/infdis/jiy612
BHL: I have added the new 2018 Kerala ref. Please remove Ref (60) accordingly. It's up to you. Or move it someplace else.

Commented [L5]: Ching et al. (2015) EID
BHL: I added the Ching 2015 Eid Ref

Commented [BHL6]: **TABLE 14.1C** should be kept in the print version. TAB14.1A & 14.1B should be moved to online materials

Commented [BHL7]: **Please delete these old refs (68), (85), or move it someplace else that make sense with the old text.**

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Commented [BHL8]: TABLE 14.2 can also be put in online materials and save us 1.5 manuscript text pages.

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Commented [L9]: Hi Benhur, for the time being, I am using the Arch Virol taxonomy. I am not sure when the new Taxonmy will be officially published. This will have a major impact on everything we do, including Figures 14.3 and 14.4
BHL: Understood. We'll have to see what Bob Lamb uses in his chapter on *Paramyxoviridae*. I already told Sean– we should be ready to change nomenclature during revisions, etc.

Commented [BHL10]: **Please delete this "CALL-OUT" if you agree with my suggestion for moving TABLE 14.2 to online materials.**

14.1). Henipavirus-infected cells also contain structures that are not seen with other paramyxoviruses—specifically a network of membrane-like reticular structures in the cytoplasm and long tubules that appear to be continuous with the plasma membrane in NiV-infected cells. Tubules can also be observed in NiV virions (Fig. 14.2A). *In situ* hybridization suggests that these reticular structures contain viral RNA and may play a role in viral transcription.(49)

<FIG14.1><FIG14.2>

<H2>Virus Morphology

Commented [BHL11]: Note to self: Do we want to talk about the superresolution NiV-F/G study in Nat Commun here, later in pathogenesis section, or at all ?

Henipavirus particles are pleomorphic, varying from spherical to filamentous and ranging in size from 40 to 1,900 nm.(49)·(69)·(104) Nucleocapsids have a diameter of 18 to 19 nm with an average pitch of 5 nm. When examined by electron microscopy (EM), HeV has a unique double-fringed appearance, caused by the presence of surface projections 15 ± 1 nm and 8 ± 1 nm in length (see Fig. 14.2B). Approximately 95% of virions contain the double fringe, and the remaining 5% display a uniform fringe length of 15 ± 1 nm. Unlike HeV, NiV possesses a single layer of surface projections with an average length of 17 ± 1 nm, and NiV particles released into the culture medium are difficult to image because they are routinely penetrated by negative stains. This suggests that the viruses may differ in the physical nature of their envelope.(69)

<H2>Genome Length and Organization

In the family *Paramyxoviridae*, the genome length of all characterized viruses is divisible by six, an observation caused by the requirement of each N protein in the viral ribonucleoprotein to bind 6 nucleotide (nt) residues.(76) This is also true for HeV and NiV despite their much larger genome sizes.(146) The genomes of the Malaysian and Bangladesh strains of NiV differ by 6 nt because of a 6-nt increase in the 3′ untranslated region of the *F* gene.(57) A minigenome replicon study confirms that NiV complies with the rule of six.(55) When the complete genome sequence of HeV was determined, its length (18,234 nt) was more than 2,700 nt, or 15% longer than the genomes of all other paramyxoviruses known at that time.(148) The size of NiV genomes at 18,246 nt to 18,252 nt are slightly larger than that of HeV.(57)(–)(59) These large genome sizes of >18 kb is a conserved feature of henipaviruses as even the recently discovered and more divergent CedV, GhV, and MojV all have genomes between 18,162 nt (CedV) to ~18,430 nt (GhV). Given the caveat that there is wide variation amongst individual species of the other paramyxovirus genera, the extra length of the henipavirus genome is primarily due to the longer 3' untranslated regions (3'UTRs) at the end of several of its six genes, and a larger *P* gene relative to the other paramyxovirus genera. A comparison of genome length and gene organisation of representative members of the *Paramyxoviridae* is shown in Fig. 14.3A. The nomenclature of each of the six major genes common to all paramyxoviruses havs been updated to reflect the latest ICTV convention. An analysis of the 3' UTR lengths between extant henipaviruses and representative species from each paramyxovirus genera that infects mammals is presented in Fig. 14.3B. The most significant differences are in the 3'UTRs of *N*, *P*, and *RBP* genes even given the wide variance seen within each genera. The significance of long 3’ UTRs, especially in HeV and NiV, is generally unknown. However, the N gene 3’UTR of NiV appears to play a role in the downregulation of N mRNAs via specific interactions with hnRNP D. [9]

<FIG14.3A><FIG14.3B>

The genome organization of henipaviruses resembles that in the genera *Respirovirus* and *Morbillivirus.* The first 12 nt of the 3′ and 5′ genomic terminal sequences of paramyxoviruses are highly conserved and complementary, containing promoter elements for replication and transcription.(76) The first 3 nt of the henipavirus genome termini are 5′-ACC-3′—a sequence that is absolutely conserved in members of the family*Paramyxoviridae* and different from that found in the family *Pneumoviridae.*

<H2>Genetic Diversity

Genome sequencing revealed that HeV isolated from equine and human sources during the outbreak in Brisbane appears identical and differs little from HeV isolated from flying foxes 2 years later.(56),(104) Sequencing of five additional horse isolates from five different locations from the 2006 to 2008 HeV occurrences has also demonstrated a very high genetic similarity.(90) Similar observations were made in Malaysia, where it was demonstrated that NiV isolated from pigs at the height of the outbreak and at its geographic focus were essentially identical to human isolates made at that time and isolates obtained from flying foxes several years later.(2),(24),(33),(58),(59)

In Bangladesh, four human isolates obtained in 2004 demonstrate significant genetic heterogeneity, which might suggest multiple spillovers of NiV from flying foxes into the human population.(57) The NiV sequences detected from human patients in India from 2001 to 2018 were more related to NiV from Bangladesh than NiV from Malaysia.(9),(23) [2] A recently whole genome sequencing study of ten NiV isolates obtained from bats in 2013 in Bangladesh at two different geographic locations revealed that the isolated viruses were very similar in sequences. This suggests that multiple strains were not co-circulating in the bat population at the time.[10] Furthermore, none of the bat NiV isolate sequences was identical with any previously detected human NiV isolate sequences, suggesting that NiV spillover into humans is a rare event and more intensive surveillance is required to appreciate the full genetic diversity of NiV in bats in an area with frequent spillover events into human populations. [10] NiV isolated from the flying fox *Pteropus lylei* in Cambodia(120) represents an evolutionary lineage that is separated from the Malaysia or Bangladesh/India cluster . Partial *N* gene sequences detected in *Pteropus lylei* in Thailand indicate the circulation of at least two lineages of NiV—one related to the Bangladesh NiV and the other more related to the Malaysian NiV.(144) On the other hand, NiV sequences detected in *Pteropus hypomelanus* in southern Thailand are clearly more related to the Malaysian NiV. [11] As the RNA-dependent RNA polymerase (RdRP), also known as the L protein, is the most highly conserved protein for paramyxoviruses, it has been used as a more reliable indicator of evolutionary relationship among different paramyxoviruses. Shown in Fig. 14.4 is

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Commented [BHL12]: **LINFA: I** am confused. I thought Genetic Diversity has been moved to Section D.5. (Under Epidemiology) according to the new outline you gave us and the figure numbering we agreed on. Please see comments below aboujt Figh. 14.4 which should be Fig. 14.11 in Section D5.

Commented [L13]: Arunkumar et al (2018) JID. DOI: 10.1093/infdis/jiy612
BHL: I have added the new 2018 Kerala ref 2. Please retain/delete old reference as you deem appropriate. But please do it using the Endnote file.

Commented [L14]: Anderson et al (2019) EID 25:166
BHL: I have added the Anderson 2019 as ref 10. All my added references are from my endnote file and follows numbering different from the original 2012 text.

Commented [L15]: Wacharapluesadee et al. Virology Journal (2016) 13:53
BHL: I have added Wacharapluesadee **2016 as ref 11. All my added references are from my endnote file and follows numbering different from the original 2012 text.**

Commented [BHL16]: **LINFA:** This is supposed to be **Fig. 14.11** if moved to Section D5 (under Epidemiology) according to the new outline we agreed on. Please clarify- see above – if this whole genetic diversity section should be moved to Section D5.

phylogenetic tree based on RdRP proteins of prototype species of each genera in the family *Paramyxoviridae* with major members of the genus *Henipavirus*, selected to represent major species origin, geographic location and time of isolation.

<FIG>

The presence of henipavirus-reactive (but not neutralizing) antibodies and viral RNA in bats from other regions of the world (see Epidemiology section below) predicts that a much greater genetic diversity of henipaviruses exists in different bat populations. This also suggests the existence of henipaviruses with different transmissibility and pathogenicity in non-bat hosts.

<H2>Virus Proteins and Their Properties

Analysis of purified viruses by polyacrylamide gel electrophoresis reveals RdRP (L), P, RBP (G), F_0, N, F_1, and F_2 proteins,(146),(147) where F_0 is the uncleaved and F_1 and F_2 the cleaved products of the *F* gene. Interestingly, F_0 is more readily detected in HeV compared with other paramyxoviruses, including NiV,(93),(146) which may suggest that HeV-F is less efficiently cleaved. Overall, the proteins of henipaviruses are typical of those of ~~*the subfamily Paramyxovirinae*~~*Paramyxoviridae*, with the exception of the P protein, which is significantly larger than cognate proteins in ~~the~~ this virus ~~sub~~family.(147) The P protein is translated from messenger RNA (mRNA) that is co-linear with genomic RNA. For most henipaviruses, the *P* gene also encodes V and W proteins, produced from mRNA in which one and two nontemplated G residues, respectively, are inserted at the RNA editing site during transcription. The P, V, and W proteins, therefore, are identical for the first 405 amino acid residues. A C protein is encoded by the 5′ end of the gene in an overlapping reading frame and is produced by an internal translational initiation mechanism, which is common to other members of ~~*Paramyxovirinae*~~*Paramyxoviridae*, except for rubulaviruses.(76) The P, V, and the C proteins predicted from the coding regions in the *P* gene are present in HeV-infected cells.(146) The functional expression of W in NiV-infected cells has recently been demonstrated.(84) It is worth to note that Cedar virus lacks both the RNA editing and expression of the V protein. 6

Paramyxovirus N, P, and RdRP proteins are necessary and sufficient for replication of viral RNA both *in vitro* and *in vivo*,(76) and this has been confirmed for henipaviruses by reverse genetics. Using a minigenome replicon containing leader and trailer sequences of the NiV genome with its entire coding region replaced with a reporter gene, it was shown that efficient genome replication was achieved only when all three proteins were expressed in the same cell.(55)12 NiV N, P, and RdRP proteins were also able to rescue a minigenome constructed from the leader and trailer sequences of the HeV genome, further demonstrating the close genetic relationship between the viruses. (BHL might want to expand more on reverse genetics of HNVs if space permits-BHL)

The RdRP protein of nonsegmented, negative-stranded RNA (NNR) viruses in the order *Mononegavirales* contains a highly conserved GDNQ motif, believed to be important for polymerase activity.(116) Henipaviruses were the first NNR viruses in which GDNQ was replaced by GDNE. It was speculated that this motif might be unique to paramyxoviruses with relatively large genomes(146),(148); however, the GDNE motif has since been found in the RdRP protein of Mossman virus that has a genome length of 16,650 nt.(96). Conversely, other henipaviruses of both Asiatic (CedV) and African lineages (GhV) also have the GDNQ motif that is common to most other NNR viruses. High-resolution cryo-EM structures of VSV-RdRP (another NNR virus, family *Rhabdoviridae*) suggest that GDN is the truly conserved catalytic motif essential for the polymerase function of RdRP.[13]

The receptor binding proteins (RBPs) of the *Paramyxovirinae* display hemagglutination (H) and neuraminidase (N) activities in a predominantly genus-specific manner. Viruses in the genera *Respirovirus, Avulavirus*, and *Rubulavirus* possess both activities, hence their RBPs were formerly termed HN proteins (76) whereas viruses in the genus *Morbillivirus* do not behave uniformly and only some possess hemagglutination activity, but nonetheless their RBPs were formerly termed H. (77) This is an unfortunate misnomer. Paramyxoviruses with RBPs bearing HN activity genuinely use sialic acid based receptors for entry whereas "H" activity of some morbilliviruses arise from their RBPs ability to bind CD46 expressed on some non-human primate red blood cells. [14] In contrast, henipavirus RBPs have neither of these activities(146),(166); rather, they utilize the host cell expressed ephrin-B2 and ephrin-B3 molecules as attachment and entry receptors (11),(13),(105),(106) [15] with the exception of MojV, which does not appear to use any known paramyxovirus receptors.[16] Recent solution structures of various henipavirus RBPs alone and in complex with the ephrin-B2 and/or ephrin-B3 receptors have revealed the details of the virus–host cell binding process, distinguishing it from other paramyxoviruses's receptor binding strategies.(20),(21),(163)[15] [14]

Proteolytic processing of paramyxovirus F proteins is essential for the generation of a fusogenic form of the protein. For most paramyxoviruses that generate systemic infections, cleavage is catalyzed by the cellular protease furin at a multibasic cleavage site.(76) Surprisingly, henipavirus F proteins are cleaved without the involvement of furin and, although cleavage occurs at a single basic residue—lysine for HeV and arginine for NiV(98)—activation of the NiV F protein does not require a basic amino acid at the cleavage site.(93) It has been shown that the lysosomal cysteine protease cathepsin L is responsible for the cleavage of the HeV F protein.(108) The cytoplasmic tails of henipavirus F proteins contain the endocytosis consensus motif YXX ø.(92),(143) Endocytosis is required for cleavage activation of the F protein,(35),(92) consistent with identification of cathepsin L as the enzyme responsible for cleavage.

<H2>Host Range

For most paramyxoviruses, host range is limited and interspecies transmission is rare. In contrast, henipaviruses display a broad species tropism. In addition to ~~at least three flying fox species, two nonpteropid fruit bat species, and an insectivorous species~~a large number of bat hosts at various geographic locations (see more detail in Epidemiology section), NiV has naturally infected pigs, humans, dogs, horses, and cats,(29),(63),(97),(120),(164) whereas HeV infects all four Australian flying fox species and has naturally infected humans,~~ and~~ horses and dogs.(42),(104) Confirmation of the wide host range of henipaviruses and the identical cell

Commented [L17]: Benhur, I forgot whether we agreed for you to include a discussion on the RBP structure evolution story here?! If so, pls insert here.

BHL: I'm stoopping here. It's 7:30 am and I need to get some sleep before resuming. Potentially the "extra:" Fig. 14.7B (Structural phylogeny of different paramyxovirus RBPs will go well here).

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tropism of HeV and NiV were obtained early using an *in vitro* cell fusion system that relies on vaccinia virus–mediated cell surface expression of G and F glycoproteins.(17)·(18)·(136) Guinea pigs, hamsters, ferrets, squirrel monkeys, and African green monkeys are also susceptible to experimental NiV infection.(19)·(46)·(88)·(94)·(157) Laboratory studies have added cats, guinea pigs, hamsters, ferrets, and African green monkeys to the list of HeV-susceptible species,(53)·(121)·(151)·(156). ~~along with ferrets (J. Pallister and L. F. Wang, unpublished results).~~Mojiang virus was isolated from rats ().

<H1>Pathogenesis and Pathology

<H2>Cell and Tissue Tropism

The primary site of NiV replication and the dynamics of virus spread in humans are unknown. However, the distribution and time of appearance of lesions throughout the vasculature and in the brain and lung in NiV encephalitis suggest that secondary infection probably arises via hematogenous spread of the virus, with secondary replication occurring in vascular endothelium.(159) Inflammation of blood vessels occurs in most organs but is particularly prominent in the brain, lung, heart, and kidney.(30)·(159)·(160) Vasculitis is limited to small arteries, arterioles, and capillaries where NiV antigen is found in both endothelial cells and the smooth muscle of the tunica media. The pattern and time of appearance of vasculitis and viral antigen distribution are consistent with endothelial cell infection occurring before infection of the smooth muscle. Syncytial endothelial cells are present in blood vessels of various organs and represent a pathognomonic but insensitive feature of henipavirus infections found in only 25% of human NiV infections.(159) Syncytial endothelial cells were also observed in the single human case of encephalitis caused by HeV.(107) NiV antigen is also found in neurons and, less frequently, in bronchiolar and renal epithelial cells. The 5-day interval between maximal vasculitis in the brain and parenchymal infection in acute NiV encephalitis suggests that primary virus replication occurs in endothelial cells, with infection of neurons occurring as a result of vascular damage. The presence of inclusion bodies and viral antigen in neurons suggests that neurologic impairment in encephalitis may be caused by both the effects of ischemia and infarction and viral infection of neurons.(30)·(114)·(159) Human HeV encephalitis has been described as widespread cortical, subcortical, and deep white matter involvement in two cases in 2008(115) and similar to those described in a previous HeV encephalitis case(82)·(107) and NiV encephalitis cases.(82)·(127)

Although NiV antigen is found less frequently in bronchiolar and renal epithelial cells compared with vascular and neuronal locations, replication in epithelial locations may play a role in virus dissemination, because NiV is found in urine and in tracheal and nasopharyngeal secretions of infected patients in the early phase of their illness.(31)·(48) Despite this, human-to-human transmission in Malaysia was extremely rare.(100)

The identification of ephrin-B2 and ephrin-B3 as functional receptors for henipaviruses in cultured cells provides an explanation for the observed distribution of viral antigen in arterial endothelial cells, smooth muscle, neurons, and some epithelial cells.(11)·(13)·(105)·(106) Ephrin-B2 is found in arteries, arterioles, capillaries in multiple organs, and tissues including arterial smooth muscle and human bronchiolar epithelial cells(135) but is absent from venous components of the vasculature.(45) Ephrin-B3 is found predominantly in the nervous system as well as the vasculature.(113) The ephrins engage Eph receptors and mediate bidirectional cell–cell signaling events and are modulators of cell remodeling events, especially within the nervous and vascular systems.(113)

<H2>Immune Response

In patients with encephalitis, anti-NiV antibodies were observed more frequently in the serum than the CSF. Immunoglobulin M (IgM) antibodies occurred more frequently than immunoglobulin G (IgG) antibodies in both locations.(30),(114),(159) The appearance of specific IgM antibodies in serum preceded their appearance in the CSF, a sequence consistent with viremia preceding central nervous system (CNS) infection. Anti-NiV antibodies were present in most patients with clinical NiV encephalitis; however, no difference was observed in clinical features, laboratory results, or mortality between seropositive and seronegative patients.(27),(48) Seroconversion of IgG against HeV was seen in two human cases of HeV infection, one fatal, in 2008,(115) following an influenza-like illnesses and before progressing to encephalitis.

<H2>Inhibition of the Interferon Response

In henipaviruses, as in other paramyxoviruses,(66) anti-interferon (IFN) activities are encoded by the *P* gene and *in vitro* studies indicate that *P* gene products inhibit both IFN induction and signaling.(37) In IFN induction, viral double-stranded RNA (dsRNA) is detected by intracellular RNA helicase enzymes(3),(79) and by toll-like receptor 3 (TLR-3).(12) Both of these dsRNA signaling pathways lead to activation of the pre-existing transcription factors interferon regulatory factor 3 (IRF-3) and nuclear factor kappa B (NF-kB),(50) and the synthesis of IFN-α and a subset of IFN-β proteins.(89) HeV inhibits dsRNA signaling by using a strategy adopted by other paramyxoviruses in which the V protein binds to the intracellular RNA helicase sensor and prevents downstream signaling(3) but does not abrogate dsRNA signaling through TLR-3.(132) In an additional strategy unique to henipaviruses, the W protein, by virtue of a nuclear localization signal located in the unique carboxy terminal, inhibits dsRNA signaling in the nucleus by targeting a process that is part of both helicase-dependent and TLR-3–dependent signaling pathways.(132)

In the IFN signaling pathway, IFN binds to cell surface receptors in a paracrine manner and initiates a signaling sequence that leads to activation of members of a family of proteins called *signal transducers and activators of transcription* (STAT).(1),(50) Henipaviruses inhibit IFN signaling by sequestering STAT proteins in high molecular weight complexes.(123),(124) The anti-IFN signaling activity is a property of the V protein, as has been observed for other paramyxoviruses, but also of the W and P proteins.(123),(124),(133) The P, V, and W proteins of henipaviruses have an N-terminal extension of 100 to 200 amino acids compared with cognate proteins in the subfamily,(59),(147) and the STAT binding domain of NiV V maps to this region.(122) The V and P proteins bind STAT in the cytoplasm, whereas the W protein, with its nuclear localization signal, co-localizes with STAT in the nucleus.(132),(133) The W protein is the most and P protein the least efficient IFN antagonist.(112) The NiV C protein also displays a modest inhibition of IFN signaling, although the mechanism and target are unknown.(112)

It should be noted that most, if not all, studies described previously were carried out *in vitro* using single-gene transfection expression systems. A recent study conducted using live virus infection indicated that IFN signaling remains functional during henipavirus infection of human cell lines, whereas IFN production was inhibited.(142)

<H2>Infections of Animals

Only limited data are available on the minimal lethal and infectious doses of henipaviruses. In golden hamsters (*Mesocricetus auratus*), the NiV lethal dose 50% (LD$_{50}$) following intraperitoneal and intranasal administration was 270 and 47,000 plaque-forming units (pfu), respectively,(157) and was calculated to be 12 pfu for HeV by intraperitoneal injection.(53) The NiV minimal infectious dose in hamsters is 100 pfu if the intraperitoneal route is used and 10^3 pfu if the virus is administered intranasally. Guinea pigs and pigs are also more resistant to infection by HeV and NiV, respectively, when the viruses are administered by the oronasal route compared with the subcutaneous route.(95),(154) In contrast, both HeV and NiV appear to be equally infectious for cats following either parenteral (5,000 tissue culture infectious dose 50% [TCID$_{50}$] virus) or oronasal (50,000 TCID$_{50}$ virus) administration.(64),(91),(95),(101),(151)

Experimental infection of horses with HeV by parenteral or oronasal routes is almost uniformly fatal, with death or euthanasia usually occurring 5 to 10 days after infection. Several horses in the original outbreak in Hendra survived infection, however, some asymptomatically.(103),(104),(156) In horses, HeV displays a predominantly respiratory tropism, and infection is characterized by pulmonary edema and congestion.(64),(104),(156) In field cases, the airways are often filled with a blood-tinged frothy exudate. Neurologic signs do exist, but they have been observed infrequently in terminally ill horses and in horses that recovered from respiratory infection.(125),(156) Infection is associated with virus replication and with the appearance of viral antigen in endothelial cells in a wide variety of organs, including lungs, lymph nodes, kidneys, spleen, bladder, and meninges. The subsequent degeneration of small blood vessels is accompanied by the appearance of syncytial endothelial cells.(63) Virus can be recovered from several internal organs, including lung, and from saliva and urine.(64),(156)

In contrast, NiV infection of pigs is frequently asymptomatic, particularly following natural infection and after experimental administration of the virus by the ocular and oronasal route.(95),(97),(150) When symptoms are present, they vary according to the age of the pig, with older animals presenting primarily with a neurologic syndrome, whereas a respiratory syndrome predominates in young animals. The virus manifests respiratory and neurologic tropisms in both asymptomatic and clinical infections.(95),(149) Neurologic signs include trembling and neurologic twitches, muscle spasms, and uncoordinated gait.(97) After experimental NiV infection of young pigs by the ocular and oronasal routes, virus replication occurs in the oropharynx and spreads sequentially to the upper respiratory tract and submandibular lymph nodes, the lower respiratory tract, and additional lymphoid tissues.(149) Here, viral antigen is widespread and syncytia are common in clinically affected animals as a result of replication in the endothelial and smooth muscle cells of medium to large veins as well as in the arteries of the central nervous, lymphoid, and respiratory systems.(63),(95),(149) NiV invades the CNS via the cranial nerves and by crossing the blood–brain barrier.(149) Virus is recovered from a range of tissues, including tonsil, nasal, and throat swabs and lung, but is recovered infrequently from urine.(34),(95),(149) Experimental HeV infection of pigs (Landrace and Gottingen minipig breeds) via oronasal or nasal inoculations has shown both to be susceptible to infection,(80) with virus detected mainly in tissues from respiratory and lymphoid systems, and could be isolated from nasal, oral, and rectal swabs, indicating the possible routes for virus shedding.

<H2>Laboratory Animal Models

In hamsters, the pathologic features following NiV infection resemble those found in humans.(157) Hamsters die 5 to 9 days after intraperitoneal administration of 100 to 10,000 infectious particles and 24 hours after the appearance of tremors and limb paralysis. In contrast, hamsters inoculated intranasally with doses as high as 10^3 to 10^6 infectious particles die between 9 and 15 days later, displaying progressive deterioration with limb paralysis, lethargy, limb twitching, and breathing difficulties. Vascular pathology is observed in a range of organs, including brain, lung, liver, kidney, and heart, and viral antigen and genome are found in endothelial cells. The brain is the most severely affected organ, with the vascular and parenchyma lesions consistent with CNS-mediated clinical signs.(159) HeV infection of hamsters also resembled the pathology seen in acute human cases, including both respiratory and brain pathology.(53) HeV-induced pathology in the hamster was similar to that of NiV(157) and consisted of endothelial infection and vasculitis with thrombosis and microinfarction, with evidence of direct parenchymal cell infection, notably in the CNS.

In cats, the first clinical signs, which are observed on days 4 to 8 after parenteral or oronasal administration of HeV and NiV, include depression, fever, and an increased rate of respiration.(65),(101),(151),(152) Most infected animals die 1 day after the appearance of respiratory distress. The disease caused by HeV and NiV in cats closely resembles that seen in HeV-infected horses, with copious frothy sanguineous fluid in the bronchi and hemorrhage or congestion of the tracheal epithelium.(64) Vaculitis affects both arteries and arterioles, and syncytial cells are observed in endothelia, predominantly in the lungs but also in gastrointestinal, spleen, and lymphoid organs. A major difference between NiV and HeV infection of cats is the extensive degree to which NiV, but not HeV, infects the respiratory epithelium.(95) Henipaviruses are found in the urine and bladder of experimentally infected cats, and NiV can transplacentally infect and replicate in fetal tissues with high levels of recoverable virus from the placenta and uterine fluid.(102)

In guinea pigs, the clinical response to henipavirus infection is frequently mild and often variable, ranging from inapparent to sudden death, with only a proportion of animals displaying signs such as transient weight loss, depression, ataxia, lethargy, and twitching.(154),(157) The vascular tropism of HeV in guinea pigs is evident in many organs.(65)

Death appears to result from vascular disease in a variety of organs.(65) Only a proportion of infected animals develop encephalitis with virus observed in blood vessels and neurons.(154)

A ferret model of henipavirus infection and pathogenesis has also been developed.(19),(110) The ferret exhibits both severe respiratory and neurologic disease as well as generalized vasculitis following an oronasal challenge with NiV with doses as low as 500 $TCID_{50}$ within 6 to 10 days postinfection. Clinical signs in affected ferrets included various combinations of severe depression, cough, serous nasal discharge, dyspnea, subcutaneous edema of the head, cutaneous ecchymoses, and obtundation with tremor and hind limb paresis depending on the challenge dose. Clinical disease in the ferret included vascular fibrinoid necrosis in multiple organs, necrotizing alveolitis, and syncytia of endothelium and alveolar epithelium. Histopathologic lesions included severe focal necrotizing alveolitis, vasculitis, degeneration of glomerular tufts, and focal necrosis in a wide range of other tissues. High levels of viral antigen were noted in blood vessel walls, and syncytial cells were frequently present. Viral antigen was present in neurons, and infectious NiV was isolated from multiple organs including the brain. Overall, NiV-mediated disease observed in the ferret model manifested with all the hallmarks seen among NiV-infected humans, and essentially identical results have been observed with HeV infection of ferrets (J. Pallister and L.-F. Wang, unpublished results).

A nonhuman primate model of henipavirus infection has been developed using the African green monkey,(46),(121) which yields a uniformly lethal disease with doses as low as approximately 2×10^4 pfu (NiV) or 4×10^5 $TCID_{50}$ of HeV. Monkeys, following intratracheal inoculation with either NiV or HeV, reveal a rapid spread of the virus (3–4 days postinfection) to numerous organ systems. NiV-infected monkeys developed a severe acute respiratory distress syndrome (ARDS)-like disease, associated with copious amounts of sanguineous fluid and froth. The lungs are enlarged with multifocal areas of congestion and hemorrhage, and immunohistochemical and histopathologic examination revealed significant amounts of polymerized fibrin and NiV antigen.(46) Endothelial syncytial cells were prominent in most of the tissues, and vasculitis was widespread. NiV antigen was present in endothelial and arterial smooth muscle cells in most examined tissues. Respiratory disease development could be seen within 7 days postinfection with either NiV or HeV following intratracheal inoculation by radiologic examination(46),(121) progressing to severe congestion and infiltration in the lung fields.

NiV and HeV could be found in virtually every organ system sampled at the time of death in the African green monkey. Immunohistochemical and histopathologic analysis revealed the presence of NiV antigen, predominantly in endothelial cells and smooth muscle cells, along with associated pathology. Most animals showed evidence of henipavirus-induced neurologic disease,(46),(121) with severe congestion and evidence of meningeal hemorrhaging and edema. NiV and HeV antigen was detected in endothelial cells in brain, with infection of neurons often widespread in the brain stem.(46),(121) NiV infection of squirrel monkeys has also been examined.(88) Although some animals demonstrated limited similarities to NiV pathogenesis in humans, only 50% of challenged animals exhibited any clinical signs, with most remaining well even following intranasal or intravenous delivery of doses as high as 10^7 pfu of NiV.

Unlike the cat, guinea pig, hamster, and squirrel monkey models of NiV or HeV infection, severe respiratory pathology, neurologic disease, and generalized vasculitis all occur in henipavirus-infected African green monkeys, providing an accurate reflection of what is observed in henipavirus-infected humans.

<H1>Epidemiology

<H2>Age

The age of patients with encephalitis in the Malaysian outbreak ranged from 9 to 76 years, with almost 50% of cases occurring in those 40 to 44 years.(27)·(28)·(48)·(111)·(159) The male-to-female ratio was approximately 3:1, and more than 80% of the patients were Chinese, with statistics reflecting the increased risk to those working with infected pigs.(48)·(111) In Bangladesh, the age of patients ranged from 4 to 60 years, and males constituted 47% and 67% of the cases in the 2001 and 2003 outbreaks, respectively.(43)·(68) The ~~recent~~ outbreak in early 2011 in Bangladesh claimed at least 35 lives, including many children and infants, with ages ranging from 2 to 56 years.(8) Among the 18 suspected patients in the 2014 oubreak in the Phillipines, the age distribution is between 21-60 years old (). In the most recent NiV outbreak in Kerala, India, the age distribution ranged from a 17 year old male to a 75 year old female among a total of 23 human cases ()

Commented [L19]: Ching et al., 2015. EID

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<H2>Morbidity and Mortality

In Malaysia, between September 1998 and June 1999, 256 patients who developed acute NiV encephalitis were admitted to Malaysian hospitals and 105 died, a mortality rate of approximately 40%.(30)·(111) The rate of subclinical infection in households and farms where cases of NiV encephalitis occurred was calculated to be 8% and 11%, respectively.(111)·(139) In Singapore, where 11 patients were confirmed to have acute NiV encephalitis, a further 2 asymptomatic abattoir workers were serologically positive, representing a rate of subclinical infection of 15%.(25) Subsequently, 89 individuals were identified on the basis of positive serology as having experienced either an asymptomatic or mildly symptomatic NiV infection.(138) This increases the number of people infected with NiV to 345 and decreases the mortality rate to approximately 30%.(160) In Bangladesh, 98 of 135 patients died in eight outbreaks from 2001 to 2008, giving a combined case fatality rate of 73%.(43)·(62)·(85) In the Philippines outbreak, the case mortality is 53% out of all the suspected cases or 81% for those with encephalitic clinical signs.() The case mortality is 94% for the Kerala outbreak in India ()There have only been seven known human cases of HeV infection in Australia in the past 16 years, four of which have been fatal (three acute and one case of relapsed encephalitis).(6)·(104)·(107)·(115)

Commented [L21]: Ching et al., 2015, EID

Commented [L22]: Arunkumar et al., 2018, JID

<H2>Origin and Spread of Epidemics

Fruit bats (flying foxes) in the genus *Pteropus*, family *Pteropodidae*, suborder *Megachiroptera* are main reservoir hosts of HeV and NiV.(33)·(56)·(164)·(165) In Australia, HeV has been shown to occur in all four flying fox species, with the crude seroprevalence of 47%, indicating an endemic pattern of infection throughout Australia.(42) Serologic tests show that

NiV or NiV-related virus is widely dispersed in ~~pteropid~~ bats ~~in~~ from Malaysia~~,~~ to the west border of Africa (Fig. 14.10). ~~especially the Island flying fox (*Pteropus hypomelanus*) and the Malayan flying fox (*Pteropus vampyrus*).(~~164) NiV was first isolated from the urine of Island flying foxes and from the saliva on partially eaten fruit(33) and has since been isolated from Lyle's flying foxes (*Pteropus lylei*) in Cambodia~~.~~(120) and *Pteropus medius* in Bangladesh() The Indian flying fox (*Pteropus giganteus*) is the ~~only~~ main pteropid species throughout Bangladesh and the Indian subcontinent with a high seroprevelence of henipavirus-specific antibody.(39),(68),(86),(87) Additional serologic and limited nucleic acid evidence has suggested that related henipaviruses are circulating in other regions, including Thailand, Indonesia, Vietnam, China, Madigascar, and several countries and regions in ~~West~~ Africa.(36),(61),(70),(81),(131),(145)

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Neither HeV nor NiV appear to cause clinical disease in flying foxes infected naturally,(42),(43),(119),(164) and experimental infection with doses of HeV, consistently shown to be lethal in horses, generates only sporadic vasculitis in the lung, spleen, meninges, kidney, and gastrointestinal tract and only in a proportion of infected bats.(156) Viral antigen is detected in the tunica media rather than endothelial cells, a fact that may spare the flying fox from the clinical effects associated with vasculitis.(37) In infected pregnant flying foxes, antigen was observed in similar locations and in the placenta.(155) The mode of transmission between flying foxes is unknown. Transplacental transmission has also been observed experimentally without apparent harm to the fetus.(155) Experimental infection of flying foxes with NiV produced a subclinical infection with a transient presence of virus within selected viscera along with periodic viral excretion in bat urine and seroconversion with neutralizing antibody present.(94)

The spillover and epidemic hosts of HeV and NiV were horses in Australia and pigs in Malaysia. All human infections with HeV in Australia and NiV in Malaysia have only occurred through transmission from these domestic animal hosts.(30),(111) No evidence exists of direct transmission from pteropid bats to humans in Australia or Malaysia, despite many opportunities in Australia for transmission to bat carers.(127),(129) In contrast, flying foxes apparently play a direct role in the transmission of NiV to humans in the many recent outbreaks of disease in Bangladesh, where epidemiologic evidence in support of a role for pigs was lacking.(43),(68) Three pathways of NiV transmission from bats to people have been identified based on epidemiologic investigations in Bangladesh.(85) Consumption of fresh date palm sap appears to be the predominant risk factor, and infrared camera studies have confirmed that *P. giganteus* bats frequently visit date palm sap trees and consume sap during collection.(126) In the 2005 NiV outbreak in Tangail District, Bangladesh, drinking raw date palm sap was the only activity significantly associated with illness (64% among cases vs. 18% among controls).(87) Another route of transmission for NiV from bats to people in Bangladesh could be via domestic animals. Contact with a sick cow in Meherpur, Bangladesh, in 2001 was strongly associated with NiV infection,(68) and contact with pigs and diseased goats have also been implicated in other occurrences of NiV in Bangladesh.(85) Although NiV has never been isolated in domestic livestock animals, serological evidence was reported for antibodies to NiV or NiV-related viruses in cattle, goats and pigs in Bangladesh. ()

Commented [L24]: Chowdhury et al., 2014 PloS NTD

Transmission via direct contact with NiV-infected bat secretions also appears possible from evidence in the Goalando outbreak in 2004, where individuals who climbed trees were more likely to develop NiV infection than controls.(99)

The mode of transmission from bats to spillover hosts in Australia and Malaysia remains to be determined. Three principal hypotheses exist. One is that masticated pellets of virus-contaminated, residual fruit pulp spat out by flying foxes are ingested by horses or pigs.(164) The second is that urine from infected animals contaminates pastures or pigsties. The third is that infected fetal tissues or fluids contaminate pastures or sties and are ingested. The latter is based largely on the fact that the HeV outbreaks have occurred during the birthing period of some species of flying fox and is supported by the isolation of virus from a pregnant flying fox and its fetus.(56)

HeV has been transmitted from horse to man on seven occasions from 1994 to 2009, twice during the initial outbreak in Brisbane,(4) twice during necropsy of horses that died in the field,(125),(130) twice during either daily nasal cavity lavage and participating in a necropsy,(115) and once from performing an endoscopy on an infected horse.(6) HeV is rarely found in the bronchi or bronchioles of infected horses, which suggests that aerosol transmission to either man or horses is less likely(64) and horse-to-horse transmission of HeV has not been demonstrated.(156) The presence of HeV in equine saliva, however, suggests that close contact with infected horses, such as might occur during manual feeding of the animals, may facilitate horse-to-human transmission.(130) The presence of virus in a wide range of tissues and in the nasal discharge commonly found at the terminal stage of infection offers a range of sources for virus transmission during necropsy.(103),(104) As shown in Fig.~~ure~~ ~~37.5~~14.1xA, high level of viral antigen can be detected in the nasal cavity of HeV-infected horses.

<FIG>

In the Malaysian NiV outbreak, contact with pigs or fresh pig products was required for transmission of the virus to humans, with greater likelihood of transfer to those in direct contact with sick or dying pigs on farms or in abattoirs.(30),(111) The presence of NiV in the respiratory epithelium of naturally and experimentally infected pigs (Fig. ~~37.5~~14.1xB) indicated that virus probably spread to humans and within the pig population by aerosol or by direct contact with oropharyngeal or nasal secretions.(95),(97),(149) The presence of the virus in a wide range of organs indicates that humans may also have been infected during processes such as slaughtering or farrowing. In Bangladesh, pigs were excluded as potential sources of NiV on epidemiolog grounds, and human-to-human transmission was observed.(54),(62) The virus may have been transmitted to human index cases directly through contact with fruit bat secretions in contaminated fruit or date palm sap before circulation in the human population.(33),(43),(68),(85) Nosocomial transmission has been detected in some of the Bangladesh and India outbreaks.(9),(23),(85) In the Philippines NiV outbreak, Of the 17 case-patients, a total of 7 (41%) had participated in horse slaughtering and horse meat consumption; 3 (18%) had only consumed horse meat and but had no history of slaughtering or meat preparation; 5 (29%) case-patients had been exposed to other human casepatients but not to any horses. So it is possible that both horse-to-human and human-to-human transmission might have occurred. () For the Kerala NiV outbreak, while it remains undefined how the index case was exposed to NiV, it was evident that human-to-human transmission was the main driver for the spread of the virus in both family/community and hospital settings. ()

Commented [L25]: Ching et al., 2015 EID

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<H1>Clinical Features

<H2>Incubation Period

Based on the time interval between the last exposure to pigs and onset of disease, the incubation period for NiV ranged from 2 to 45 days; however, for 90% of patients, it was 2 weeks or less.(27),(28),(48) An estimate of 2 to 3 weeks was made based on the time interval between importation of pigs from NiV-affected areas of Malaysia and development of human disease at a Singaporean abattoir.(25) A mean incubation period of 9.4 days was calculated for four patients who had a fixed period of exposure.(27) In the occurrence of HeV in Australia in 2008, a detailed examination of exposure histories from two infected patients (one fatal) suggested a likely incubation period of 9 to 16 days, with exposure occurring some 3 days before the onset of symptoms of HeV infection in the horse.(115)

<H2>Acute Clinical Features

The first two patients infected with HeV presented with myalgia, headaches, lethargy, and vertigo. One patient recovered; however, the other developed pneumonitis, respiratory failure, renal failure, and arterial thrombosis, and died of cardiac arrest 7 days after admission to the hospital. Findings at autopsy were consistent with a viral infection; both lungs were congested, hemorrhagic, and filled with serous fluid, and the histology revealed focal necrotizing alveolitis with many giant cells, some syncytial formation, and viral inclusions.(130) A third case of HeV infection presented first with meningitis and a 12-day history of sore throat, headache, drowsiness, vomiting, and neck stiffness. After an apparent full recovery, this patient developed fatal encephalitis 13 months later and was admitted to the hospital with a generalized tonic-clonic seizure after 2 weeks of irritable mood and low back pain. Recurrent focal motor seizures occurred over the next 7 days, as did secondarily generalized seizures and low-grade fever, followed by dense right hemiplegia, signs of brain stem involvement, and depressed consciousness requiring intubation. The patient remained comatose and died 25 days after admission.(107) Two patients in 2008(115) presented with initial influenza-like illness, although soon after apparent clinical improvement and an absence of fever, encephalitis developed in both. Magnetic resonance imaging (MRI) revealed widespread cortical, subcortical, and deep white matter involvement, similar to the previous late-onset case of HeV encephalitis(107) and to NiV encephalitis cases.(78),(127)

With NiV, the mean duration of illness from the onset of symptoms to the nadir was 3 to 31 days, with an average of 6.9 days.(48) Most patients presented with acute encephalitis characterized by fever, headache, drowsiness, dizziness, myalgia, and vomiting, and more than 50% had a reduced level of consciousness.(27),(30),(48),(114) The major clinical signs included drowsiness, areflexia, segmental myoclonus, tachycardia, hypertension, pinpoint pupils, and an abnormal doll's eye reflex. Such clinical features as these suggested involvement of the brain stem and upper cervical spinal cord, and were observed more frequently in patients with a reduced level of consciousness.(48),(139) Patients who retained normal levels of consciousness throughout their illness recovered fully; however, only 15% with reduced levels of consciousness survived. Such neurologic manifestations are consistent with vasculitis-induced thrombosis in the brain and the direct infection of neurons.(29),(30),(114),(159) The multiple discrete lesions 1 to 5 mm in diameter in the cerebral white matter detected by MRI may be the site of such microinfarctions and are distinct from lesions caused by other viruses.(30),(82),(114),(127),(159) Although the predominant clinical features of NiV encephalitis derive from CNS involvement, a proportion of patients displayed pulmonary involvement, which presented as an atypical pneumonia with fever, cough, and headache.(27),(48),(114) The clinical presentation of NiV infections in Bangladesh is also predominantly a severe respiratory disease.(67)

<H2>Outcome of Infection

Most patients who survived acute NiV encephalitis made a full recovery; however, approximately 20% had residual neurologic deficits.(27),(48),(83) Neurologic sequelae included cognitive difficulties, tetraparesis, cerebellar signs, nerve palsies, and clinical depression. A few patients remained in a vegetative state. In patients with encephalitis who recovered, most brain lesions revealed by MRI disappeared or became smaller over a period of 12 to 18 months, although some remained unchanged during this period.(83) Approximately 7.5% of patients who recovered from acute encephalitis and 3.4% of those who experienced nonencephalitic or asymptomatic infection developed late neurologic disease.(48),(127),(138),(161) Relapse encephalitis and late-onset encephalitis presented several months to 4 years after the initial infection. Relapsed cases had elevated IgG, but not IgM, and no vasculitis, and unlike the situation in acute encephalitis, virus was not isolated from throat and nasal secretions.(30)(–)(32),(127),(159),(161)

The clinical features associated with relapse and late-onset encephalitis resembled those found with acute NiV encephalitis, although decreased incidence was seen of fever, coma, segmental myoclonus, and meningism and an increased occurrence of seizures and focal cortical signs compared with the acute manifestation of the disease.(138) The clinical, radiologic, and pathologic features of relapse NiV encephalitis resembled those of the patient who became infected with HeV, suffered mild, transient aseptic meningitis, and recovered but died of a fatal meningoencephalitis 13 months later.(107),(158) Most patients with relapse and late-onset encephalitis had only one neurologic episode, although some patients experienced two episodes separated by a mean of 7.6 months (6 weeks to 1 year).(138) The mortality rate associated with relapse and late-onset encephalitis at 18% was lower than that associated with acute encephalitis, at 30% to 40%. However, 61% of patients with relapse and late onset had further neurologic sequelae compared with 22% after acute encephalitis. Among NiV survivors in Bangladesh, some 30% have moderate to severe persistent neurologic dysfunction for years following acute infection.(128)

The demographics, clinical features, serology, and MRI of patients with relapsed and late-onset encephalitis were similar, suggesting that the two diseases have identical pathogenesis(138) and that the initial infection in late-onset encephalitis patients may not have been sufficiently severe to cause neurologic symptoms. MRI abnormalities similar to those observed in patients with acute encephalitis were also seen in 16% of asymptomatic patients, although the lesions were fewer in number.(139) The involvement of the cortex in relapse and late-onset encephalitis suggests a different pathologic mechanism compared with acute encephalitis. Relapse and late-onset encephalitis are considered to be caused by the recrudescence and rapid replication of virus that had persisted following acute or asymptomatic NiV infection.(138) NiV, however, was not isolated from CSF and brain tissue of patients with relapse and late-onset encephalitis.(138)

<H1>Diagnosis

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<H2>Laboratory Diagnosis

Virus isolation, EM, immuno-EM, immunohistochemistry (IHC), serology, and polymerase chain reaction (PCR) played key roles in the initial discovery of HeV(104) and NiV,(29) and they remain essential elements in a repertoire of procedures for the rapid and specific diagnosis of henipavirus infections in humans and animals.

During investigation of a suspected disease outbreak, attempts to grow henipaviruses may be initiated in a BSL3 laboratory. However, if a cytopathic effect (CPE) is observed and the growth of henipavirus is confirmed by PCR or immune staining, infected cultures should be handled under BSL4 conditions and subsequent work with live virus restricted to BSL4. Both HeV and NiV replicate in various cell lines—a feature that contributed to the efficiency with which they were isolated during the initial disease outbreak investigations.(29)·(104) Vero cells are commonly used, generating titers of virus as high as 10^8 infectious virions per milliliter.(28)·(34) In fatal cases, attempts should be made to isolate virus from brain, lung, kidney, and spleen.(34) For tissue specimens containing a high virus load, direct examination by immuno-EM and IHC can be very useful in providing early diagnosis. Various antibody reagents have been developed for this purpose, including polyclonal antisera, monospecific antibodies raised against recombinant antigens,(147) and monoclonal antibodies (mAb) raised against whole virions or vaccinia virus–expressed viral proteins.(71)·(140)·(141)·(153) Using HeV- or NiV-specific mAb, it is possible to differentiate between the two viruses.(141)·(153) Quantitative real-time PCR (TaqMan assay) has been the method of choice to detect viral materials in infected tissues because of its speed, specificity, and sensitivity. The first-generation henipavirus TaqMan assays are either HeV specific(134) or NiV specific.(52) Recently, several consensus henipavirus real-time PCR assays have been developed that target different conserved regions of the viral genome.(41) It should be cautioned that the current PCR tests may not work with new henipaviruses yet to be discovered, especially those from African bats, owing to expected greater genetic divergence than those detected in Australia and Asia.

For henipaviruses, serologic tests are important both during outbreak investigation and for disease surveillance. The virus neutralization test (VNT) is accepted as the reference standard.(34) Few laboratories, however, can conduct neutralization tests because of the requirement to handle live virus at BSL4. For surveillance and diagnostic purposes, three types of tests that do not require BSL4 containment have been developed:

1. *Enzyme-linked immunosorbent assay (ELISA):* Several ELISA-based tests have been reported for the detection of henipavirus antibodies.(34),(40),(73) For diagnosis of human infections, two different ELISA tests have been applied: an IgM capture ELISA for early diagnosis of infection and an indirect ELISA for detection of IgG antibodies.(34)
2. *Liquid protein array multiplex test:* A Luminex-based test based on recombinant soluble G proteins of HeV and NiV was developed that is capable of mimicking VNT with great sensitivity and differentiating between antibody responses of HeV versus NiV infection.(15)
3. *Pseudotype virus:* Different pseudotype systems carrying the henipavirus F and G proteins have been developed as a surrogate VNT for detecting henipavirus-specific antibodies.(72),(74),(137) Incorporation of reporter genes in these systems resulted in greater sensitivity and reproducibility.(72),(137)

<H1>Prevention and Control

<H2>Treatment

Ribavirin, which inhibits replication of HeV *in vitro*,(162) was used during the NiV outbreak in Malaysia in an open-label study in which 140 patients with encephalitis were given the drug, and 54 patients who presented before ribavirin became available or who refused treatment acted as controls.(26) Mortality in the treated group was 32% compared with 54% in the control group, representing a 35% reduction ($P = 0.011$). Duration of ventilation and total hospital stay were both significantly shorter in the ribavirin group ($P = 0.0002$ and <0.0001, respectively). In the absence of other therapies, ribavirin may be an option for treatment of henipavirus infections. However, two HeV-infected patients in 2008(115) were given a high-dose intravenous regimen of ribavirin, although basal concentrations appeared inadequate given the results of *in vitro* susceptibility testing of HeV, and the efficacy of ribavirin as therapy or prophylaxis in people remains at best uncertain. Chloroquine, an antimalarial drug, was first demonstrated to block the critical proteolytic processing needed for HeV F maturation and function.(109) Not surprisingly, the drug was later shown to inhibit NiV and HeV infection in cell culture experiments.(117) Chloroquine was administered along with ribavirin to one HeV-infected individual in 2009(6) with no apparent clinical benefit.

In vivo, ribavirin only delayed but did not prevent deaths caused by NiV and had no effect on HeV infection in a hamster model.(44),(47) Ribavirin treatment also only delayed disease onset by 1 to 2 days in African green monkeys challenged with HeV with no significant benefit for disease progression or outcome.(121) Chloroquine administration, either alone or in combination with ribavirin, had no therapeutic benefit in ferrets challenged with NiV or hamsters challenged with either NiV or HeV.(44),(110)

<H2>Vaccines and Passive Immunotherapy

No henipavirus vaccines are available at this time; however, studies suggest that vaccination may offer several viable anti-henipavirus strategies.(22),(38) NiV F and G glycoproteins expressed from vaccinia virus elicit neutralizing antibodies in mice and hamsters,(51),(136) with higher antibody titers generated in response to the G glycoprotein compared with the F glycoprotein. Hamsters were protected from lethal challenge with NiV following vaccination with vaccinia virus expressing either NiV F or G glycoprotein.(51) The challenge virus was capable of hyperimmunizing vaccinated animals, indicating that although the virus replicated, the presence of neutralizing antibodies ameliorated replication to an extent that limited infection and prevented clinical disease. Further, the protection afforded hamsters by passive transfer of anti-G and anti-F antibodies before a lethal NiV or HeV challenge confirmed both the importance of a humoral protective immune response to NiV.(51),(53)

HeV G glycoprotein has been expressed in a soluble form (sG-HeV) that retains many native characteristics and can elicit a potent cross-reactive neutralizing antibody response in rabbits.(14) Rabbit anti-HeV G antibodies neutralize both HeV and NiV in cell culture, displaying a slightly higher titer against the homologous virus. The nature and location of the neutralizing epitopes on the F glycoprotein have not been reported, although preliminary information is available for the HeV G glycoprotein.(153) Four neutralizing epitopes have been mapped on the globular head of the HeV G protein. Two are located on the base of the head and two on the top, in locations resembling those identified as neutralizing sites in other paramyxoviruses.

Immunization and challenge studies using recombinant sG-HeV in the cat model have demonstrated that the protein can illicit a completely protective immune response against NiV challenge,(101) even at a low-dose formulation with CpG and Alhydrogel and a two-dose protocol followed by oronasal challenge with 50,000 TCID$_{50}$ of NiV.(91) Further sG-HeV immunization studies have been completed in the ferret with a HeV challenge (J. Pallister and L-F. Wang, in submission) and in the African green monkey with a NiV challenge (T. Geisbert and C. Broder, in submission). In both of these investigations, complete protection from henipavirus-induced disease was achieved. The potential application of sG-HeV as an equine vaccine is being evaluated in Australia (D. Middleton, personal communication). These data suggest that a single vaccine (sG-HeV) may be effective against both HeV and NiV. Analysis of the antibody responses in sera from naturally infected or immunized sources has also shown that HeV-infected sources had high levels of NiV G cross-reactive antibodies, whereas NiV-infected individuals had limited cross-reactive antibodies to HeV G. Together, these data suggested that the HeV G stimulates a more cross-reactive immune response.(15)

The sG-HeV glycoprotein was also used to isolate HeV G-specific human mAbs. One human mAb (m102.4) was HeV and NiV cross-reactive and possessed extremely potent virus neutralizing activity.(167),(168) *In vivo* studies have demonstrated that m102.4 can protect animals from a lethal challenge with henipavirus as a postexposure application in the ferret model with NiV(19) and in the African green monkey model with HeV (T. Geisbert and C. Broder, in submission). In August 2009, m102.4 was used on a compassionate basis to save the life of an HeV-infected individual while in a coma (G. Playford, personal communication). Unfortunately, delivery and intravenous administration of only 100 mg of available antibody occurred after the onset of encephalitis, and the individual died shortly thereafter. During the 2010 HeV emergence, 11 people had potential exposure and 2 individuals considered at high risk. In this instance, the m102.4 antibody was given to 2 individuals prior to HeV diagnosis or the onset of clinical disease(5) at doses sufficient to achieve a high serum concentration. Both individuals have remained healthy. Altogether, these findings highlight the therapeutic potential of antibody-based passive transfer modalities for treating henipavirus exposure.

<H2>Peptide Inhibitors

The first potential henipavirus-specific therapeutic was shown to be a heptad peptide-based fusion inhibitor(17) analogous to the human immunodeficiency virus type 1 (HIV-1)-specific peptide, enfuvirtide (Fuzeon) approved by the Food and Drug Administration (FDA) in March 2003. The henipavirus F_1 glycoprotein resembles other fusion glycoproteins in having α-helical heptad repeat (HR) domains proximal to both the fusion peptide at the amino (N) terminus and the transmembrane domain near the carboxy (C) terminus of the protein. The HR domains are involved in the formation of a trimer-of-hairpins structure during or immediately following the fusion of virus and cell membranes that occurs during infection. Addition of exogenous peptide from either HR domain blocks formation of the trimer-of-hairpins and abrogates membrane fusion and entry of the viral genome into the cell.(16)·(17)·(38) These observations were followed up with testing cholesterol tagged HR-derived peptides in the hamster model of NiV infection.(118) The *in vivo* efficacy of peptide fusion inhibitors of henipavirus infection merits further investigation.

<H1>Perspective and Geographic Considerations

The high virulence of the henipaviruses and the requirement for BSL4 facilities have hampered investigations into the biology and pathogenesis of these novel paramyxoviruses. Recent investigations into the structure and function of henipavirus proteins expressed from cloned genes have provided insight into the functions of many henipavirus proteins in infected cells. It remains to be determined if all of the functional characteristics of the henipavirus proteins determined *in vitro* accurately reflect the role that they play in the cells of both terrestrial and chiropteran hosts.

Many questions relating to the ecology and biology of henipaviruses remain unanswered. Little doubt exists that *Pteropus* species of fruit bats are the major reservoir host of these viruses. With the wide geographic range of *Pteropus* species as overlapping populations, extending from islands in the South Pacific through Australia, and southern Asia to Pakistan, and with additional species on islands off the eastern coast of Africa, together with the cross-reactive serologic evidence of henipavirus presence,(61)·(81) it would seem that several other related viruses may remain to be identified.(43)·(56) The emergence of these and related viruses is probably associated with the destruction of the flying fox native habitats, driving the animals to seek food from orchards and ornamental trees in urban and periurban areas.

Thus, with continued deforestation, undoubtedly further outbreaks of HeV, NiV, and novel related members of the genus will occur. The mechanisms by which henipaviruses are transmitted between fruit bats and maintained within their colonies, as well as the pathways leading to the infection of spillover hosts, remain to be elucidated.

<H1>References

1. Aaronson DS, Horvath CM. A road map for those who don't know JAK-STAT. Science 2002;296(5573):1653–1655.
2. AbuBakar S, Chang LY, Ali AR, et al. Isolation and molecular identification of Nipah virus from pigs. Emerg Infect Dis 2004;10(12):2228–2230.
3. Andrejeva J, Childs KS, Young DF, et al. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. Proc Natl Acad Sci U S A 2004;101(49):17264–17269.
4. Anonymous. Hendra virus—Australia (Queensland). ProMED archive no. 20041214.3307. Brookline, MA: International Society for Infectious Diseases; 2004.
5. Anonymous. Hendra virus, equine—Australia (05): (QL) human exposure. ProMED archive no. 20100527.1761. Brookline, MA: International Society for Infectious Diseases; 2010.
6. Anonymous. Hendra virus, human, equine—Australia (05): Queensland. ProMED archive no. 20090910.3189. Brookline, MA: International Society for Infectious Diseases; 2009.
7. Anonymous. Nipah encephalitis, human—Bangladesh (03). ProMED archive no. 20110218.0539. Brookline, MA: International Society for Infectious Diseases; 2011.
8. Anonymous. Nipah encephalitis, human—Bangladesh: Rangpur (05). ProMED archive no. 20110308.0756. Brookline, MA: International Society for Infectious Diseases; 2011.
9. Arankalle VA, Bandyopadhyay BT, Ramdasi AY, et al. Genomic characterization of Nipah virus, West Bengal, India, during an intrafamilial outbreak. Emerg Infect Dis 2011;17(5):907–909.
10. Bishop KA, Broder CC. Hendra and Nipah: lethal zoonotic paramyxoviruses. In: Scheld WM, Hammer SM, Hughes JM, eds. Emerging Infections. Washington, DC: American Society for Microbiology; 2008:155–187.
11. Bishop KA, Stantchev TS, Hickey AC, et al. Identification of Hendra virus G glycoprotein residues that are critical for receptor binding. J Virol 2007;81(11):5893–5901.
12. Boehme KW, Compton T. Innate sensing of viruses by toll-like receptors. J Virol 2004;78(15):7867–7873.
13. Bonaparte MI, Dimitrov AS, Bossart KN, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. Proc Natl Acad Sci U S A 2005;102(30):10652–10657.
14. Bossart KN, Crameri G, Dimitrov AS, et al. Receptor binding, fusion inhibition and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. J Virol 2005;79(11):6690–6702.
15. Bossart KN, McEachern JA, Hickey AC, et al. Neutralization assays for differential henipavirus serology using Bio-Plex protein array systems. J Virol Methods 2007;142(1–2):29–40.

16. Bossart KN, Mungall BA, Crameri G, et al. Inhibition of Henipavirus fusion and infection by heptad-derived peptides of the Nipah virus fusion glycoprotein. Virol J 2005;2:57.
17. Bossart KN, Wang LF, Eaton BT, et al. Functional expression and membrane fusion tropism of the envelope glycoproteins of Hendra virus. Virology 2001;290(1):121–135.
18. Bossart KN, Wang LF, Flora MN, et al. Membrane fusion tropism and heterotypic functional activities of the Nipah virus and Hendra virus envelope glycoproteins. J Virol 2002;76(22):11186–11198.
19. Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute nipah virus infection. PLoS Pathog 2009;5(10):e1000642.
20. Bowden TA, Aricescu AR, Gilbert RJ, et al. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. Nat Struct Mol Biol 2008;15(6):567–572.
21. Bowden TA, Crispin M, Harvey DJ, et al. Dimeric architecture of the Hendra virus attachment glycoprotein: evidence for a conserved mode of assembly. J Virol 2010;84(12):6208–6217.
22. Broder CC. Therapeutics and vaccines against Hendra and Nipah viruses. In: Levine MM, Dougan G, Good MF, et al, eds. New Generation Vaccines. 4th ed. New York: Informa Healthcare USA; 2010:885–894.
23. Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006;12(2):235–240.
24. Chan YP, Chua KB, Koh CL, et al. Complete nucleotide sequences of Nipah virus isolates from Malaysia. J Gen Virol 2001;82(Pt 9):2151–2155.
25. Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000;181(5):1760–1763.
26. Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001;49(6):810–813.
27. Chong HT, Kunjapan SR, Thayaparan T, et al. Nipah encephalitis outbreak in Malaysia, clinical features in patients from Seremban. Can J Neurol Sci 2002;29(1):83–87.
28. Chua KB. Nipah virus outbreak in Malaysia. J Clin Virol 2003;26(3):265–275.
29. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000;288(5470):1432–1435.
30. Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999;354(9186):1257–1259.
31. Chua KB, Lam SK, Goh KJ, et al. The presence of Nipah virus in respiratory secretions and urine of patients during an outbreak of Nipah virus encephalitis in Malaysia. J Infect 2001;42(1):40–43.
32. Chua KB, Lam SK, Tan CT, et al. High mortality in Nipah encephalitis is associated with presence of virus in cerebrospinal fluid. Ann Neurol 2000;48(5):802–805.
33. Chua KB, Lek Koh C, Hooi PS, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes Infect 2002;4(2):145–151.
34. Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001;3(4):289–295.

35. Diederich S, Moll M, Klenk HD, et al. The Nipah virus fusion protein is cleaved within the endosomal compartment. J Biol Chem 2005;280(33):29899–29903.
36. Drexler JF, Corman VM, Gloza-Rausch F, et al. Henipavirus RNA in African bats. PLoS One. 2009;4(7):e6367.
37. Eaton BT, Broder CC, Middleton D, et al. Hendra and Nipah viruses: different and dangerous. Nat Rev Microbiol 2006;4(1):23–35.
38. Eaton BT, Broder CC, Wang LF. Hendra and Nipah viruses: pathogenesis and therapeutics. Curr Mol Med 2005;5(8):805–816.
39. Epstein JH, Prakash V, Smith CS, et al. Henipavirus infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008;14(8):1309–1311.
40. Eshaghi M, Tan WS, Mohidin TB, et al. Nipah virus glycoprotein: production in baculovirus and application in diagnosis. Virus Res 2004;106(1):71–76.
41. Feldman KS, Foord A, Heine HG, et al. Design and evaluation of consensus PCR assays for henipaviruses. J Virol Methods 2009;161(1):52–57.
42. Field H, Young P, Yob JM, et al. The natural history of Hendra and Nipah viruses. Microbes Infect 2001;3(4):307–314.
43. Field HE, Mackenzie JS, Daszak P. Henipaviruses: emerging paramyxoviruses associated with fruit bats. Curr Top Microbiol Immunol 2007;315:133–159.
44. Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010;91(Pt 3):765–772.
45. Gale NW, Baluk P, Pan L, et al. Ephrin-B2 selectively marks arterial vessels and neovascularization sites in the adult, with expression in both endothelial and smooth-muscle cells. Dev Biol 2001;230(2):151–160.
46. Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010;5(5):e10690.
47. Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006;50(5):1768–1772.
48. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med 2000;342(17):1229–1235.
49. Goldsmith CS, Whistler T, Rollin PE, et al. Elucidation of Nipah virus morphogenesis and replication using ultrastructural and molecular approaches. Virus Res 2003;92(1):89–98.
50. Goodbourn S, Didcock L, Randall RE. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J Gen Virol 2000;81(Pt 10):2341–2364.
51. Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004;78(2):834–840.
52. Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004;120(2):229–237.
53. Guillaume V, Wong KT, Looi RY, et al. Acute Hendra virus infection: analysis of the pathogenesis and passive antibody protection in the hamster model. Virology 2009;387(2):459–465.

54. Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infect Dis 2007;13(7):1031–1037.
55. Halpin K, Bankamp B, Harcourt BH, et al. Nipah virus conforms to the rule of six in a minigenome replication assay. J Gen Virol 2004;85(Pt 3):701–707.
56. Halpin K, Young PL, Field HE, et al. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. J Gen Virol 2000;81(Pt 8):1927–1932.
57. Harcourt BH, Lowe L, Tamin A, et al. Genetic characterization of Nipah virus, Bangladesh, 2004. Emerg Infect Dis 2005;11(10):1594–1597.
58. Harcourt BH, Tamin A, Halpin K, et al. Molecular characterization of the polymerase gene and genomic termini of nipah virus. Virology 2001;287(1):192–201.
59. Harcourt BH, Tamin A, Ksiazek TG, et al. Molecular characterization of Nipah virus, a newly emergent paramyxovirus. Virology 2000;271(2):334–349.
60. Harit AK, Ichhpujani RL, Gupta S, et al. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. Indian J Med Res 2006;123(4):553–560.
61. Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PLoS One 2008;3(7):e2739.
62. Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010;138(11):1630–1636.
63. Hooper P, Zaki S, Daniels P, et al. Comparative pathology of the diseases caused by Hendra and Nipah viruses. Microbes Infect 2001;3(4):315–322.
64. Hooper PT, Ketterer PJ, Hyatt AD, et al. Lesions of experimental equine morbillivirus pneumonia in horses. Vet Pathol 1997;34(4):312–322.
65. Hooper PT, Westbury HA, Russell GM. The lesions of experimental equine morbillivirus disease in cats and guinea pigs. Vet Pathol 1997;34(4):323–329.
66. Horvath CM. Silencing STATs: lessons from paramyxovirus interferon evasion. Cytokine Growth Factor Rev 2004;15(2–3):117–127.
67. Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008;46(7):977–984.
68. Hsu VP, Hossain MJ, Parashar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004;10(12):2082–2087.
69. Hyatt AD, Zaki SR, Goldsmith CS, et al. Ultrastructure of Hendra virus and Nipah virus within cultured cells and host animals. Microbes Infect 2001;3(4):297–306.
70. Iehle C, Razafitrimo G, Razainirina J, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007;13(1):159–161.
71. Imada T, Abdul Rahman MA, Kashiwazaki Y, et al. Production and characterization of monoclonal antibodies against formalin-inactivated Nipah virus isolated from the lungs of a pig. J Vet Med Sci 2004;66(1):81–83.
72. Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009;160(1–2):7–13.
73. Kashiwazaki Y, Na YN, Tanimura N, et al. A solid-phase blocking ELISA for detection of antibodies to Nipah virus. J Virol Methods 2004;121(2):259–261.
74. Khetawat D, Broder CC. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. Virol J 2010;7:312.

75. Lamb RA, Collins PL, Kolakofsky D, et al. Family Paramyxoviridae. In: Fauquet CM, Mayo J, Maniloff J, et al, eds. Virus Taxonomy: 8th Report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press; 2005:655–668.
76. Lamb RA, Parks GD. *Paramyxoviridae:* the viruses and their replication. In: Knipe DM, Griffin DE, Lamb RA, et al, eds. Fields Virology. Philadelphia: Lippincott Williams & Wilkins; 2007:1449–1496.
77. Langedijk JP, Daus FJ, van Oirschot JT. Sequence and structure alignment of Paramyxoviridae attachment proteins and discovery of enzymatic activity for a morbillivirus hemagglutinin. J Virol 1997;71(8):6155–6167.
78. Lee KE, Umapathi T, Tan CB, et al. The neurological manifestations of Nipah virus encephalitis, a novel paramyxovirus. Ann Neurol 1999;46(3):428–432.
79. Levy DE, Marie IJ. RIGging an antiviral defense—it's in the CARDs. Nat Immunol 2004;5(7):699–701.
80. Li M, Embury-Hyatt C, Weingartl HM. Experimental inoculation study indicates swine as a potential host for Hendra virus. Vet Res 2010;41(3):33.
81. Li Y, Wang J, Hickey AC, et al. Antibodies to Nipah or Nipah-like viruses in bats, China. Emerg Infect Dis 2008;14(12):1974–1976.
82. Lim CC, Lee KE, Lee WL, et al. Nipah virus encephalitis: serial MR study of an emerging disease. Radiology 2002;222(1):219–226.
83. Lim CC, Lee WL, Leo YS, et al. Late clinical and magnetic resonance imaging follow up of Nipah virus infection. J Neurol Neurosurg Psychiatry 2003;74(1):131–133.
84. Lo MK, Miller D, Aljofan M, et al. Characterization of the antiviral and inflammatory responses against Nipah virus in endothelial cells and neurons. Virology 2010;404(1):78–88.
85. Luby SP, Gurley ES, Hossain MJ. Transmission of human infection with Nipah virus. Clin Infect Dis 2009;49(11):1743–1748.
86. Luby SP, Hossain MJ, Gurley ES, et al. Recurrent zoonotic transmission of Nipah virus into humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009;15(8):1229–1235.
87. Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006;12(12):1888–1894.
88. Marianneau P, Guillaume V, Wong T, et al. Experimental infection of squirrel monkeys with nipah virus. Emerg Infect Dis 2010;16(3):507–510.
89. Marie I, Durbin JE, Levy DE. Differential viral induction of distinct interferon-alpha genes by positive feedback through interferon regulatory factor-7. EMBO J 1998;17(22):6660–6669.
90. Marsh GA, Todd S, Foord A, et al. Genome sequence conservation of Hendra virus isolates during spillover to horses, Australia. Emerg Infect Dis 2010;16(11):1767–1769.
91. McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008;26(31):3842–3852.
92. Meulendyke KA, Wurth MA, McCann RO, et al. Endocytosis plays a critical role in proteolytic processing of the hendra virus fusion protein. J Virol 2005;79(20):12643–12649.

93. Michalski WP, Crameri G, Wang L, et al. The cleavage activation and sites of glycosylation in the fusion protein of Hendra virus. Virus Res 2000;69(2):83–93.
94. Middleton DJ, Morrissy CJ, van der Heide BM, et al. Experimental Nipah virus infection in pteropid bats (Pteropus poliocephalus). J Comp Pathol 2007;136(4):266–272.
95. Middleton DJ, Westbury HA, Morrissy CJ, et al. Experimental Nipah virus infection in pigs and cats. J Comp Pathol 2002;126(2–3):124–136.
96. Miller PJ, Boyle DB, Eaton BT, et al. Full-length genome sequence of Mossman virus, a novel paramyxovirus isolated from rodents in Australia. Virology 2003;317(2):330–344.
97. Mohd Nor MN, Gan CH, Ong BL. Nipah virus infection of pigs in peninsular Malaysia. Rev Sci Tech 2000;19(1):160–165.
98. Moll M, Diederich S, Klenk HD, et al. Ubiquitous activation of the nipah virus fusion protein does not require a basic amino acid at the cleavage site. J Virol 2004;78(18):9705–9712.
99. Montgomery JM, Hossain MJ, Gurley E, et al. Risk factors for Nipah virus encephalitis in Bangladesh. Emerg Infect Dis 2008;14(10):1526–1532.
100. Mounts AW, Kaur H, Parashar UD, et al. A cohort study of health care workers to assess nosocomial transmissibility of Nipah virus, Malaysia, 1999. J Infect Dis 2001;183(5):810–813.
101. Mungall BA, Middleton D, Crameri G, et al. Feline model of acute Nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. J Virol 2006;80(24):12293–12302.
102. Mungall BA, Middleton D, Crameri G, et al. Vertical transmission and fetal replication of Nipah virus in an experimentally infected cat. J Infect Dis 2007;196(6):812–816.
103. Murray K, Rogers R, Selvey L, et al. A novel morbillivirus pneumonia of horses and its transmission to humans. Emerg Infect Dis 1995;1(1):31–33.
104. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. Science 1995;268(5207):94–97.
105. Negrete OA, Levroney EL, Aguilar HC, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. Nature 2005;436(7049):401–405.
106. Negrete OA, Wolf MC, Aguilar HC, et al. Two key residues in EphrinB3 are critical for its use as an alternative receptor for Nipah virus. PLoS Pathog 2006;2(2):e7.
107. O'Sullivan JD, Allworth AM, Paterson DL, et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. Lancet 1997;349(9045):93–95.
108. Pager CT, Dutch RE. Cathepsin L is involved in proteolytic processing of the Hendra virus fusion protein. J Virol 2005;79(20):12714–12720.
109. Pager CT, Wurth MA, Dutch RE. Subcellular localization and calcium and pH requirements for proteolytic processing of the Hendra virus fusion protein. J Virol 2004;78(17):9154–9163.
110. Pallister J, Middleton D, Crameri G, et al. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. J Virol 2009;83(22):11979–11982.
111. Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998–1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000;181(5):1755–1759.

112. Park MS, Shaw ML, Munoz-Jordan J, et al. Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus V, W, and C proteins. J Virol 2003;77(2):1501–1511.
113. Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. Cell 2008;133(1):38–52.
114. Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999;354(9186):1253–1256.
115. Playford EG, McCall B, Smith G, et al. Human Hendra virus encephalitis associated with equine outbreak, Australia, 2008. Emerg Infect Dis 2010;16(2):219–223.
116. Poch O, Blumberg BM, Bougueleret L, et al. Sequence comparison of five polymerases (L proteins) of unsegmented negative-strand RNA viruses: theoretical assignment of functional domains. J Gen Virol 1990;71(Pt 5):1153–1162.
117. Porotto M, Orefice G, Yokoyama CC, et al. Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy. J Virol 2009;83(10):5148–5155.
118. Porotto M, Rockx B, Yokoyama CC, et al. Inhibition of Nipah virus infection in vivo: targeting an early stage of paramyxovirus fusion activation during viral entry. PLoS Pathog 2010;6(10):e1001168.
119. Rahman SA, Hassan SS, Olival KJ, et al. Characterization of Nipah virus from naturally infected Pteropus vampyrus bats, Malaysia. Emerg Infect Dis 2010;16(12):1990–1993.
120. Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005;11(7):1042–1047.
121. Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010;84(19):9831–9839.
122. Rodriguez JJ, Cruz CD, Horvath CM. Identification of the nuclear export signal and STAT-binding domains of the Nipah virus V protein reveals mechanisms underlying interferon evasion. J Virol 2004;78(10):5358–5367.
123. Rodriguez JJ, Parisien JP, Horvath CM. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. J Virol 2002;76(22):11476–11483.
124. Rodriguez JJ, Wang LF, Horvath CM. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. J Virol 2003;77(21):11842–11845.
125. Rogers RJ, Douglas IC, Baldock FC, et al. Investigation of a second focus of equine morbillivirus infection in coastal Queensland. Aust Vet J 1996;74(3):243–244.
126. Salah Uddin Khan M, Hossain J, Gurley ES, et al. Use of infrared camera to understand bats' access to date palm sap: implications for preventing Nipah virus transmission. Ecohealth 2010;7(4):517–525.
127. Sarji SA, Abdullah BJ, Goh KJ, et al. MR imaging features of Nipah encephalitis. AJR Am J Roentgenol 2000;175(2):437–442.
128. Sejvar JJ, Hossain J, Saha SK, et al. Long-term neurological and functional outcome in Nipah virus infection. Ann Neurol 2007;62(3):235–242.

129. Selvey L, Taylor R, Arklay A, et al. Screening of bat carers for antibodies to equine morbillivirus. Commun Dis Intell 1996;20(22):477–478.
130. Selvey LA, Wells RM, McCormack JG, et al. Infection of humans and horses by a newly described morbillivirus. Med J Aust 1995;162(12):642–645.
131. Sendow I, Field HE, Curran J, et al. Henipavirus in Pteropus vampyrus bats, Indonesia. Emerg Infect Dis 2006;12(4):711–712.
132. Shaw ML, Cardenas WB, Zamarin D, et al. Nuclear localization of the Nipah virus W protein allows for inhibition of both virus- and toll-like receptor 3-triggered signaling pathways. J Virol 2005;79(10):6078–6088.
133. Shaw ML, Garcia-Sastre A, Palese P, et al. Nipah virus V and W proteins have a common STAT1-binding domain yet inhibit STAT1 activation from the cytoplasmic and nuclear compartments, respectively. J Virol 2004;78(11):5633–5641.
134. Smith IL, Halpin K, Warrilow D, et al. Development of a fluorogenic RT-PCR assay (TaqMan) for the detection of Hendra virus. J Virol Methods 2001;98(1):33–40.
135. Su AI, Wiltshire T, Batalov S, et al. A gene atlas of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci U S A 2004;101(16):6062–6067.
136. Tamin A, Harcourt BH, Ksiazek TG, et al. Functional properties of the fusion and attachment glycoproteins of Nipah virus. Virology 2002;296:190–200.
137. Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009;160(1–2):1–6.
138. Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002;51(6):703–708.
139. Tan KS, Sarji SA, Tan CT, et al. Patients with asymptomatic Nipah virus infection may have abnormal cerebral MR imaging. Neurol J Southeast Asia 2000;5:69–73.
140. Tanimura N, Imada T, Kashiwazaki Y, et al. Monoclonal antibody-based immunohistochemical diagnosis of malaysian Nipah virus infection in pigs. J Comp Pathol 2004;131(2–3):199–206.
141. Tanimura N, Imada T, Kashiwazaki Y, et al. Reactivity of anti-Nipah virus monoclonal antibodies to formalin-fixed, paraffin-embedded lung tissues from experimental Nipah and Hendra virus infections. J Vet Med Sci 2004;66(10):1263–1266.
142. Virtue ER, Marsh GA, Wang LF. Interferon signaling remains functional during henipavirus infection of human cell lines. J Virol 2011;85(8):4031–4034.
143. Vogt C, Eickmann M, Diederich S, et al. Endocytosis of the Nipah virus glycoproteins. J Virol 2005;79(6):3865–3872.
144. Wacharapluesadee S, Hemachudha T. Duplex nested RT-PCR for detection of Nipah virus RNA from urine specimens of bats. J Virol Methods 2007;141(1):97–101.
145. Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005;11(12):1949–1951.
146. Wang L, Harcourt BH, Yu M, et al. Molecular biology of Hendra and Nipah viruses. Microbes Infect 2001;3(4):279–287.
147. Wang LF, Michalski WP, Yu M, et al. A novel P/V/C gene in a new member of the Paramyxoviridae family, which causes lethal infection in humans, horses, and other animals. J Virol 1998;72(2):1482–1490.

148. Wang LF, Yu M, Hansson E, et al. The exceptionally large genome of Hendra virus: support for creation of a new genus within the family Paramyxoviridae. J Virol 2000;74(21):9972–9979.
149. Weingartl H, Czub S, Copps J, et al. Invasion of the central nervous system in a porcine host by nipah virus. J Virol 2005;79(12):7528–7534.
150. Weingartl HM, Berhane Y, Caswell JL, et al. Recombinant nipah virus vaccines protect pigs against challenge. J Virol 2006;80(16):7929–7938.
151. Westbury HA, Hooper PT, Brouwer SL, et al. Susceptibility of cats to equine morbillivirus. Aust Vet J 1996;74(2):132–134.
152. Westbury HA, Hooper PT, Selleck PW, et al. Equine morbillivirus pneumonia: susceptibility of laboratory animals to the virus. Aust Vet J 1995;72(7):278–279.
153. White JR, Boyd V, Crameri GS, et al. Location of, immunogenicity of and relationships between neutralization epitopes on the attachment protein (G) of Hendra virus. J Gen Virol 2005;86(Pt 10):2839–2848.
154. Williamson MM, Hooper PT, Selleck PW, et al. A guinea-pig model of Hendra virus encephalitis. J Comp Pathol 2001;124(4):273–279.
155. Williamson MM, Hooper PT, Selleck PW, et al. Experimental hendra virus infectionin pregnant guinea-pigs and fruit Bats (Pteropus poliocephalus). J Comp Pathol 2000;122(2–3):201–207.
156. Williamson MM, Hooper PT, Selleck PW, et al. Transmission studies of Hendra virus (equine morbillivirus) in fruit bats, horses and cats. Aust Vet J 1998;76(12):813–818.
157. Wong KT, Grosjean I, Brisson C, et al. A golden hamster model for human acute Nipah virus infection. Am J Pathol 2003;163(5):2127–2137.
158. Wong KT, Robertson T, Ong BB, et al. Human Hendra virus infection causes acute and relapsing encephalitis. Neuropathol Appl Neurobiol 2009;35(3):296–305.
159. Wong KT, Shieh WJ, Kumar S, et al. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol 2002;161(6):2153–2167.
160. Wong KT, Shieh WJ, Zaki SR, et al. Nipah virus infection, an emerging paramyxoviral zoonosis. Springer Semin Immunopathol 2002;24(2):215–228.
161. Wong SC, Ooi MH, Wong MN, et al. Late presentation of Nipah virus encephalitis and kinetics of the humoral immune response. J Neurol Neurosurg Psychiatry 2001;71(4):552–554.
162. Wright PJ, Crameri G, Eaton BT. RNA synthesis during infection by Hendra virus: an examination by quantitative real-time PCR of RNA accumulation, the effect of ribavirin and the attenuation of transcription. Arch Virol 2005;150(3):521–532.
163. Xu K, Rajashankar KR, Chan YP, et al. Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. Proc Natl Acad Sci U S A 2008;105(29):9953–9958.
164. Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7(3):439–441.
165. Young PL, Halpin K, Selleck PW, et al. Serologic evidence for the presence in Pteropus bats of a paramyxovirus related to equine morbillivirus. Emerg Infect Dis 1996;2(3):239–240.

166. Yu M, Hansson E, Langedijk JP, et al. The attachment protein of Hendra virus has high structural similarity but limited primary sequence homology compared with viruses in the genus Paramyxovirus. Virology 1998;251(2):227–233.

167. Zhu Z, Bossart KN, Bishop KA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. J Infect Dis 2008;197(6):846–853.

168. Zhu Z, Dimitrov AS, Bossart KN, et al. Potent neutralization of Hendra and Nipah viruses by human monoclonal antibodies. J Virol 2006;80(2):891–899.

Figure 37.1. Syncytia induced in vero cells 24 hours after infection by Hendra virus (HeV) (A) and Nipah virus (NiV) (B). Methanol-fixed infected cells were labeled with rabbit monospecific antiserum to the HeV P protein and fluorescein-conjugated goat antirabbit immunoglobulin G. P protein is detected in extensive perinuclear ribonucleoprotein complexes (*small arrow*) and in discrete regularly shaped arrays (*large arrow*) distributed throughout the cytoplasm and believed to be sites of virus egress from the cell. Nuclei are indicated by *chevrons.*

Figure 37.2. A: Electron micrograph of Nipah virus (NiV)-infected Vero cells showing tubule-like structures, both in the cytoplasm and in a maturing virus particle (*arrows*). **B:** Electron micrograph of negatively stained Hendra virus (HeV) displaying the double fringe at the virus envelope (*small arrow*) and the herringbone nucleocapsids (*large arrow*). (Courtesy of Dr. Alex Hyatt, CSIRO Australian Animal Health Laboratory.)

Figure 37.3. Genome size and organization of Hendra virus compared with type species of each of the other four classified genera in the subfamily *Paramyxovirinae.* Genome lengths (in nucleotides) are given in brackets after each virus.

Figure 37.4. Phylogenetic relationship between the N protein sequences of henipavirus isolates from different geographic locations. The virus nomenclature abbreviation follows the following format: virus/country origin/host/year/isolate name. For example, NiV/KH/BA/2004/KHM represents a Nipah virus isolated from bats in Cambodia in 2004 with KHM as its isolate name.

Figure 37.5. Immunoperoxidase detection of viral antigen in henipavirus-infected tissues. **A:** Staining of Hendra virus (HeV) antigen (N protein-specific antibody) within the wall of superficial arteriole in the submucosa of the nasal cavity in a HeV-infected horse. **B:** Immunolabeling (using anti–Nipah virus rabbit serum) within the lung of a pig infected with Nipah virus. Involvement of bronchial epithelium and airway debris is noted. (Courtesy of Dr. Deborah Middleton, CSIRO Australian Animal Health Laboratory.)

Table 37.1. Summary of Henipaviruses Isolated from Different Species and Geographic Locations

		Isolation details			
Virus	Isolate name and	Year	Country	Host species/tissue	Reference

	number			
Hendra	Horse-1	1994 Australia	Horse/spleen, lung	(104)
	VR-1 Bat-1-1	1994 Australia 1996 Australia	Human/lung, liver, kidney, spleen Grey-headed flying fox (*Pteropus poliocephalus*)/uterine fluid	(104) (56)
	Bat-1-2 Bat-2 Murwillumbah Clifton Beach Peachester Redlands Proserpine	1996 Australia 1996 Australia 2006 Australia 2007 Australia 2008 Australia 2008 Australia 2008 Australia	Grey-headed flying fox (*P. poliocephalus*)/fetus Black flying fox (*Pteropus alecto*)/fetal lung Horse/lung Horse/lung Horse/blood Horse/lung Horse/lung	(56) (56) (90) (90) (90) (90) (90)
Nipah	PKL	1999 Malaysia	Human/cerebral spinal fluid	(29), (30)
	EKK	1999 Malaysia	Human/cerebral spinal fluid	(29), (30)
	WWS	1999 Malaysia	Human/cerebral spinal fluid	(29), (30)
	UMMC1	1999 Malaysia	Human/cerebral spinal fluid	(24)
	UMMC2	1999 Malaysia	Human/throat secretion	(24)
	UM-0128	1999 Malaysia	Human	(2)
	VRI-0626	1999 Malaysia	Pig/lung	(2)
	VRI-1413	1999 Malaysia	Pig/lung	(2)
	VRI-2794	1999 Malaysia	Pig/lung	(2)
	B13/6-18 B13/6-43	2000 Malaysia 2000 Malaysia	Bat/pooled urine Bat/pooled urine	(33) (33)
	JA13/6-4	2000 Malaysia	Bat/partially eaten jambu air fruit	(33)
	Rajbari-1	2004 Bangladesh	Human/oropharyngeal	(57)
	Rajbari-2	2004 Bangladesh	Human/cerebral spinal fluid	(57)

	Faridpur	2004	Bangladesh	Human/urine	(57)
	Rajshahi	2004	Bangladesh	Human/urine	(57)
	CSUR381	2004	Cambodia	Flying fox (*Pteropus lylei*)/urine	(120)
	CSUR382	2004	Cambodia	Flying fox (*P. lylei*)/urine	(120)

1. Summary of Hendra virus incidents in horses. *Business Queensland* [Web Page]. 2018; https://www.business.qld.gov.au/industries/service-industries-professionals/service-industries/veterinary-surgeons/guidelines-hendra/incident-summary. Accessed 20 Sep 2018.
2. Arunkumar G, Chandni R, Mourya DT, et al. Outbreak investigation of Nipah Virus Disease in Kerala, India, 2018. *J Infect Dis.* 2018.
3. Ching PK, de los Reyes VC, Sucaldito MN, et al. Outbreak of henipavirus infection, Philippines, 2014. *Emerg Infect Dis.* 2015;21(2):328-331.
4. Amarasinghe GK, Arechiga Ceballos NG, Banyard AC, et al. Taxonomy of the order Mononegavirales: update 2018. *Arch Virol.* 2018;163(8):2283-2294.
5. Maes P, Amarasinghe GK, Ayllon MA, et al. Taxonomy of the order Mononegavirales: second update 2018. *Arch Virol.* 2019.
6. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog.* 2012;8(8):e1002836.
7. Drexler JF, Corman VM, Muller MA, et al. Bats host major mammalian paramyxoviruses. *Nat Commun.* 2012;3:796.
8. Wu Z, Yang L, Yang F, et al. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis.* 2014;20(6):1064-1066.
9. Hino K, Sato H, Sugai A, Kato M, Yoneda M, Kai C. Downregulation of Nipah virus N mRNA occurs through interaction between its 3' untranslated region and hnRNP D. *J Virol.* 2013;87(12):6582-6588.
10. Anderson DE, Islam A, Crameri G, et al. Isolation and Full-Genome Characterization of Nipah Viruses from Bats, Bangladesh. *Emerg Infect Dis.* 2019;25(1):166-170.
11. Wacharapluesadee S, Samseeneam P, Phermpool M, et al. Molecular characterization of Nipah virus from Pteropus hypomelanus in Southern Thailand. *Virol J.* 2016;13:53.
12. Freiberg A, Dolores LK, Enterlein S, Flick R. Establishment and characterization of plasmid-driven minigenome rescue systems for Nipah virus: RNA polymerase I- and T7-catalyzed generation of functional paramyxoviral RNA. *Virology.* 2008;370(1):33-44.
13. Liang B, Li Z, Jenni S, et al. Structure of the L Protein of Vesicular Stomatitis Virus from Electron Cryomicroscopy. *Cell.* 2015;162(2):314-327.
14. Lee B, Ataman ZA. Modes of paramyxovirus fusion: a Henipavirus perspective. *Trends Microbiol.* 2011;19(8):389-399.
15. Lee B, Pernet O, Ahmed AA, Zeltina A, Beaty SM, Bowden TA. Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. *Proc Natl Acad Sci U S A.* 2015;112(17):E2156-2165.
16. Rissanen I, Ahmed AA, Azarm K, et al. Idiosyncratic Mojiang virus attachment glycoprotein directs a host-cell entry pathway distinct from genetically related henipaviruses. *Nat Commun.* 2017;8:16060.

1

From: Lee, Benhur on behalf of Lee, Benhur <benhur.lee@mssm.edu>
To: LinFa Wang Linfa
Cc: Broder, Christopher
Subject: Your Sections
Date: Tuesday, February 19, 2019 8:18:31 AM
Attachments: CH14-Henipavirus-LW190215 vBHL.docx
HNV_Fields Ref.enlp.zip

Dear LinFa,

I started reading through your sections and adding/editing what I could but ran into some problems and inconsistencies with our agreed upon outline, I had to stop. I simply can’t do everything myself - I also have meetings to chair and conferences to go to.

For now, I’m sending back to you what I have done and ask for your help in giving me back a document that I can deal with more easily. I almost finished everything up to my own section on Pathogenesis _ I can't add my own section yet because of the **referencing conflicts** - in fact I hardly have had time for my own section.

(1) I have added all the new references you requested and then some more (these are are highlighted in GREEN in the main text). This was easy using my endnote file.

- The most important thing is that all the old references in big brackets/parentheses (ref)(ref)(ref)(ref) are not linked to the master endnote file I sent out. So, when I configure my bibliography, I can only see the new refs I added in, which is about 16 for now.
- I simply do **not** have the time or energy to go through 168 (ref)(ref)(ref)(ref) by myself and individually replace them with linked citations from my endnote file (I’m sure you weren't expecting me to).
- When Chris gave me his section, I could easily format and configure the bibliography, which will help when I put everything together. That was the plan.
- PLEASE replace all the (ref) (ref) (ref) (ref) in the main text with the refs from my Endnote file. Or have someone do it for you. You and Chris will see what I mean when you open the document and see my comments/notes.

(2) To make things easier to see, I have accepted all your comments (on the side column) but left your original changes (it appears orange/peach colored in my word document. My changes/edits are dark red/maroon. It’ll be obvious. I added the most text about the 3’IUTR and on the RBP section. Then, I ran out of steam. It’s extremely distracting and hard not to be able to format all those (ref) (ref) (ref) (ref) !

(3) I made some suggestions about what Tables to put online, etc.

(4) Finally, parts of what you wrote does not conform to the section outline you sent us, and which I painstakingly clarified and confirmed multiple times by sending out clearly color coded outline and Figure/Table numbering. For example, I thought the genetic diversity section was suppose to be in Section D5 (Epidemiology), so your **Fig. 14.4 in the text** is actually **Fig. 14.11** in my master powerpoint. I thought we al agreed to the figure and Table numbering and order.

Basically, I was a bit frustrated and confused to continue. I felt like all my efforts in organizing the figures and sticking to your outline have been wasted. I am more than happy to edit and compile, but we must all agree to the same rules and do our parts.

I am sending back **(A)** your doc which I edited so far, and **(B)** the latest master endnote file, which you should use to replace all (ref) (ref) (ref) (ref) in your document. Chris, I hope you understand what I mean when you open the document.

There is no way I can compile the final documents unless everyone uses the same endnote file. I had no problems with compilingeveryone's Tables, so I was surprised at receiving your doc where all the (ref) (ref) (ref) (ref) have not been replaced.

Benhur **(One word doc and one Endnote file attached).**

Table 14.3. Henipavirus Cell Tropism and Receptor Expression

Cell line/ Primary cells	Species/Cell Type	Virus	EFNB2[a]	EFNB3[a]	Infection Permissive[b]	References[c]
HUMAN						
HeLa-CCL2	Human Cervical Cancer	NiV HeV	+	-	+	Bonaparte, 2005[1] Yoneda, 2006[2]
HeLa-USU	Human Cervical Cancer	NiV HeV	-	-	-	Bonaparte, 2005[1]
HeLa-USU-EFNB2	HeLa-USU stably expressing EFNB2	HeV CedV	+ +	- -	+ +	Marsh, 2012[3] Laing, 2018[4]
HeLa-USU-EFNB3	HeLa-USU stably expressing EFNB3	HeV CedV	- -	+ +	+ -	Marsh, 2012[3] Laing, 2018[4]
Vero (various subclones)	African green monke kidney epithelial	NiV HeV CedV GhV	++	+	+	Murray, 1995[5] Chua, 2000[6] Bossart, 2005[1] Erbar, 2008[7] Marsh, 2012[3] Pernet, 2014[8]
293T	Human embryonic kidney fibroblast		+	+	+	Yoneda, 2006[9] Talekar, 2011[10] Sawatsky, 2007[11] Martinez-Gil, 2017[12]
A549	Human lung adenocarcinoma	NiV HeV	+	+	+	Aljofan , 2009[13] Bauer , 2014[14] Dietzel ., 2015[15]
PCI 13	Human head and neck carcinoma	NiV HeV	+	+	+	Bonaparte, 2005[1] Bossart, 2005[16]
U373-MG	Human astroglioma	NiV	+	++	+	Bonaparte, 2005[1] Bossart, 2005[16] Mathieu, 2011[17]
U87-MG	Human glioblastoma	NiV GhV	+	+	+	Bender, 2016[18] Lee, 2015[19] Pernet, 2014[8] Khetawat, 2010[20]

HBMEC (Primary)	Human brain microvascular endothelial	NiV	+	-	+	Wong , 2002[21] Erbar . 2008[7]
HUVEC (Primary)	Human umbilical cord endothelial	NiV HeV	+	+	+	Mathieu , 2012 Park , 2015
HuNSC Neurons/ Astrocytes (Primary)	Human neural stem cells derived neurons and astrovytes	NiV, HeV	+	+	+	Palomares, 2013[22] Park, 2015[23]
NHBE (Primary)	(Normal) Human bronchial epithelial	NiV, HeV	+	+	+	Escaffre , 2013[24] Escaffre , 2016[25]
SAEC (Primary)	Human small airway epithleilial	NiV HeV	+	+	+	Escaffre , 2013[24] Escaffre , 2016[25]
Hu PBL (Primary)	Human peripheral blood lymphocytes	NiV	(+/-)[d]	(+/-)[d]	-	Mathieu , 2011[17]
HuMono/ Mac/DCs (Primary)	Human monocytes, macrophages, dendritic cells	NiV	Mono/ Mac (+/-)[d] DC (+/-)[d]	Mono/ Mac (+/-)[d] DC (-)	Mono (-) Mac (-) DC (+/-)	Mathieu , 2011[17]
HuOE (Primary)	Human olfactory epithelial	NiV HeV	(?)	+	+	Borisevich, 2017[26] Tanos, 2017[27]
Cell line/ Primary cells	**Species/Cell Type**	**Virus**	**EFNB2[a]**	**EFNB3[a]**	**Infection Permissive[b]**	**References[c]**
NON-HUMAN						
BHK-21	Baby hamster kidney fibroblast	NiV	+	(?)	+	Yoneda . 2006[2] Aljofan , 2009[13]
CHO	Chinese Hamster Ovary	NiV HeV	-	-	-	Negrete, 2005[28] Negrete, 2006[29] Yoneda, 2006[2]
CHO-B2	CHO stably expressing ephrin-B2	NiV HeV	+	-	+	Negrete, 2005[28] Negrete, 2006[29]
CHO-B3	CHO stably expressing	NiV HeV	-	+	+	Negrete, 2005[28] Negrete, 2006[29]

	ephrin-B3					
CRFK	Cat (Feline) kidney epithelial	NiV HeV	+	+	+	Sawatsky , 2007[11]
PBMEC (Primary)	Porcine brain microvascular endothelial	NiV	+	+	+	Erbar . 2008[7] Erabar , 2010[30]
PAEC (Primary)	Pig Aorta Endothelial	NiV	-	-	-	Erbar . 2008[7]
PK13	Pig kidney fibroblast	NiV	-	-	-	Negrete . 2005[28] Chua , 2007[31]
Rat cortical neurons	Rat cortical neurons (Primary)	NiV	+	+	+	Negrete , 2005[28] Talekar , 2011[10]
4/4RM4	Rat pleural mesothelial	NiV	+	ND	+	Yoneda . 2006[2] Hudson , 2014[32]
L2	Rat lung epithelial	NiV	+	ND	+	Yoneda . 2006[2] Yoneda , 2009[33]
208f	Rat embryonic fibroblast	NiV	+	ND	-	Yoneda . 2006[2] Yoneda , 2009[33]
P815	Mouse mast cell	NiV	+	ND	-	Yoneda . 2006[2] Yoneda , 2009[33]
MyEnd	Mouse myocardial endothelial	NiV	(?)	(?)	-	Erbar 2008[7]
PaLuT02	Immortalized *P. alecto* (fruit bat) lung cells	NiV HeV	+	+	+	Virtue, 2011[34] Bossart, 2008[35]
PaKi	Primary *P. alecto* (fruit bat) kidney cells	HeV	+	+	+	Virtue, 2011[34] Bossart, 2008[35]
PaBrH04	Immortalized *P. alecto* (fruit bat) brain cells	NiV HeV	+	+	+	Crameri, 2009[36] Bossart, 2008[35]
MMEC	Mouse microvascular endothelium	NiV HeV	(?)	(?)	(+/-)[d]	Aljofan , 2009[13]
BAEC	Bovine Aoritc Endothelium	NiV, HeV	(?)	(?)	(+/-)[d]	Aljofan , 2009[13]

[a] Expression inferred from (1) transcriptional data (microarray, next-generation sequencing, RT-PCR, etc.) provided in the cited references, or (2) indepedently verified through the Gene Expression Omnibus (GEO) Database (https://www.ncbi.nlm.nih.gov/geo/), or (3) competition with soluble ephrinB ligands or interference with cognate henipavirus receptor binding proteins. [b] For clarity and uniformity, only infection-permissive (not fusion-permissive) data are indicated; infection includes use of live virus and/or pseudotyped virus. [c] References cited support expression, infection data, or cell-type origin where relevant, and are representative, not comprehensive. [d] Indicates >10-100-fold below positive control when quantitative data is avialable. ND, not determined; (?), not specifically mentioned.

REFERENCES

1. Bonaparte MI, Dimitrov AS, Bossart KN, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A.* 2005;102(30):10652-10657.
2. Yoneda M, Guillaume V, Ikeda F, et al. Establishment of a Nipah virus rescue system. *Proc Natl Acad Sci U S A.* 2006;103(44):16508-16513.
3. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog.* 2012;8(8):e1002836.
4. Laing ED, Amaya M, Navaratnarajah CK, et al. Rescue and characterization of recombinant cedar virus, a non-pathogenic Henipavirus species. *Virol J.* 2018;15(1):56.
5. Murray K, Selleck P, Hooper P, et al. A morbillivirus that caused fatal disease in horses and humans. *Science.* 1995;268(5207):94-97.
6. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science.* 2000;288(5470):1432-1435.
7. Erbar S, Diederich S, Maisner A. Selective receptor expression restricts Nipah virus infection of endothelial cells. *Virol J.* 2008;5:142.
8. Pernet O, Wang YE, Lee B. Henipavirus receptor usage and tropism. *Curr Top Microbiol Immunol.* 2012;359:59-78.
9. Yoneda M. Nipah and Hendra virus infectious diseases. *Nihon Rinsho.* 2016;74(12):1973-1978.
10. Talekar A, Pessi A, Porotto M. Infection of primary neurons mediated by nipah virus envelope proteins: role of host target cells in antiviral action. *J Virol.* 2011;85(16):8422-8426.
11. Sawatsky B, Grolla A, Kuzenko N, Weingartl H, Czub M. Inhibition of henipavirus infection by Nipah virus attachment glycoprotein occurs without cell-surface downregulation of ephrin-B2 or ephrin-B3. *J Gen Virol.* 2007;88(Pt 2):582-591.
12. Martinez-Gil L, Vera-Velasco NM, Mingarro I. Exploring the Human-Nipah Virus Protein-Protein Interactome. *J Virol.* 2017;91(23).
13. Aljofan M, Porotto M, Moscona A, Mungall BA. Development and validation of a chemiluminescent immunodetection assay amenable to high throughput screening of antiviral drugs for Nipah and Hendra virus. *J Virol Methods.* 2008;149(1):12-19.
14. Bauer A, Neumann S, Karger A, et al. ANP32B is a nuclear target of henipavirus M proteins. *PLoS One.* 2014;9(5):e97233.
15. Dietzel E, Kolesnikova L, Sawatsky B, et al. Nipah Virus Matrix Protein Influences Fusogenicity and Is Essential for Particle Infectivity and Stability. *J Virol.* 2015;90(5):2514-2522.
16. Bossart KN, Crameri G, Dimitrov AS, et al. Receptor binding, fusion inhibition, and

induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol.* 2005;79(11):6690-6702.

17. Mathieu C, Pohl C, Szecsi J, et al. Nipah virus uses leukocytes for efficient dissemination within a host. *J Virol.* 2011;85(15):7863-7871.
18. Bender RR, Muth A, Schneider IC, et al. Receptor-Targeted Nipah Virus Glycoproteins Improve Cell-Type Selective Gene Delivery and Reveal a Preference for Membrane-Proximal Cell Attachment. *PLoS Pathog.* 2016;12(6):e1005641.
19. Lee B, Pernet O, Ahmed AA, Zeltina A, Beaty SM, Bowden TA. Molecular recognition of human ephrinB2 cell surface receptor by an emergent African henipavirus. *Proc Natl Acad Sci U S A.* 2015;112(17):E2156-2165.
20. Khetawat D, Broder CC. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol J.* 2010;7:312.
21. Wong KT, Shieh WJ, Kumar S, et al. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol.* 2002;161(6):2153-2167.
22. Palomares K, Vigant F, Van Handel B, et al. Nipah virus envelope-pseudotyped lentiviruses efficiently target ephrinB2-positive stem cell populations in vitro and bypass the liver sink when administered in vivo. *J Virol.* 2013;87(4):2094-2108.
23. Yun T, Park A, Hill TE, et al. Efficient reverse genetics reveals genetic determinants of budding and fusogenic differences between Nipah and Hendra viruses and enables real-time monitoring of viral spread in small animal models of henipavirus infection. *J Virol.* 2015;89(2):1242-1253.
24. Escaffre O, Borisevich V, Carmical JR, et al. Henipavirus pathogenesis in human respiratory epithelial cells. *J Virol.* 2013;87(6):3284-3294.
25. Escaffre O, Borisevich V, Vergara LA, Wen JW, Long D, Rockx B. Characterization of Nipah virus infection in a model of human airway epithelial cells cultured at an air-liquid interface. *J Gen Virol.* 2016;97(5):1077-1086.
26. Borisevich V, Ozdener MH, Malik B, Rockx B. Hendra and Nipah Virus Infection in Cultured Human Olfactory Epithelial Cells. *mSphere.* 2017;2(3).
27. Tanos T, Saibene AM, Pipolo C, Battaglia P, Felisati G, Rubio A. Isolation of putative stem cells present in human adult olfactory mucosa. *PLoS One.* 2017;12(7):e0181151.
28. Negrete OA, Levroney EL, Aguilar HC, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature.* 2005;436(7049):401-405.
29. Negrete OA, Wolf MC, Aguilar HC, et al. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog.* 2006;2(2):e7.
30. Erbar S, Maisner A. Nipah virus infection and glycoprotein targeting in endothelial cells. *Virol J.* 2010;7:305.
31. Chua KB, Wong EM, Cropp BC, Hyatt AD. Role of electron microscopy in Nipah virus outbreak investigation and control. *Med J Malaysia.* 2007;62(2):139-142.
32. Hudson AL, Weir C, Moon E, et al. Establishing a panel of chemo-resistant mesothelioma models for investigating chemo-resistance and identifying new treatments for mesothelioma. *Sci Rep.* 2014;4:6152.
33. Yoneda M, Fujita K, Sato H, Kai C. Reverse genetics of Nipah virus to probe viral pathogenicity. *Methods Mol Biol.* 2009;515:329-337.
34. Virtue ER, Marsh GA, Baker ML, Wang LF. Interferon production and signaling pathways are antagonized during henipavirus infection of fruit bat cell lines. *PLoS One.* 2011;6(7):e22488.

35. Bossart KN, Tachedjian M, McEachern JA, et al. Functional studies of host-specific ephrin-B ligands as Henipavirus receptors. *Virology.* 2008;372(2):357-371.
36. Crameri G, Todd S, Grimley S, et al. Establishment, immortalisation and characterisation of pteropid bat cell lines. *PLoS One.* 2009;4(12):e8266.

TABLE 14.5 Summary of Advanced Henipavirus Vaccination Development Initiatives Evaluated in Animal Challenge Models

Platform	Viral antigen target or immunogen	Animal challenge model
Active vaccination		
Recombinant vaccinia virus	Nipah F and/or G glycoprotein	Hamster[a] (NiV)
Recombinant canarypox virus	Nipah F and/or G glycoprotein	Pig[b] (NiV)
Recombinant VSV	Nipah F and/or G glycoprotein	Ferret[c] (NiV), Hamster[d] (NiV), nonhuman primate[e] (NiV)
Recombinant AAV	Nipah G glycoprotein	Hamster[f] (NiV, HeV)
Recombinant measles virus	Nipah G glycoprotein	Hamster[g], Nonhuman primate (NiV, HeV)
Recombinant subunit	Hendra soluble G glycoprotein	Cat[h] (NiV), Ferret[i] (HeV, NiV), nonhuman primate[j] (HeV, NiV) Horse[k] (HeV)
Passive immunization		
Human monoclonal antibody m102.4	Hendra / Nipah G glycoprotein	Ferret[l] (NiV) Nonhuman primate[m] (HeV, NiV)

[a] Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vaccinia viruses were protected against disease following intraperitoneal challenge with 10^3 PFU of NiV.[1]

[b] Pigs immunized with NiV F and/or G glycoprotein encoding recombinant canarypox viruses were protected against intranasal challenge with $2.5x10^5$ PFU of NiV.[2].

[c] Ferrets immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intranasal challenge with $5x10^3$ PFU of NiV.[3]

[d] Hamsters immunized with NiV F and/or G glycoprotein encoding recombinant vesicular stomatitis virus (VSV) vectors were protected against lethal intraperitoneal challenge with 10^5 $TCID_{50}$ of NiV;[4] or $6.8x10^4$ $TCID_{50}$ of NiV.[5]

[e] African green monkeys immunized with a NiV G encoding recombinant VSV vector were protected against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV.[6,7]

[f] Hamsters immunized with a NiV G encoding recombinant adeno-associated virus (AAV) vector were protected against lethal intraperitoneal with 10^4 PFU of NiV.[8]

[g] Hamsters and African green monkeys immunized with a NiV G encoding recombinant measles virus vector were protected against lethal intraperitoneal challenge with 10^3 $TCID_{50}$ of NiV (hamsters) or 10^5 $TCID_{50}$ of NiV (AGMs).[9]

[h] Hendra virus soluble G glycoprotein (HeV-sG) used to immunize cats protects against lethal subcutaneous (500 $TCID_{50}$)[10] or oronasal ($5x10^4$ $TCID_{50}$) NiV challenge.[11]

[i] HeV-sG used to immunize ferrets protects against lethal oronasal challenge with $5x10^3$ $TCID_{50}$ of HeV[12] or $5x10^3$ $TCID_{50}$ of NiV challenge.[13]

[j] HeV-sG used to immunize African green monkeys protects against lethal intratracheal challenge with 10^5 $TCID_{50}$ of NiV[14] or $5x10^5$ PFU of HeV.[15]

[k] HeV-sG used to immunize horses protects against lethal oronasal challenge with $2x10^6$ $TCID_{50}$ of HeV[16].

[l] A NiV and HeV cross-reactive G glycoprotein specific neutralizing human mAb (m102.4) protects ferrets against lethal oronasal challenge with $5x10^3$ $TCID_{50}$ of NiV[17] or $5x10^3$ $TCID_{50}$ of HeV (J. Pallister and C. Broder, unpublished) by post-exposure infusion.

[m] Human mAb m102.4 protects African green monkeys by post-exposure infusion following lethal intratracheal challenge with $4x10^5$ $TCID_{50}$ of HeV[18] or lethal intratracheal challenge with $5x10^5$ PFU of NiV.[19,20]

1. Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. *J Virol.* 2004;78(2):834-840.
2. Weingartl HM, Berhane Y, Caswell JL, et al. Recombinant nipah virus vaccines protect pigs against challenge. *J Virol.* 2006;80(16):7929-7938.
3. Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. *Virol J.* 2013;10:353.
4. Lo MK, Bird BH, Chattopadhyay A, et al. Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters. *Antiviral Res.* 2014;101:26-29.
5. DeBuysscher BL, Scott D, Marzi A, Prescott J, Feldmann H. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. *Vaccine.* 2014;32(22):2637-2644.
6. Prescott J, DeBuysscher BL, Feldmann F, et al. Single-dose live-attenuated vesicular stomatitis virus-based vaccine protects African green monkeys from Nipah virus disease. *Vaccine.* 2015;33(24):2823-2829.
7. DeBuysscher BL, Scott D, Thomas T, Feldmann H, Prescott J. Peri-exposure protection against Nipah virus disease using a single-dose recombinant vesicular stomatitis virus-based vaccine. *NPJ Vaccines.* 2016;1.
8. Ploquin A, Szecsi J, Mathieu C, et al. Protection against henipavirus infection by use of recombinant adeno-associated virus-vector vaccines. *J Infect Dis.* 2013;207(3):469-478.
9. Yoneda M, Georges-Courbot MC, Ikeda F, et al. Recombinant measles virus vaccine expressing the Nipah virus glycoprotein protects against lethal Nipah virus challenge. *PLoS One.* 2013;8(3):e58414.
10. Mungall BA, Middleton D, Crameri G, et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol.* 2006;80(24):12293-12302.
11. McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. *Vaccine.* 2008;26(31):3842-3852.
12. Pallister J, Middleton D, Wang LF, et al. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine.* 2011;29(34):5623-5630.
13. Pallister JA, Klein R, Arkinstall R, et al. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J.* 2013;10:237.
14. Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci Transl Med.* 2012;4(146):146ra107.

15. Mire CE, Geisbert JB, Agans KN, et al. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol.* 2014;88(9):4624-4631.
16. Middleton D, Pallister J, Klein R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis.* 2014;20(3):372-379.
17. Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute nipah virus infection. *PLoS Pathog.* 2009;5(10):e1000642.
18. Bossart KN, Geisbert TW, Feldmann H, et al. A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. *Sci Transl Med.* 2011;3(105):105ra103.
19. Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med.* 2014;6(242):242ra282.
20. Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Sci Rep.* 2016;6:30916.

From: Lee, Benhur on behalf of Lee, Benhur <benhur.lee@mssm.edu>
To: Broder, Christopher; LinFa Wang Linfa
Subject: Table 14.3 Henipavirus Cell Tropism and Receptor Expression
Date: Sunday, February 17, 2019 12:07:46 AM
Attachments: Table 14.3.Henipavirus Cell Tropism.docx
TABLE 14-5-Formatted-Broder.docx
HNV Fields Ref.enlp.zip

Hi Folks,

I forgot to send you my completed **Table 14.3** on HNV Cell tropism and Receptor Expression.

Please check the Table if you have time. Refs have been formatted. I will remove the name/date when we are ready to put everything together (since only Ref numbers are allowed in the Tables).

- Please see the **footnotes (a, b, c, d)** to the Table for an explanation of how to interpret the various items in the Table.
- It's obviously not comprehensive. I had to make judgement calls about what to put in and what to leave out. My criteria is for the table to be useful, so there is a combination of positive and negative data.
- It's divided into human and non-human cell lines/primary cell types, and then listed alphabetically.
- Let me know if I missed anything major.

LinFa, can you put the reference number in (or ask your asst to do it ;-) for your tables. It'll take me too much time to replace all the (name/date) references that you have in your Tables by myself.

Chris, I was so pleased that I could format your Table 14.5 with no problems using the Master endnote file (**HNV_Fields Ref.enlp**). So, the system works! I have attached your formatted Table 14.5. Hopefully everything looks correct.

Finally, I have added the refs you all have asked me to. The Master endnote file I'm attaching to this email has **1,208 references**.

Thanks!

Benhur

From: Lee, Benhur on behalf of Lee, Benhur <benhur.lee@mssm.edu>
To: Broder, Christopher
Cc: LinFa Wang Linfa
Subject: Master Endnote file
Date: Friday, February 15, 2019 4:17:30 PM
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O.K, I hope this is what you are asking for.

This is the Endnote "Package" file (i.e. it is self-contained **".enlp"** that does not require accompanying "Endnote Library.Data Folders", rdg, tdb, etc.). It is NOT the default ",enl" endnote file which Endnote generates.

Feel free to search directly from this **.enlp** file to add anything that is not here. Right now, it has 1,193 ref with no duplications and contains PubMed Refs with Nipah OR Hendra in any field.

Thanks!

benhur

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". . . all progress, both theoretical and practical, has resulted from a single human activity: the quest for what I call good explanations."
--David Deutsch, The Beginning of Infinity (2012)

> On Feb 15, 2019, at 10:21 AM, Broder, Christopher <christopher.broder@usuhs.edu> wrote:
>
> Do we need to add edits to the WORD file they sent us? As you (LF) have done?
>
> I was under the impression that we need to make a new WORD file of the Text body, there
> will be so many edits and sections and reff changes.
>
> My sections are considerably re-written. As soon as I get a master Endnote file from you
> I will start putting in all the correct reffs to the sections i am writing now/.
>
> On Thu, Feb 14, 2019 at 9:18 PM Wang Linfa <linfa.wang@duke-nus.edu.sg>

wrote:

> Dear Benhur,
>
> See attached with all my edits and changes of the text.
>
> I will look into the Endnot files later …. Has to go to meetings now.
>
> Thanks
>
> LF
>
> ***Linfa (Lin-Fa) WANG, PhD FTSE***

January 2019

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs)—diagnostics, therapeutics, and vaccines—against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (*Pteropus* species and several other bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species (including horses, cats, and dogs) were found to be infected with NiV on the farms involved in the outbreak. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen—Hendra virus (HeV)—which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat; disease also occurred in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

NiV infection in humans results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India. Some differences have been noted in the clinical features of infection, with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of MCMs to enable effective and timely emergency response to infectious disease epidemics. NiV infection is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this roadmap ranges from basic research to late-stage development of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include minimizing zoonotic NiV transmission, improving use of personal protective equipment (PPE), ensuring adequate hand hygiene and environmental hygiene, promoting effective community engagement, implementing adequate infection prevention and control practices, developing adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and promoting workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap but need to be addressed as part of a broader public health control strategy. Further research of NiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV (or related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

VISION

Ready availability and accessibility of r~~R~~obust MCMs to detect, prevent, and control human outbreaks of NiV infection (and other closely related henipaviruses) ~~that are readily available and accessible~~ for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research represents a substantial challenge, since economic incentives to invest in Nipah research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with small and sporadic outbreaks. The development of a sustainable value proposition for industry and international philanthropic public-private partnerships is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate.
- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (biosafety level 4 [BSL-4]) conditions, which can increase the cost of MCM development.
- To date, NiV spillovers to human communities have been identified most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the *Pteropus* genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to the lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- While ferrets, Syrian hamsters, and interferon-alpha/beta receptor knockout (IFNAR-KO) mice are well-established animal models for NiV research, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection occurs in relatively small, focal outbreaks, the low disease incidence poses a major

challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop and manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries and to global stakeholders to ensure they understand the potential health, societal, and economic benefits of devoting resources to improving NiV surveillance, detection, prevention, and control measures.
- Obtaining additional prospective serosurveillance data of henipavirus exposure from susceptible animal species and proximate human populations in areas of predicted risk should be explored as a strategy to assess the potential of human spillover and to build preparedness for detection of human cases and for limiting exposure.
- Standardized and well-characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains for R&D of MCMs for NiV infection. Assays that can be used at lower biosafety levels are an important priority. WHO international standards should be used (when available) as calibrators and reported in units/ml to harmonize assay results.
- Clear criteria for downselection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or other regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.

- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, depend on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of *Pteropus* bats and potentially other bat species.
- Additional research is needed to optimize relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) for promoting development and evaluation of MCMs, particularly if investigators are required to use an alternative pathway (such as the United States Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after-challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunologic clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurologic sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes that may affect the epidemiologic and clinical features of disease over time and thereby influence MCM development.

- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole-genome sequencing of NiV are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume date palm sap, healthcare workers, and workers at the human-animal interface) and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains to facilitate R&D of NiV MCMs.

2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and that may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.
- **Generate** research tools to promote R&D of MCMs for NiV infection (e.g., standardized and validated assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability of henipaviruses to improve understanding of their global distribution.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for downselection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.

- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an "affordable and fair" price is one that can reasonably be paid by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

DIAGNOSTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific, and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and investigation into the cause of infection is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.

- Owing to the biosafety precautions necessary when working with NiV, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods is problematic, given the rapid disease progression of NiV infection.
- Currently, no approved tests for diagnosis of NiV infection are commercially available.
- *Pteropus* species (and perhaps other bat species) appear to carry other henipaviruses in addition to NiV and HeV, some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate using serologic assays which henipaviruses are in circulation. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.
- A TPP for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples to assess and validate diagnostic tests. As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6) identify who would have access to the samples; (7) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature), and have a high sensitivity and specificity for different NiV strains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

- Improved diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, particularly given the costs of regulatory approval.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotype neutralization assays and antigen-capture enzyme-linked immunosorbent assays [ELISAs]) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries: Bangladesh and India.

2. By 2021, identify funding and initiate creation of the virtual reference repository in Bangladesh and India, with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.

2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnose NiV infection during future outbreaks.

Priority Areas/Activities

Research

- **Create** a virtual repository of clinical reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- **Develop and evaluate** point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed.

Policy and commercialization

- **Develop** guidance on optimal strategies for the deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

THERAPEUTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment of patients and PEP for exposed persons.
- In NiV-endemic areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis but do not have NiV infection. Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore, accurate and rapid diagnosis is critical.
- Studies in animals often evaluate the usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well a therapeutic agent will work in the field.
- NiV can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control–programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.
- A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that are detected annually.

Key needs

- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas, particularly during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).
- A transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials, given the limited number of cases that could potentially be enrolled in trials each year.

Knowledge gaps

- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to further assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed that the therapeutic window for m102.4 against a strain from Bangladesh/India was shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection and strengthen the therapeutic pipeline.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship of promising therapeutic candidates.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will affect the response to therapeutic candidates and results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials to inform best-practice guidelines and evidence-based policy decisions.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
3. By 2019, identify an approach for downselecting promising therapeutic candidates for further study in clinical trials, given limited annual case numbers for enrollment.
4. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
5. By 2020, generate a reliable source of m102.4 (or other promising agent[s] or agent combinations) to be ~~used~~ assessed in clinical trials.

Strategic Goal 2: Develop and evaluate therapeutic agents for the treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as m102.4 and favipiravir) for treating and preventing NiV infection, including conducting studies in animal models and clinical trials as appropriate and feasible.
- **Continue to expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Develop, evaluate, and license** safe and effective therapeutic agents for the treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in affected areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- **Generate** a reliable source of m102.4 to be used in clinical trials.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

VACCINES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development, and few companies are willing to invest in the generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could diminish the acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for the timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on the use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptance and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen, and therefore multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed involving target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations and identify additional areas of potential NiV spillover.
- Additional sociological research is needed to explore perceptions and concerns of at-risk populations regarding NiV vaccine implementation.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the need for licensure and future use of NiV vaccines. This consultation should include representatives from in-country regulators, other regulatory agencies (such as the FDA or the European Medicines Agency), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of the humoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serologic assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.

- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Evaluate** the feasibility of generating multivalent vaccines (i.e., that protect against more than one disease) and determine whether or not such vaccines would be useful in future NiV control efforts.

Product development

- **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptance and promotion within the community.
- **Consider** developing a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for faster spread.

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

WHO. Nipah baseline situational analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017 [Full text]

Other Publications:

Arunkumar G, Chandni R, D T Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Med 2016;9(7):630-4 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 29 Nov 2016; site accessed 31 October 2018 [Web page]

Baseler L, Scott DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov 3;10(11):e0005120 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Pathog 2009 Oct 30;5(10):e1000642 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (US Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 20 Mar 2014 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2):235–240 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-283 [Full text]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of infectious diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PKG, de los Reyes V, Sucaldito M, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015 Feb;21(2):328-31 [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. Malaysian J Pathol 2010; 32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microb Infect 2013 Jun;2(6):e37 [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Curr Opin Virol 2017 Feb;22:97-104 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8:7604 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024 [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Abstract]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global virology I — identifying and investigating viral diseases. New York, NY: Springer US; 2015:125-46

Deka MA, Morshed N. Mapping disease transmission risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2).pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug;14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-7 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37. [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The role of animals in emerging viral diseases. Amsterdam: Elsevier; 2014:293-315 [Abstract]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis. 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2):e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lancet editors. Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137):2295 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55. [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013 Oct;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug; 15(8): 1229–1235 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015 Mar;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-227 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis. 2014;20:372–9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;6:30916 [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13;10:353 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9. Epub 2000 May 15 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sep 1;157(9):1635-41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Rahman SA. National guideline for management, prevention and control of Nipah virus infection including encephalitis. Dec 2011 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-4 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Schmidt R, Beltzig LC, Sawatsky B, et al. Generation of therapeutic antisera for emerging viral infections. NPJ Vaccines 2018 Oct 5;3:42 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013; 8(7): e69544. Published online 2013 Jul 22 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Epub ahead of print]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Steffen DL, Xu K, Nikolov DB, et al. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Sun B, Jia L, Liang B, et al. Phylogeography, transmission, and viral proteins of Nipah virus. Virol Sin 2018; Oct 11; Epub ahead of print [Full text]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

Yu J, Lv X, Yang Z, et al. The main risk factors of Nipah disease and its risk analysis in China. Viruses 2018 Oct 19;10(10). pii:E572 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, influenza, Nipah, and Semliki Forest chimeric viruses with vesicular stomatitis virus: actions in the brain. J Virol 2017 Feb 28;91(6):e02154-16 [Full text]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah Virus, Thailand. Emerg Infect Dis 2005 Dec; 11(12):1949–1951 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

January 2019

A Research and Development (R&D) Roadmap for Nipah Virus

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The work for this project was completed with generous support from the Wellcome Trust. Dr. de Wit is supported by the intramural research program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA.

The authors would also like to acknowledge Mr. Carlos R. Cruz; CIDRAP, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

Word Count: 3,870 (text only, not including References or Tables)

Introduction

Nipah virus (NiV), a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chua 1999, Chew 2000, Parashar 2000). At least 265 human cases of encephalitis were identified in Malaysia and most had close contact with pigs (Chua 2010, Parashar 2000). In addition, 11 abattoir workers in Singapore became ill following contact with pigs imported from Malaysia; eight patients presented with signs of encephalitis and three presented with atypical pneumonia (one of which later also had evidence of encephalitis) (Paton 1999). No new outbreaks have been reported in these countries since May 1999; however, NiV infection was subsequently recognized in Bangladesh in 2001 (Hsu 2004) and nearly annual outbreaks have occurred in that country since then (WHO 2018). Outbreaks also have been recognized in eastern India and, in 2018, an outbreak occurred in the southern Indian state of Kerala (Arunkumar 2018, Sharma 2018, Chattu 2018). To date, nearly 350 cases of NiV infection have been identified in Bangladesh and India (WHO 2018). Possible emergence of NiV in a third geographic area is evidenced by an outbreak of an unidentified henipavirus that occurred among horses and humans in the Philippines in 2014 (Ching 2015). Although detailed genomic information for the virus is limited, experts agree that the causative agent was either NiV or a virus closely related to NiV. The Henipavirus genus includes another pathogen, Hendra virus, which predominantly causes infection in horses in Australia and can also lead to human disease, usually following contact with infected horses (Ksiazek 2011).

The most common clinical syndrome associated with NiV infection is severe, rapidly progressive encephalitis (Goh 2000, Hossain 2008). The main presenting features include fever, headache, dizziness, vomiting, and altered mental status. NiV infection may result in late-onset

encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae (Tan 2002, Goh 2000). Some patients also have respiratory involvement or respiratory illness alone, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The overall case-fatality rate for the Malaysia outbreak was approximately 40%, whereas case-fatality rates during outbreaks in Bangladesh and India have generally ranged from 65% to 100% (Hossain 2008, WHO 2018). Genomic sequencing has demonstrated that there are multiple strains of NiV, which may account for the differences noted in clinical features and outcomes between Malaysia and Bangladesh (Mire 2016).

Fruit bats of the Pteropus genus are considered the primary natural reservoir for NiV. In the Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and possibly limited human-to-human NiV transmission (Chua 2010). Additionally, several other domestic animal species in the outbreak area (including horses, cats, and dogs) were found to be infected with NiV (Chua 2000). In outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap (Islam 2016, Luby 2006, Rahman 2012) and subsequent person-to-person transmission. While person-to-person transmission was apparently minimal in the Malaysia outbreak, it has played a larger role in amplifying outbreaks in Bangladesh and India (Gurley 2007, Luby 2009, Chadha 2006, Arunkumar 2018). This may, in part, be related to the greater propensity toward respiratory disease in NiV strains found in South Asia and transmission via droplet spread (Spiropoulou 2018). A recent study demonstrated that hamsters could be infected when exposed to NiV via a small-particle aerosol; however, the epidemiologic significance of this remains unclear (Escaffre 2018). Given the potential for respiratory transmission of NiV and the recent experience with

Ebola in West Africa, some have raised concerns about the possibility of larger NiV outbreaks if the virus takes hold in a crowded or urban area (Donaldson 2018) or about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Pteropus fruit bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Other fruit bats of the Pteropodidae family also may harbor NiV, and such bats can be found across Africa and parts of the Middle East. Evidence of NiV infection (via virus isolation, serologic testing, or nucleic acid detection using real-time polymerase chain reaction [RT-PCR]) has been found in Pteropus bat species and other bat species in geographically diverse regions, including South Asia (Bangladesh and India); Southeast Asia (Cambodia, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam); the Western Pacific region (Papua New Guinea); and Africa (Madagascar and Ghana) (Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2018, Yob 2001). This wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.

WHO has determined that Nipah virus is a priority disease for epidemic preparedness (WHO 2018). To improve epidemic response for this infection, in the fall of 2017, WHO embarked on development of a research and development (R&D) roadmap for NiV disease; this report provides a summary of the key elements of that roadmap, including important challenges and barriers that need to be addressed to ensure that diagnostics, therapeutics, and vaccines are

developed, manufactured, and readily available when needed *[provide link to full roadmap here when available]*.

The NiV R&D roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one medical countermeasure category), diagnostics, therapeutics, and vaccines. The strategic goals and milestones identified in each section are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updates to the roadmap as needed. The roadmap is specific to R&D for disease in humans; however, further research of NiV in animal species, including development of appropriate medical countermeasures targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV in humans and virus transmission can occur at the human-animal interface.

Cross-Cutting Issues

A major challenge for development of diagnostics, therapeutics, and vaccines for NiV is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Advocacy to policy makers in affected countries and to global stakeholders is needed to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures. As part of this process, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species (e.g., bats, swine, and equids) and proximate human populations in areas of predicted risk. Human health, animal health, and wildlife officials should be engaged in these efforts as part of a long-term collaborative One Health approach (Chattu 2018, Kulkarni 2013).

Another important issue is the need to optimize relevant animal challenge models for promoting development and evaluation of medical countermeasures for NiV infection, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval (Rockx 2014). For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for therapeutic or vaccine development; (3) determine when after challenge medical countermeasures should be administered in animal models to best mimic realistic timing of such use in humans; (4) bridge data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human doses; and (5) identify the best models for studying chronic (relapsing) infection (Dhondt 2013, Johnston 2015, Mathieu 2015). Currently, the African green monkey is regarded as the most relevant animal model for evaluating candidate therapeutics and vaccines intended for use in humans (Bossart 2012, Geisbert 2010, Johnston 2015), and studies involving nonhuman primates may be required for licensure of therapeutics and vaccines via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns, however, constrain the use of this animal model. In addition to optimizing animal models, standardized and well-characterized assays, reagents, antibodies, nucleic acids, and NiV challenge strains are needed to move R&D of medical countermeasures for NiV infection forward (Satterfield 2016).

To inform development of optimal NiV medical countermeasures, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals.

This includes issues such as evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection.

To respond to these challenges, the strategic goals and milestones for cross-cutting issues in the NiV R&D roadmap focus primarily on developing a sustainable value proposition and identifying funding sources to promote R&D, availability, and accessibility of NiV medical countermeasures, optimizing animal models for research, and generating standardized and well-characterized assays, reagents, antibodies, nucleic acids, and NiV challenge strains to facilitate R&D of NiV medical countermeasures (Table 1).

Diagnostics

Currently, no approved tests for diagnosis of NiV infection are commercially available. The most commonly used methods for diagnosing NiV infection include virus isolation, enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies, and real time-polymerase chain reaction (RT-PCR) nucleic acid testing. Virus isolation requires biosafety level 4 (BSL-4) capabilities and is generally only performed in reference laboratories for specific purposes, such as to confirm a new outbreak or to identify the virus in new host species (Wang 2012). During the Malaysia outbreak, the US Centers for Disease Control and Prevention (CDC) developed a capture ELISA for detection of IgM antibodies and an indirect ELISA for detection of IgG antibodies (Daniels 2001); since then, additional ELISA tests have been developed (Wang 2012) and serologic testing is often used for case confirmation during outbreaks (Chadha 2006, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009). RT-PCR, which can be performed on throat and nasal swabs, cerebrospinal fluid, urine, and blood, is also often used for diagnostic testing (Chadha 2006, Guillaume 2004, Lo 2012). Quantitative RT-qPCR tests have been developed for quantifying viral replication; such tools can be used to measure viral load reduction when screening vaccine candidates and potential therapeutics (Jensen 2018). More recently, pseudotyped-based serum neutralization assays for NiV detection that use vesicular stomatitis virus systems have been developed; these assays potentially can be used under BSL2 conditions in laboratories with limited diagnostic capabilities and appear to offer improved sensitivity and specificity (Kaku 2009, Kaku 2012, Tamin 2009). Antigen-capture ELISAs using monoclonal antibodies also have been developed, which could result in more rapid diagnosis (Chiang 2010). These newer technologies hold promise; however, more information is needed regarding their performance characteristics, particularly under field conditions. Development and validation of immunohistochemical staining tests can facilitate studies on pathogenesis of NiV infections and may also be useful for primary diagnosis in low-resource settings.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation, which can delay outbreak detection and implementation of infection control measures. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. To address these issues, rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes, once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of such a repository requires addressing the following: (1) determining what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outlining the purposes of sample collection; (3) determining what organizations will be responsible for activities related to creating and maintaining the repositories; (4) establishing standardized protocols for sample collection and maintenance; (5) establishing an appropriate governance structure; (6) identifying who would have access to the samples; (7) prioritizing use of samples and sample distribution; and (8) ensuring that material transfer agreements are in place. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

To address these issues, the strategic goals and milestones for diagnostic testing outlined in the NiV R&D roadmap focus on creation of a virtual reference repository, development of highly sensitive and specific point-of-care or near-patient NiV diagnostic tests, and enhancement of laboratory diagnostic preparedness to promote early detection of NiV in areas of known or potential spillover risk (Table 2).

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients; findings from treated patients were compared to those of 54 historical controls who became ill before ribavirin was available or who refused treatment (Chong 2001).

Results demonstrated a significantly lower mortality (36%) among treated patients; however, the use of historical controls may have biased this finding. No additional clinical studies using ribavirin have been conducted, and limited studies in animal models have not demonstrated efficacy of ribavirin following NiV or Hendra virus challenge (Rockx 2010, Georges-Courbot 2006). Chloroquine also has been studied in animal models, but has not shown any therapeutic benefit, either alone or in combination with ribavirin (Freiberg 2010, Pallister 2009). Based on these findings, these approaches are not considered viable options for treatment of NiV infection.

A more promising therapeutic option is the development of monoclonal antibodies (mAbs) against viral envelope proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). Therapy with anti-NiV mAbs may be appropriate for early treatment and for post-exposure prophylaxis (PEP) to prevent illness in exposed persons, such as healthcare workers, family caregivers, and persons exposed to infected livestock. Researchers have demonstrated that the human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009), and it has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Recently, a phase 1 clinical trial of m102.4 with 40 human participants was completed in Australia, but results are not yet available (ANZCTR). M102.4 was deployed for use during the 2018 NiV outbreak in Kerala, India, but was not used because the outbreak was brought under control quickly (Spiropoulou 2018). One recent study in nonhuman primates showed that the therapeutic window for m102.4 may vary by virus strain, which is a consideration for further preclinical and clinical study of this agent (Mire 2016). It may also be worthwhile to explore development of alternate mAbs against NiV or to evaluate cocktails of mAbs.

Other potential options for NiV treatment include favipiravir (a small-molecule antiviral that inhibits RNA-dependent RNA polymerase), fusion inhibitory peptides (peptides that inhibit virus-mediated membrane fusion), and remdesivir (also known as GS-5734, which is a monophosphoramidate prodrug of an adenosine analogue). Researchers recently demonstrated efficacy of favipiravir in hamsters challenged with a lethal dose of NiV (Dawes 2018), supporting proof-of-concept for favipiravir as a possible therapy against NiV infection. Studies in animal models also have been conducted for fusion inhibitory peptides; for example, investigators recently reported that lipopeptides delivered via the respiratory route can prevent lethal NiV infection in hamsters and nonhuman primates (Mathieu 2018). Remdesivir is a small-molecule antiviral that is currently being evaluated in clinical studies as a therapeutic option for treating Ebola virus disease. Investigators recently demonstrated in vitro inhibition by remdesivir against a number of different viruses, including paramyxoviruses (Lo 2017). While these agents are promising, further preclinical data are needed to assess their utility. In addition, efforts are needed to expand the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents that may be more aligned with optimal and desired characteristics for anti-NiV therapy.

Key additional considerations for moving NiV therapeutics forward include: (1) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising novel treatments for NiV disease; (2) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (3) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in both endemic and at-risk areas. A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that are detected each year. To address this concern, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how to best allocate scarce resources. Additionally, clinicians in endemic areas need to maintain high vigilance for cases of acute febrile encephalitic syndrome, particularly in association with respiratory symptoms, to facilitate early diagnosis and case identification. Additional training of clinicians along with effective and appropriate risk communication strategies need to be implemented in endemic areas.

To address these issues, the goals and milestones for therapeutics in the NiV R&D roadmap focus on continuing to move promising agents forward into clinical trials and on enhancing preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks (Table 3).

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Satterfield 2016). A Hendra virus subunit vaccine involving soluble glycoprotein (sG) has been developed and is commercially available for use in horses (Middleton 2014), which supports this option as a promising approach for developing a human NiV vaccine.

The Coalition for Epidemic Preparedness Innovations (CEPI), which is a partnership of government agencies, industry, academia, philanthropy, and intergovernmental institutions aimed at providing funding for R&D of new vaccines against pathogens with epidemic potential, has recently provided financial support to promote the development of several NiV candidate vaccines. In May 2018, CEPI awarded a US$ 25 million contract to Profectus BioSciences, Inc. and Emergent BioSolutions Inc. to develop a human NiV vaccine that is based on the same technology as that used for the Hendra virus vaccine for horses (CEPI 2018). In September 2018, CEPI announced plans to award approximately US$ 2 million to the Jenner Institute at the University of Oxford and Janssen Vaccines & Prevention BV for preclinical development of a replication-deficient simian adenoviral-vectored vaccine, derived from an adenovirus originally isolated from chimpanzees (CEPI 2018). These efforts, and potentially additional future efforts funded by CEPI, will support preclinical and early clinical development of NiV vaccines.

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012, Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research in this area should focus primarily on the humoral immune response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

The immediate goals and milestones in the vaccine section of the NiV R&D roadmap focus on moving vaccine candidates forward into clinical trials and promoting development of guidance on requirements for clinical trials, regulatory pathways, and other considerations relevant to licensure of a vaccine against NiV (Table 4).

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures forward. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 31 October 2018 [Web page]

Arunkumar G, Chandni R, DT Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

CEPI (Coalition for Epidemic Preparedness Innovations). CEPI awards $25 million contract to Profectus BioSciences and Emergent BioSolutions to develop Nipah virus vaccine. 2018 May 24 [Full text]

CEPI. CEPI awards contract worth up to USD$19 million to Oxford University and Janssen Vaccines to develop MERS, Lassa, and Nipah vaccines. 2018 Sep 27 [Full text]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in Fruit Bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C$_{12}$U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis 2014;20:372-9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6): 30916 [Full text]

Pallister J, Middleton D, Crameri G. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. J Virol 2009 Nov;83(22):11979-82 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. List of Blueprint priority diseases; accessed October 28, 2018 [Webpage]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed October 26, 2018 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Tables

Table 1: Goals and Milestones for NiV Infection Cross-Cutting Issues

Goals and Milestones for NiV Infection Cross-Cutting Issues	
STRATEGIC GOALS	**MILESTONES**
1. Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.	1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas. 2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.
2. Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.	1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation. 2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.
3. Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.	1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and NiV challenge strains to facilitate R&D of NiV MCMs. 2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.
Abbreviations: NiV, Nipah virus; MCM, medical countermeasure; R&D, research and development.	

Table 2: Goals and Milestones for NiV Diagnostics

Goals and Milestones for NiV Diagnostics	
STRATEGIC GOALS	**MILESTONES**
1. Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.	1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India). 2. By 2021, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.

2. Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.	1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays. 2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays. 3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP. 4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
3. Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.	1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnose NiV infection during future outbreaks.
Abbreviations: NiV, Nipah virus; NRA, national regulatory authority; TPP, target product profile.	

Table 3: Goals and Milestones for NiV Therapeutics

Goals and Milestones for NiV Therapeutics	
STRATEGIC GOALS	**MILESTONES**
1. Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.	1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol. 2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol. 3. By 2019, identify an approach for downselecting promising therapeutic candidates for further study in clinical trials, given limited annual case numbers for enrollment. 4. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol. 5. By 2020, generate a reliable source of m102.4 (or other promising agent[s] or agent combinations) to be assessed in clinical trials.

2. Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.	1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates. 2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection. 3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
Abbreviations: NiV, Nipah virus; PEP, post-exposure prophylaxis.	

Table 4: Goals and Milestones for NiV Vaccines

Goals and Milestones for NiV Vaccines	
STRATEGIC GOALS	**MILESTONES**
1. Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.	1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure. 2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as the FDA or the EMA), national and international public health agencies and organizations, and local and international researchers.
2. Develop and evaluate NiV vaccines for prevention of NiV disease in humans.	1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates. 2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines. 3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.
Abbreviations: FDA, US Food and Drug Administration; EMA, European Medicines Agency; NiV, Nipah virus; NRA, national regulatory authority; WHO, World Health Organization.	

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Date:	Friday, January 11, 2019 8:05:42 PM
Attachments:	Nipah RD Roadmap.January2019.docx Nipah Roadmap manuscript.20190111.docx

Dear Nipah virus R&D taskforce members:

First, thanks to all of you who reviewed the manuscript and roadmap and provided input. We very much appreciate your thoughtful comments and time.
We have incorporated your feedback and have sent the documents to WHO for their final review. I have attached them here as an FYI. The roadmap contains track changes so our WHO colleagues can see the edits and make final decisions regarding the content.

We hope that that the roadmap will be published in the next few weeks and that we can submit the manuscript for publication soon. We're currently planning to submit to Lancet ID, but the final decision and next steps rest with WHO. We will likely need you to sign off on the manuscript once it is accepted for publication.

We'll keep you posted as this effort progresses.

Again, thank you for your time and support.

Kind regards,

Kris

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A Research and Development (R&D) Roadmap for Nipah Virus

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The work for this project was completed with generous support from the Wellcome Trust. Dr. de Wit is supported by the intramural research program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA.

The authors would also like to acknowledge Mr. Carlos R. Cruz; CIDRAP, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

Word Count: 3,695 (text only, not including References or Tables)

Introduction

Nipah virus (NiV), a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chua 1999, Chew 2000, Parashar 2000). At least 265 human cases of encephalitis were identified in Malaysia and most had close contact with pigs (Chua 2010, Parashar 2000). In addition, 11 abattoir workers in Singapore became ill following contact with pigs imported from Malaysia; eight patients presented with signs of encephalitis and three presented with atypical pneumonia (one of which later also had evidence of encephalitis) (Paton 1999). No new outbreaks have been reported in these countries since May 1999; however, NiV infection was subsequently recognized in Bangladesh in 2001 (Hsu 2004) and nearly annual outbreaks have occurred in that country since then (WHO 2018). Outbreaks also have been recognized in eastern India and, in 2018, an outbreak occurred in the southern Indian state of Kerala (Arunkumar 2018, Sharma 2018, Chattu 2018). To date, nearly 350 cases of NiV infection have been identified in Bangladesh and India (WHO 2018). Possible emergence of NiV in a third geographic area is evidenced by an outbreak of an unidentified henipavirus that occurred among horses and humans in the Philippines in 2014 (Ching 2015). Although detailed genomic information for the virus is limited, experts agree that the causative agent was either NiV or a virus closely related to NiV. The Henipavirus genus includes another pathogen, Hendra virus, which predominantly causes infection in horses in Australia and can also lead to human disease, usually following contact with infected horses (Ksiazek 2011).

The most common clinical syndrome associated with NiV infection is severe, rapidly progressive encephalitis (Goh 2000, Hossain 2008). The main presenting features include fever, headache, dizziness, vomiting, and altered mental status. NiV infection may result in late-onset

encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae (Tan 2002, Goh 2000). Some patients also have respiratory involvement or respiratory illness alone, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The overall case-fatality rate for the Malaysia outbreak was approximately 40%, whereas case-fatality rates during outbreaks in Bangladesh and India have generally ranged from 65% to 100% (Hossain 2008, WHO 2018). Genomic sequencing has demonstrated that there are multiple strains of NiV, which may account for the differences noted in clinical features and outcomes between Malaysia and Bangladesh (Mire 2016).

Fruit bats of the Pteropus genus are considered the primary natural reservoir for NiV. In the Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and possibly limited human-to-human NiV transmission (Chua 2010). Additionally, several other domestic animal species in the outbreak area (including horses, cats, and dogs) were found to be infected with NiV (Chua 2000). In outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap (Islam 2016, Luby 2006, Rahman 2012) and subsequent person-to-person transmission. While person-to-person transmission was apparently minimal in the Malaysia outbreak, it has played a larger role in amplifying outbreaks in Bangladesh and India (Gurley 2007, Luby 2009, Chadha 2006, Arunkumar 2018). This may, in part, be related to the greater propensity toward respiratory disease in NiV strains found in South Asia and transmission via droplet spread (Spiropoulou 2018). ~~The potential for droplet spread is supported by a~~ A recent study ~~that~~ demonstrated that hamsters could be infected when exposed to NiV via a small-particle aerosol; however, the epidemiologic significance of this remains unclear (Escaffre 2018). Given the potential for respiratory transmission of NiV and the recent experience with Ebola in West Africa, some have raised concerns about the possibility of larger NiV outbreaks if the virus takes hold in a crowded or urban area (Donaldson 2018) or about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Pteropus fruit bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Other fruit bats of the Pteropodidae family also may harbor NiV, and such bats can be found across Africa and parts of the Middle East. Evidence of NiV infection (via virus isolation, serologic testing, or nucleic acid detection using real-time polymerase chain reaction [RT-PCR]) has been found in Pteropus bat species and other bat species in geographically diverse regions, including South Asia (Bangladesh and India); Southeast Asia (Cambodia, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam); the Western Pacific region (Papua New Guinea); and Africa (Madagascar and Ghana) (Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2018, Yob 2001). This wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.

WHO has determined that Nipah virus is a priority disease for epidemic preparedness (WHO 2018). To improve epidemic response for this infection, in the fall of 2017, WHO embarked on development of a research and development (R&D) roadmap for NiV disease; this report provides a summary of the key elements of that roadmap, including important challenges and barriers that need to be addressed to ensure that diagnostics, therapeutics, and vaccines are developed, manufactured, and readily available when needed *[provide link to full roadmap here when available]*.

The NiV R&D roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one medical countermeasure category), diagnostics, therapeutics, and vaccines. The strategic goals and milestones identified in each section are focused on key achievements for the next 5 years; ~~the roadmap milestones will be tracked over time,~~ the roadmap milestones will be tracked over time, with periodic assessment of progress and updates to the roadmap as needed. The roadmap is specific to R&D for disease in humans; however, further research of NiV in animal species, including development of appropriate medical countermeasures targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV in humans and virus transmission can occur at the human-animal interface.

Cross-Cutting Issues

A major challenge for development of diagnostics, therapeutics, and vaccines for NiV is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Advocacy to policy makers in affected countries and to global stakeholders is needed to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures. As part of this process, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species (e.g., bats, swine, and equids) and proximate human populations in areas of predicted risk. Human health, animal health, and wildlife officials should be engaged in these efforts as part of a long-term collaborative One Health approach (Chattu 2018, Kulkarni 2013).

Another important issue is the need to optimize relevant animal challenge models for promoting development and evaluation of medical countermeasures for NiV infection, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval (Rockx 2014). For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for therapeutic or vaccine development; (3) determine when after challenge medical countermeasures should be administered in animal models to best mimic realistic timing of such use in humans; (4) bridge data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human doses; and (5) identify the best models for studying chronic (relapsing) infection ~~(Bossart 2012, Debuysscher 2013, ~~(Dhondt 2013, Johnston 2015, Mathieu 2015~~, Satterfield 2016~~). Currently, the African green monkey is regarded as the most relevant animal model for evaluating candidate therapeutics and vaccines intended for use in humans (Bossart 2012, Geisbert 2010, Johnston 2015), and studies involving nonhuman primates may be required for licensure of therapeutics and vaccines via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns, however, constrain the use of this animal model. In addition to optimizing animal models, standardized and well-characterized assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains are needed to move R&D of medical countermeasures for NiV infection forward (Satterfield 2016).

To inform development of optimal NiV medical countermeasures, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. This includes issues such as evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection.

To respond to these challenges, the strategic goals and milestones for cross-cutting issues in the NiV R&D roadmap focus primarily on developing a sustainable value proposition and identifying funding sources to promote R&D, availability, and accessibility of NiV medical countermeasures, optimizing animal models for research, and generating standardized and well-characterized assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains to facilitate R&D of NiV medical countermeasures (Table 1).

Diagnostics

Currently, no approved tests for diagnosis of NiV infection are commercially available. The most commonly used methods for diagnosing NiV infection include virus isolation, enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies, and real time-polymerase chain reaction (RT-PCR) nucleic acid testing. Virus isolation requires biosafety level 4 (BSL-4) capabilities and is generally only performed in reference laboratories for specific purposes, such as to confirm a new outbreak or to identify the virus in new host species (Wang 2012). During the Malaysia outbreak, the US Centers for Disease Control and

Prevention (CDC) developed a capture ELISA for detection of IgM antibodies and an indirect ELISA for detection of IgG antibodies (Daniels 2001); since then, additional ELISA tests have been developed (Wang 2012) and serologic testing is often used for case confirmation during outbreaks (Chadha 2006, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009). RT-PCR, which can be performed on throat and nasal swabs, cerebrospinal fluid, urine, and blood, is also often used for diagnostic testing (Chadha 2006, Guillaume 2004, Lo 2012). Quantitative RT-qPCR tests have been developed for quantifying viral replication; such tools can be used to measure viral load reduction when screening vaccine candidates and potential therapeutics (Jensen 2018). More recently, pseudotyped-based serum neutralization assays for NiV detection that use vesicular stomatitis virus systems have been developed; these assays potentially can be used under BSL2 conditions in laboratories with limited diagnostic capabilities and appear to offer improved sensitivity and specificity (Kaku 2009, Kaku 2012, Tamin 2009). Antigen-capture ELISAs using monoclonal antibodies also have been developed, which could result in more rapid diagnosis (Chiang 2010). These newer technologies hold promise; however, more information is needed regarding their performance characteristics, particularly under field conditions. Development and validation of immunohistochemical staining tests can facilitate studies on pathogenesis of Nipah infections and may also be useful for primary diagnosis in low-resource settings.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation, which can delay outbreak detection and implementation of infection control measures. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. To address these issues, rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes, once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of such a repository requires addressing the following: (1) determining what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outlining the purposes of sample collection; (3) determining what organizations will be responsible for activities related to creating and maintaining the repositories; (4) establishing standardized protocols for sample collection and maintenance; (5) establishing an appropriate governance structure; (6) identifying who would have access to the samples; (7) prioritizing use of samples and sample distribution; and (8) ensuring that material transfer agreements are in place. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

To address these issues, the strategic goals and milestones for diagnostic testing outlined in the NiV R&D roadmap focus on creation of a virtual reference repository, development of highly sensitive and specific point-of-care or near-patient NiV diagnostic tests, and enhancement of laboratory diagnostic preparedness to promote early detection of NiV in areas of known or potential spillover risk (Table 2).

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients; findings from treated patients were compared to those of 54 historical controls who became ill before ribavirin was available or who refused treatment (Chong 2001). Results demonstrated a ~~statistically~~ significantly lower mortality ~~reduction in mortality~~ among treated patients (36%); however, the use of historical controls may have biased this finding. No additional clinical studies using ribavirin have been conducted, and limited studies in animal models have not demonstrated efficacy of ribavirin following NiV or Hendra virus challenge (Rockx 2010, Georges-Courbot 2006). Chloroquine also has been studied in animal models, but has not shown any therapeutic benefit, either alone or in combination with ribavirin (Freiberg 2010, Pallister 2009). Based on these findings, these approaches are not considered viable options for treatment of NiV infection.

A more promising therapeutic option is the development of monoclonal antibodies (mAbs) against viral envelope proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). Therapy with anti-NiV mAbs may be appropriate for early treatment and for post-exposure prophylaxis (PEP) to prevent illness in exposed persons, such as healthcare workers, family caregivers, and persons exposed to infected livestock. Researchers have demonstrated that the human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009), and it has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Recently, a phase 1 clinical trial of m102.4 with 40 human participants was completed in Australia, but results are not yet available (ANZCTR). M102.4 was deployed for use during the 2018 NiV outbreak in Kerala, India, but was not used because the outbreak was brought under control quickly

(Spiropoulou 2018). One recent study in nonhuman primates showed that the therapeutic window for m102.4 may vary by virus strain, which is a consideration for further preclinical and clinical study of this agent (Mire 2016). It may also be worthwhile to explore development of alternate mAbs against NiV or to evaluate cocktails of mAbs.

Other potential options for NiV treatment include favipiravir (a small-molecule antiviral that inhibits RNA-dependent RNA polymerase), fusion inhibitory peptides (peptides that inhibit virus-mediated membrane fusion), and remdesivir (also known as GS-5734, which is a monophosphoramidate prodrug of an adenosine analogue). Researchers recently demonstrated efficacy of favipiravir in hamsters challenged with a lethal dose of NiV (Dawes 2018), supporting proof-of-concept for favipiravir as a possible therapy against NiV infection. Studies in animal models also have been conducted for fusion inhibitory peptides; for example, investigators recently reported that lipopeptides delivered via the respiratory route can prevent lethal NiV infection in hamsters and nonhuman primates (Mathieu 2018). Remdesivir is a small-molecule antiviral that is currently being evaluated in clinical studies as a therapeutic option for treating Ebola virus disease. Investigators recently demonstrated in vitro inhibition by remdesivir against a number of different viruses, including paramyxoviruses (Lo 2017). While these agents are promising, further preclinical data are needed to assess their utility. In addition, efforts are needed to expand the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents that may be more aligned with optimal and desired characteristics for anti-NiV therapy.

Key additional considerations for moving NiV therapeutics forward include: (1) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising novel treatments for NiV disease; (2)

ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (3) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in both endemic and at-risk areas. A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that are detected each year. To address this concern, a transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials and how to best allocate scarce resources. Additionally, clinicians in endemic areas need to maintain high vigilance for cases of acute febrile encephalitic syndrome, particularly in association with respiratory symptoms, to facilitate early diagnosis and case identification. Additional training of clinicians, along with effective and appropriate risk communication strategies need to be implemented in endemic areas.

To address these issues, the goals and milestones for therapeutics in the NiV R&D roadmap focus on continuing to move promising agents forward into clinical trials and on enhancing preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Satterfield 2016). A Hendra virus subunit vaccine involving soluble glycoprotein (sG) has been developed and is commercially available for use in horses (Middleton 2014), which supports this option as a promising approach for developing a human NiV vaccine.

The Coalition for Epidemic Preparedness Innovations (CEPI), which is a partnership of government agencies, industry, academia, philanthropy, and intergovernmental institutions aimed at providing funding for R&D of new vaccines against pathogens with epidemic potential, has recently provided financial support to promote the development of several NiV candidate vaccines. In May 2018, CEPI awarded a US$ 25 million contract to Profectus BioSciences, Inc. and Emergent BioSolutions Inc. to develop a~~n~~ human NiV vaccine that is based on the same technology as that used for the Hendra virus vaccine for horses (CEPI 2018). In September 2018, CEPI announced plans to award approximately US$ 2 million to the Jenner Institute at the University of Oxford and Janssen Vaccines & Prevention BV for preclinical development of a replication-deficient simian adenoviral-vectored vaccine, derived from an adenovirus originally isolated from chimpanzees (CEPI 2018). These efforts, and potentially additional future efforts funded by CEPI, will support preclinical and early clinical development of NiV vaccines.

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012, Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research in this area should focus primarily on the humoral immune response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

The immediate goals and milestones in the vaccine section of the NiV R&D roadmap focus on moving vaccine candidates forward into clinical trials and promoting development of guidance on requirements for clinical trials, regulatory pathways, and other considerations relevant to licensure of a vaccine against NiV.

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures forward. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 31 October 2018 [Web page]

Arunkumar G, Chandni R, DT Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

CEPI (Coalition for Epidemic Preparedness Innovations). CEPI awards $25 million contract to Profectus BioSciences and Emergent BioSolutions to develop Nipah virus vaccine. 2018 May 24 [Full text]

CEPI. CEPI awards contract worth up to USD$19 million to Oxford University and Janssen Vaccines to develop MERS, Lassa, and Nipah vaccines. 2018 Sep 27 [Full text]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

~~DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]~~

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in Fruit Bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C_{12}U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis 2014;20:372-9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6): 30916 [Full text]

Pallister J, Middleton D, Crameri G. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. J Virol 2009 Nov;83(22):11979-82 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. List of Blueprint priority diseases; accessed October 28, 2018 [Webpage]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed October 26, 2018 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Tables

Table 1: Goals and Milestones for NiV Infection Cross-Cutting Issues

Goals and Milestones for NiV Infection Cross-Cutting Issues Nipah Virus Roadmap	
STRATEGIC GOALS	**MILESTONES**
1. Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.	1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
	2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.
2. Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.	1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
	2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Table 2: Goals and Milestones for NiV Diagnostics

Goals and Milestones for NiV Diagnostics Nipah Virus Roadmap	
STRATEGIC GOALS	**MILESTONES**
1. Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.	1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).
	2. By 2021, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.
2. Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are	1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.
	2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
	3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.	4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
3. Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.	1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnose NiV infection during future outbreaks.

Table 3: Goals and Milestones for NiV Therapeutics

Goals and Milestones for NiV Therapeutics **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.	1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol. 2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol. 3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol. ~~3.~~4. By 2019, identify an approach for downselecting promising therapeutic candidates for further study in clinical trials, given limited annual case numbers for enrollment. ~~4.~~5. By 2020, generate a reliable source of m102.4 (or other promising agent[s] or agent combinations) to be assessed in clinical trials.
2. Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.	1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
	2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
	3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Table 4: Goals and Milestones for NiV Vaccines

Goals and Milestones for NiV Vaccines **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.	1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
	2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as the FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.
2. Develop and evaluate NiV vaccines for prevention of NiV disease in humans.	1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
	2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
	3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

December 2018

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs)—diagnostics, therapeutics, and vaccines—against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (*Pteropus* species and several other bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species (including horses, cats, and dogs) were found to be infected with NiV on the farms involved in the outbreak. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen—Hendra virus (HeV)—which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat; disease also occurred in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

NiV infection in humans results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India. Some differences have been noted in the clinical features of infection, with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of MCMs to enable effective and timely emergency response to infectious disease epidemics. NiV infection is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this roadmap ranges from basic research to late-stage development of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include minimizing zoonotic NiV transmission, improving use of personal protective equipment (PPE), ensuring adequate hand hygiene and environmental hygiene, promoting effective community engagement, implementing adequate infection prevention and control practices, developing adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and promoting workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap but need to be addressed as part of a broader public health control strategy. Further research of NiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV (or related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

VISION

Ready availability and accessibility of r~~R~~obust MCMs to detect, prevent, and control human outbreaks of NiV infection (and other closely related henipaviruses) ~~that are readily available and accessible~~ for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research represents a substantial challenge, since economic incentives to invest in Nipah research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with small and sporadic outbreaks. The development of a sustainable value proposition for industry and international philanthropic public-private partnerships is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate.
- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (biosafety level 4 [BSL-4]) conditions, which can increase the cost of MCM development.
- To date, NiV spillovers to human communities have been identified most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the *Pteropus* genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to the lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- While ferrets, Syrian hamsters, and interferon-alpha/beta receptor knockout (IFNAR-KO) mice are well-established animal models for NiV research, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection occurs in relatively small, focal outbreaks, the low disease incidence poses a major

challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop and manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries and to global stakeholders to ensure they understand the potential health, societal, and economic benefits of devoting resources to improving NiV surveillance, detection, prevention, and control measures.
- Obtaining additional prospective serosurveillance data of henipavirus exposure from susceptible animal species and proximate human populations in areas of predicted risk should be explored as a strategy to assess the potential of human spillover and to build preparedness for detection of human cases and for limiting exposure.
- Standardized and well-characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains for R&D of MCMs for NiV infection. Assays that can be used at lower biosafety levels are an important priority. WHO international standards should be used (when available) as calibrators and reported in units/ml to harmonize assay results.
- Clear criteria for downselection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or other regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.

- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, depend on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of *Pteropus* bats and potentially other bat species.
- Additional research is needed to optimize relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) for promoting development and evaluation of MCMs, particularly if investigators are required to use an alternative pathway (such as the United States Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after-challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunologic clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurologic sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes that may affect the epidemiologic and clinical features of disease over time and thereby influence MCM development.

- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole-genome sequencing of NiV are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume date palm sap, healthcare workers, and workers at the human-animal interface) and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains to facilitate R&D of NiV MCMs.

2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and that may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.
- **Generate** research tools to promote R&D of MCMs for NiV infection (e.g., standardized and validated assays, reagents, antibodies, nucleic acids, and ~~stocks of~~ NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability of henipaviruses to improve understanding of their global distribution.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for downselection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.

- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an "affordable and fair" price is one that can reasonably be paid by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

DIAGNOSTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific, and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and investigation into the cause of infection is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.

- Owing to the biosafety precautions necessary when working with NiV, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods is problematic, given the rapid disease progression of NiV infection.
- Currently, no approved tests for diagnosis of NiV infection are commercially available.
- *Pteropus* species (and perhaps other bat species) appear to carry other henipaviruses in addition to NiV and HeV, some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate using serologic assays which henipaviruses are in circulation. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.
- A TPP for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples to assess and validate diagnostic tests. As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6) identify who would have access to the samples; (7) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature), and have a high sensitivity and specificity for different NiV strains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

- Improved diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, particularly given the costs of regulatory approval.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotype neutralization assays and antigen-capture enzyme-linked immunosorbent assays [ELISAs]) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries: Bangladesh and India.
2. By 2021, identify funding and initiate creation of the virtual reference repository in Bangladesh and India, with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.

2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnose NiV infection during future outbreaks.

Priority Areas/Activities

Research

- **Create** a virtual repository of clinical reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- **Develop and evaluate** point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed.

Policy and commercialization

- **Develop** guidance on optimal strategies for the deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

THERAPEUTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment of patients and PEP for exposed persons.
- In NiV-endemic areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis but do not have NiV infection. Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore, accurate and rapid diagnosis is critical.
- Studies in animals often evaluate the usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well a therapeutic agent will work in the field.
- NiV can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control–programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.
- A limiting constraint to assessing the effectiveness of promising therapies is the number of patients with NiV infection who can be enrolled in clinical trials, given the small number of cases that are detected annually.

Key needs

- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas, particularly during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).
- A transparent and collaborative process is needed to determine which agents are most appropriate for study in future clinical trials, given the limited number of cases that could potentially be enrolled in trials each year.

Knowledge gaps

- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to further assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed that the therapeutic window for m102.4 against a strain from Bangladesh/India was shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection and strengthen the therapeutic pipeline.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship of promising therapeutic candidates.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will affect the response to therapeutic candidates and results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials to inform best-practice guidelines and evidence-based policy decisions.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
4. By 2019, identify an approach for downselecting promising therapeutic candidates for further study in clinical trials, given limited annual case numbers for enrollment.
5. By 2020, generate a reliable source of m102.4 (or other promising agent[s] or agent combinations) to be ~~used~~ assessed in clinical trials.

Strategic Goal 2: Develop and evaluate therapeutic agents for the treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as m102.4 and favipiravir) for treating and preventing NiV infection, including conducting studies in animal models and clinical trials as appropriate and feasible.
- **Continue to expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Develop, evaluate, and license** safe and effective therapeutic agents for the treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in affected areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- **Generate** a reliable source of m102.4 to be used in clinical trials.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

VACCINES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development, and few companies are willing to invest in the generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could diminish the acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for the timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on the use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen, and therefore multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed involving target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations and identify additional areas of potential NiV spillover.
- Additional sociological research is needed to explore perceptions and concerns of at-risk populations regarding NiV vaccine implementation.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the need for licensure and future use of NiV vaccines. This consultation should include representatives from in-country regulators, other regulatory agencies (such as the FDA or the European Medicines Agency), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of the humoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serologic assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.

- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Evaluate** the feasibility of generating multivalent vaccines (i.e., that protect against more than one disease) and determine whether or not such vaccines would be useful in future NiV control efforts.

Product development

- **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptance and promotion within the community.
- **Consider** developing a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for faster spread.

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

WHO. Nipah baseline situational analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017 [Full text]

Other Publications:

Arunkumar G, Chandni R, D T Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Med 2016;9(7):630-4 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 29 Nov 2016; site accessed 31 October 2018 [Web page]

Baseler L, Scott DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov 3;10(11):e0005120 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Pathog 2009 Oct 30;5(10):e1000642 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (US Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 20 Mar 2014 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2):235–240 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-283 [Full text]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of infectious diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PKG, de los Reyes V, Sucaldito M, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015 Feb;21(2):328-31 [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. Malaysian J Pathol 2010; 32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microb Infect 2013 Jun;2(6):e37 [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Curr Opin Virol 2017 Feb;22:97-104 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8:7604 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024 [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Abstract]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global virology I — identifying and investigating viral diseases. New York, NY: Springer US; 2015:125-46

Deka MA, Morshed N. Mapping disease transmission risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2).pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug;14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-7 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37. [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The role of animals in emerging viral diseases. Amsterdam: Elsevier; 2014:293-315 [Abstract]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis. 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2):e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lancet editors. Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137):2295 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55. [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013 Oct;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug; 15(8): 1229–1235 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015 Mar;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-227 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis. 2014;20:372–9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;6:30916 [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13;10:353 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9. Epub 2000 May 15 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sep 1;157(9):1635-41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Rahman SA. National guideline for management, prevention and control of Nipah virus infection including encephalitis. Dec 2011 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-4 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Schmidt R, Beltzig LC, Sawatsky B, et al. Generation of therapeutic antisera for emerging viral infections. NPJ Vaccines 2018 Oct 5;3:42 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013; 8(7): e69544. Published online 2013 Jul 22 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Epub ahead of print]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Steffen DL, Xu K, Nikolov DB, et al. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Sun B, Jia L, Liang B, et al. Phylogeography, transmission, and viral proteins of Nipah virus. Virol Sin 2018; Oct 11; Epub ahead of print [Full text]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

Yu J, Lv X, Yang Z, et al. The main risk factors of Nipah disease and its risk analysis in China. Viruses 2018 Oct 19;10(10). pii:E572 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, influenza, Nipah, and Semliki Forest chimeric viruses with vesicular stomatitis virus: actions in the brain. J Virol 2017 Feb 28;91(6):e02154-16 [Full text]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah Virus, Thailand. Emerg Infect Dis 2005 Dec; 11(12):1949–1951 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

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Date:	Wednesday, December 19, 2018 12:57:13 PM
Attachments:	Nipah Roadmap manuscript.V2.docx Nipah RD Roadmap.December2018.docx
Importance:	High

Dear Nipah virus R&D taskforce members: Thank you to those who submitted comments on the versions that were sent earlier. I have attached a revised manuscript and a slightly revised roadmap; both incorporate feedback that we received.

Please take another look, if you have time. IF WE DON'T HEAR FROM YOU BY JANUARY 7, WE WILL ASSUME THAT THESE VERSIONS ARE ACCEPTABLE TO YOU.

After that date, I will forward the manuscript and roadmap to WHO for their final review and approval process. Any changes to the roadmap will be at the discretion of WHO. They will also be moving the manuscripts forward. We'll keep you posted on that process. I assume that, at some point, we'll need to obtain an authorship form from you, but that can wait for now.

Thank you again for your time and effort regarding this important work!

Happy holidays to all of you!

Warm regards,

Kris

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CEPI New vaccines for a safer world

Application for funding from CEPI (CEPI-CfP-001)

Project description template step 2

1. Project summary

1.1. Project Title

A Subunit Vaccine (HeV-sG) to Protect Against Nipah and Hendra Diseases

1.2. Project summary (500 words)

*Please give a summary of the proposed project, including rationale, technology, vaccine candidate, objectives, expected outcomes and **total project costs***

Nipah virus (NiV) and Hendra virus (HeV) are closely related paramyxoviruses associated with significant morbidity and mortality in both animals and humans. Outbreaks of NiV (Malaysia, Bangladesh, India) and HeV (Australia) lead to death in 40 to 90% of infected subjects. There are currently no approved products that prevent or treat NiV or HeV infections in humans. A recombinant subunit vaccine derived from the ectodomain of HeV attachment G glycoprotein (HeV-sG) has demonstrated complete protection against lethal NiV and HeV disease in three animal species, including African Green Monkeys (AGM). In addition, a horse vaccine composed of the same HeV-sG has been marketed in Australia by Zoetis since 2012. We and the cooperating partners are currently developing the HeV-sG vaccine to protect humans against both NiV and HeV disease. To date, a Master Cell Bank (MCB) has been manufactured and released, a cGMP process for vaccine manufacture has been developed, a GLP toxicology study is nearing completion, a study in AGMs to confirm protective efficacy against lethal challenge has been completed, and stability studies of the development/toxicology HeV-sG candidate vaccine are in progress. Here we propose to advance development of this dually protective vaccine to the point of establishing an emergency use stockpile by:

- Producing and releasing drug substance (DS) and drug product (DP) under cGMPs.
    - Production at 1,000L scale is calculated to yield approximately 120 gm of purified product
    - 1,000 vials will be filled to support phase 1 evaluation
    - 50,000 vials at optimum dose filled to support phase 2 evaluation and investigational vaccine stockpiling
- Submitting an IND.
- Performing Phase 1 and 2 clinical studies to demonstrate human safety and immunogenicity.

Total project cost to this point in the proposal is estimated to be $XXXX

Optional activities that could be performed to accelerate licensure:

- Performing challenge studies under the “animal rule” to bridge human immune responses to protective responses in AGMs.
- Producing and releasing two additional at-scale Lots of vaccine to demonstrate manufacturing and clinical consistency.
- Submitting a BLA.

1.3. Development status

NiV and HeV must be handled under biological safety level 4 (BSL-4) conditions due to the severe disease they cause in humans, and the US NIH and US CDC list them as Category C biothreat agents. Only NiV is included in the current CEPI call for proposals, however, the ability of HeV-sG to protect against both NiV and HeV disease provides an additional benefit. These emerging viruses, unlike other Category A biothreat viral agents such as Smallpox or Ebola virus, can easily be isolated from their natural reservoir, grown in cell culture to high titer (1-5), and spread in livestock that are known to transmit these viruses to humans, where subsequent person-to-person transmission can facilitate an outbreak (6-8). Transmission via aerosol and a high rate of lethality heighten their potential as biothreat agents.

Currently, there is no approved human prophylactic or therapeutic against NiV or HeV, disease. During the 2009 outbreak of HeV in Australia, four people exposed at a Queensland horse nursery were offered treatment with Ribavirin (The Sydney Morning Herald, August 12, 2009). Unfortunately, the Ribavirin treatment had no impact on disease presentation or progression, and the infected veterinarian died on September 1, 2009. The other three individuals were later found negative for HeV. Chloroquine and Ribavirin will delay disease progression to death in NiV infected hamsters by approximately five days, but do not show any effect in HeV-infected hamsters (9) or AGMs (10). Vaccination, on the other hand, offers a viable anti-henipavirus strategy (4, 11) that could provide significant public health benefit against natural outbreak or deliberate misuse as a bio-weapon. A HeV-sG vaccine for veterinary use (Equivac® HeV), which is identical to the vaccine proposed here for human use, has been marketed by Zoetis in Australia since November 2012 under the authority of the Australian Pesticides and Veterinary Medicines Authority (APVMA). This is the first commercialized vaccine against any BLS-4 agent. The APVMA granted full registration of Equivac® HeV in August 2015. Of note, the human immune response to accidental inoculation (N=2) with the veterinary vaccine has been shown to be potent and to cross-neutralize both NiV and HeV (I. Smith and C. Broder, unpublished).

Profectus BioSciences, Inc. is a vaccine developer with the personnel and resources to manage HeV-sG vaccine manufacture, clinical evaluation, and regulatory approval. The company possesses intellectual property and expertise in the fields of live replication competent recombinant vaccines based on attenuated vesiculoviruses, vaccines and molecular adjuvants based on plasmid DNA vectors, as well as soluble subunit vaccines. Profectus performs GMP, GLP, and GCP activities through outsourcing, and has the personnel and key quality system policies and procedures needed to support the manufacture, testing, and conduct of human clinical trials utilizing GMP, GLP and GCP-compliant contract service providers. Development of the HeV-sG vaccine as well as a human monoclonal anti-NiV/HeV antibody (m102.4) for post-exposure treatment have been supported by two NIAID RO1 awards under the Partnerships for Biodefense program that include Drs. Broder (Uniformed Services University) and Geisbert (University of Texas Medical Branch at Galveston) as essential personnel. In the case of HeV-sG, the RO1 funding supports development of this vaccine through MCB production, upstream process development (USP), downstream process development (DSP), and toxicology testing, but does not support manufacture under cGMPs. Therefore, this application proposes the manufacture of a GMP Lot of HeV-sG of sufficient size to support phase 1 clinical testing, phase 2 clinical testing, and the stockpiling of approximately 150,000 doses of investigational vaccine for emergency use. Manufacturing will be performed with Profectus operational and quality oversight using Profectus-approved CMOs. The clinical trials will be performed in partnership with PATH, using PATH clinical research, operations, and data management infrastructure and the Profectus clinical assay laboratory.

Should CEPI choose to support the most expedient path to licensure, two additional at-scale Lots of candidate vaccine could be produced and released to demonstrate manufacturing and clinical consistency. In addition, “animal rule” studies designed in consultation with the US FDA to bridge the human immune response to the protective response in AGMs could be conducted in partnership with Dr. Geisbert at the BSL-4 laboratories at UTMB.

2. About the applicant organisation/consortium

2.1. About the applicant organization and cooperating partner(s)

Profectus BioSciences, Inc. The Profectus Vaccines Group was spun-out of Wyeth Vaccines Research in 2008 with the mission of developing and commercializing vaccines to treat and prevent chronic viral diseases (HIV, HCV, HPV, HSV), and viral infections of biodefense and public health importance (filoviruses, arenaviruses, alphaviruses, henipaviruses), areas outside the immediate focus of Wyeth (now Pfizer). To enable Profectus, Wyeth transferred:

- A broad intellectual property (IP) portfolio in vectored and subunit vaccines.
- The experienced senior staff essential for the research, development, and cGMP manufacture of vaccines. These individuals each possess 10 or more years of experience in a major pharma environment and include:
    - John H. Eldridge, Ph.D., Chief Scientific Officer
    - David Clarke, Ph.D., Senior Director of Virology/Vector Design
    - Rong Xu, Ph.D., Director of Immunology
    - Susan Sciotto-Brown, Senior Director Regulatory Affairs
    - Tracy Chen, Ph.D., Executive Director of Outsourcing and Operations
    - Marc Tremblay, M.S., Senior Director of Quality
    - Alan Gordon, Esq., Corporate Counsel
    - Also on staff at Profectus is Dr. Antony Dimitrov, subject matter expert on NiV and HeV, and Project Leader
- Proprietary and patented manufacturing methods, equipment, and certified cell banks needed to manufacture vaccines for human use.

Profectus performs GMP, GLP, and GCP activities through outsourcing, and has the personnel, key quality systems, policies and procedures needed to support the manufacture, testing, and conduct of human clinical trials utilizing GMP, GLP and GCP-compliant contract service providers. Over the past 8 years Profectus has successfully produced/maintained 6 vaccine components under cGMPs, written 8 INDs, and conducted 7 clinical trials in the US and EU. Partners and collaborators include, the Galveston National Laboratory at UTMB, Yale University, the Institute of Human Virology, the Center for HIV/AIDS Vaccine Immunology, the National Cancer Institute, the NIH Division of AIDS, the Bill and Melinda Gates Foundation, the International AIDS Vaccine Initiative, the HIV Vaccines Trials Network, and the AIDS Clinical Trials Group.

University of Texas Medical Branch, Galveston National Laboratory (UTMB). Dr. Thomas Geisbert at UTMB has extensive experience conducting experiments utilizing small animals and nonhuman primates (NHPs) in high biocontainment, with over 28 years' biosafety-level 4 (BSL-4) experience at USAMRIID and at the Galveston National Laboratory (GNL). He has experience managing large research programs, including a current US National Institutes of Health-funded Center of Excellence for Translational Research grant. Dr. Geisbert's research interests have focused on the study of haemorrhagic fever viruses and he has published extensively in this area. His research group was the first to demonstrate complete protection of NHPs against Ebola, Marburg, and Lassa viruses using a novel vaccine based on recombinant vesicular stomatitis virus (VSV). In addition, Dr. Geisbert has extensive experience with developing appropriate animal models that mimic human pathology, including the ferret and AGM models for NiV and HeV.

PATH. For more than 20 years, PATH has successfully developed and delivered lifesaving vaccines for the most vulnerable children and communities around the world, spanning the spectrum of discovery to development to delivery. PATH's Center for Vaccine Innovation and Access (CVIA) brings together global expertise across every stage of the long and complex vaccine process, from early discovery and clinical testing of vaccine candidates, to regulatory approval and manufacturing, to introduction and delivery. PATH has led numerous global partnerships to advance vaccine development, including the Malaria Vaccine Initiative, which helped develop the first malaria vaccine candidate recommended for pilot implementation, and the award-winning Meningitis Vaccine Project, which developed the first vaccine tailor-made for use in sub-Saharan Africa. CVIA's current portfolio encompasses more than two dozen vaccines either in development or already in use to protect against 16 different diseases.

Catalent: Catalent Pharma Solutions (Catalent) is located at 726 Heartland Trail, Madison, WI and is a contract manufacturer of Master Cell Banks and Bulk Drug Substance (BDS) such as monoclonal, fusion and recombinant mammalian derived proteins for clinical Phase I and II use. These proteins are produced utilizing aseptic processing to minimize bioburden and cross contamination during the biological manufacturing processes.

GRAM: Grand Rapid Aseptic Manufacturing (GRAM) is located 140 Front Avenue SW, Suite 3, Grand Rapids, MI and is founded in 2010. It is a full-service parenteral contract manufacturing organization supporting new product development and cGMP manufacturing. GRAM is currently approved by FDA to manufacture 4 commercial drug products with the first approval granted in 2013. The current portfolio is 57% biologics and 43% small molecules with 80% being sterilized by aseptic filtration and 20% by terminal sterilization. GRAM manufactures drug products in vials and syringes to support clinical trial from Phase 1 to Phase 3.

Uniformed Services University, Henry M. Jackson Foundation (USU-HMJF). The mission of the Uniformed Services University of Health Sciences is to educate, train, and comprehensively prepare uniformed services health professionals, scientists, and leaders to support the Military and Public Health Systems, the National Security and National Defense Strategies of the United States, and the readiness of our Uniformed Services. The mission of the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. is to advance military medical research and improve health worldwide.

Duke-NUS Medical School. SingHealth Investigational Medicine Unit, Singapore General Hospital, Block 7 Level 7, Outram Road, Singapore 169608. & Duke-NUS Medical School, 8 College Road, Singapore 169857. Dr. Low is a senior consultant infectious disease physician at the Singapore General Hospital and a Associate Professor of Emerging Infectious Diseases at the Duke-NUS Medical School. Her research addresses translational therapeutics and vaccinology for acute viral diseases, focusing primarily on dengue and influenza in the first instance. She has been the clinical PI for 5 vaccine trials, 2 of which were first-in-man trials, as well as the clinical PI for a therapeutic trial for acute dengue. Importantly, she co-led the trial that authenticated antibody-dependent enhancement of yellow fever vaccination in those with cross-reactive flaviviral antibodies, the findings of which were published last year in Nature Microbiology.

2.2. The experience and track record of applicant organisation, principal investigator and cooperating partner(s)

Pre-clinical studies

In the interest of brevity, the pre-clinical R&D experience of the Profectus, UTMB, USU-HMJF collaborators relevant to this proposal is presented in **Table 1**.

Table 1. Relevant PBS, UTMB, USU-HMJF Pre-Clinical Experience

Field	Animal Model	Vaccine Type			Challenge	Immune Measures			
						Humoral		CMI	
		pDNA	Viral Vector	Protein Subunit		ELISA	Neut	ELISpot	ICS
Arenaviruses (LASV, MACV, JUNV, GTO)	Mouse		√			√			
	NHP		√		√	√			
Nipah/Hendra				√					
	Ferret				√				
	NHP				√	√			

Filoviruses (EBOV, SUDV, MARV, BUDV)	Mouse		√			√	√	√	√
	GP				√				
	NHP				√	√	√	√	√
Chikungunya	Mouse		√		√	√	√		
Zika	Mouse		√		√	√	√		
Malaria	Mouse	√	√			√		√	
	NHP					√		√	
HPV	Mouse	√	√		√	√		√	
HIV	Mouse	√	√	√		√	√	√	√
	GP						√		
	NHP				√	√	√	√	√

The UTMB Galveston National Laboratory (GNL). The GNL provides BSL-4, BSL-3 and BSL-2 laboratories designed to allow the safe conduct of research into emerging infectious diseases and agents of bioterrorism to develop vaccines, diagnostics, and therapeutics against them. The seven-story GNL and the adjacent Keiller Building together offer 14,330 square feet of BSL-4 space, 29,713 square feet of BSL-3 facilities and 51,903 square feet for BSL-2 level laboratories. Additionally, there are BSL-3 and BSL-4 aerobiology facilities.

The GNL's mission is fulfilled through a system of specialized research cores providing the following services: aerosolization studies of BSL-3 and BSL-4 level pathogens in small animals and NHPs; animal model development; host-pathogen studies; screening methodology development through *in vitro* studies; technical assistance in histological and tissue processing methods; and diagnostic expertise in the event of disease outbreaks or bioterrorism. The GNL also provides a national resource for studying and developing protocols against arthropod vectors; state-of-the-art equipment, particularly that available via an Imaging Core having expertise in the analysis of human and animal tissues and *in vivo* whole-animal imaging; plus, a full-service immunology core in which knockout and transgenic mouse models are developed for immunological studies. Other cores provide administrative and regulatory (GLP) oversight, as well as ensure security, safety and efficient operation of the GNL. In addition, the GNL features full-service animal care procedural and maintenance facilities, including laminar flow isolator caging systems, sterilization equipment, procedure rooms, cage-washing and autoclaving.

The GNL BSL-4 laboratory consists of approximately 12,000 square feet of space equally divided between wet space and ABSL-4 facilities. The BSL-4 laboratory consists of three modules that can be operated in concert or independently. Each module consists of a wet lab (~1,000 square feet), a necropsy room (approximately 250 square feet), a virus storage and centrifuge room (approximately 250 square feet) and one to three animal rooms. Animal housing can accommodate rodents, ferrets, and nonhuman primates (NHPs). CDC inspections and select agent permits are current. The first NHP studies were performed in the GNL BSL-4 laboratories in April 2011. Seven different viruses, including Lassa, Nipah and Hendra viruses, have been used, and Dr. Geisbert's team is adept at performing procedures on animals that involve inoculating filoviruses, monitoring animals for signs of disease, collecting specimens, and evaluating clinical data during the disease course.

Phase I/II clinical vaccine trials

Profectus phase I/II clinical trials relevant to the current proposal are presented in **Table 2**, PATH phase I/II clinical trials are presented in **Table 3**, and Duke-NUS phase I/II clinical trials are listed in **Table 4**.

Table 2. Relevant PBS Phase I/II Clinical Vaccine Trials – High Income Countries

Field	**IND Number**	**Vaccine Type(s)**			**Number of Subjects**	**Immune Measures**			
						Humoral		**CMI**	
		pDNA	**Viral Vector**	**Protein Subunit**		**ELISA**	**Neut**	**ELISpot**	**ICS**
HIV	BB-IND-14116	√			120	√	√	√	√
HIV	BB-IND-13449	√			120	√	√	√	√

HIV	BB-IND-14665		√		60	√	√	√	√
HIV	BB-IND-14976	√	√		100	√	√	√	√
HIV	BB-IND-14770	√	√		120	√	√	√	√
HIV	BB-IND-15459	√	√		30	√	√	√	√
HIV	BB-IND-16505			√	60	√	√	√	√
Ebola	BB-INF-16670		√		39	√	√	√	√

PATH has expertise in early and late stage clinical development of vaccine candidates for use in resource-limited settings. Over the past 15 years, PATH has overseen the conduct of approximately 70 Phase I and II trials, including approximately 40 in high-income countries and approximately 30 in low- and middle-income countries. The following is an illustrative list highlighting PATH's diverse early-stage clinical trial experience.

Table 3. Select PATH Phase I/II Clinical Vaccine Trials – All Countries

Short Title	Indication	Phase	Number of Subjects	Population	Countries	Clinical Trial Registry Number
Multivalent Inactivated Enterotoxigenic Escherichia Coli (ETEC) ETVAX (ETVAX/dmLT)	Enterotoxigenic E Coli	I	495	Adults, Toddlers, Infants	Bangladesh	NCT02531802
Shigella Sonnei 53G Challenge	Shigella	II	45	Adults	USA	NCT02816346
Serum Institute of India's 10-valent Pneumococcal Conjugate Vaccine (SIILPCV10)	Pneumococcal	I	30	Adults	India	CTRI/2013/09/003961
Malaria Vaccine in Healthy Adults (Ad35.CS.01 + RTS,S prime-boost)	Malaria	I	168	Adults	USA	NCT01366534
Monovalent Live attenuated H7N3 Influenza vaccine (A/17/Mallard/Netherlands/00/95	Influenza	I	40	Adults	Russia	NCT01511419
Influenza A/H5N1 Vaccine Clinical Trial (IVACFLU-A/H5N1) - Phase 1	Influenza	I	76	Adults	Vietnam	NCT02171819
Trivalent Split, Inactivated Influenza Vaccine	Influenza	I	60	Adults	Serbia	NCT02585700
Needle-free Jet Injection of Reduced-dose, Intradermal, Influenza Vaccine in >= 6 to < 24-month-old Children	Influenza	I/IIa	450	Children	Dominican Republic	NCT00386542
Live Attenuated Shigella Vaccine (WRSS1)	Shigella	I/II	103	Toddlers	Bangladesh	NCT01813071
S.Pneumoniae Whole Cell Vaccine (PATH-wSP)	Pneumococcal	I/II	304	Adults, Toddlers	Kenya	NCT02097472
Monovalent P2VP8 Subunit Parenteral Rotavirus Vaccine	Rotavirus	I/II	204	Toddlers, infants	South Africa	NCT02109484
10-Valent Pneumococcal Conjugate Vaccine (SIILPCV10)	Pneumococcal	I/II	346	Adults, Toddlers, Infants	The Gambia	NCT02308540
Trivalent P2-VP8 Subunit Parenteral Rotavirus Vaccine	Rotavirus	I/II	660	Adults, Toddlers, Infants	South Africa	NCT02646891
Live attenuated Shigella Vaccine (WRSS1)	Shigella	I/II	64	Toddlers	Bangladesh	NCT02934178
Meningococcal A conjugate vaccine (MenAfriVac®)	Meningitis	II	1200	Infants	Ghana	ISRCTN No. 82484612
Polyvalent ACYW Conjugate Meningococcal Vaccine	Meningitis	II	601	Toddlers	Mali, The Gambia	ISRCTN No. 78147026
GSK Biologicals' Malaria Vaccine 257049	Malaria	II	480	Infants	Malawi	NCT01231503
GSK Biologicals' 2189242A Vaccine (Nasopharyngeal Carriage, Safety & Immunogenicity)	Pneumococcal	II	1320	Children	The Gambia	NCT01262872
Intranasal Seasonal Trivalent Live-Attenuated Influenza Vaccine	Influenza	II	300	Children	Bangladesh	NCT01625689

Vaccine for Pneumonia in Adults and Toddlers in Kenya (Dose-finding)	Pneumococcal	II	147	Adults, Toddlers	Kenya	NCT02543892
Inactivated Whole Cell Shigella Flexneri 2a Vaccine With and Without dmLT	Shigella	II	120	Adults	USA	NCT03038243

Table 4. Relevant Duke-NUS Phase I/II Clinical Vaccine Trials – Middle Income Countries

	Study Title	Publication
1	A Phase 1 Trial to Evaluate the Safety and Immune Responses of a Virus-like Influenza Particle (VLP) in healthy adults (Funding source: Experimental Trial Centre) $200,000, 03/12-12/12, Clinical PI	Safety and immunogenicity of a virus-like particle pandemic influenza A (H1N1) 2009 vaccine: Results from a double-blinded, randomized Phase I clinical trial in healthy Asian volunteer. *Vaccine. 2014 Sep 3;32(39):5041-8.* **Low JG**, Lee LS, Ooi EE, Ethirajulu K, Yeo P, Matter A, Connolly JE, Skibinski DA, Saudan P, Bachmann M, Hanson BJ, Lu Q, Maurer-Stroh S, Lim S, Novotny-Diermayr V
2	Adult Dengue Platelet Study (ADEPT) (NMRC/TCR/005) $413,001, 01/09-12/14, Site-PI	Prophylactic platelet transfusion versus supportive care in adult dengue with severe thrombocytopenia: a randomised, open-label, superiority trial. The Lancet 2017 Apr 22;389(10079):1611-1618 David C Lye, Sophia Archuleta, Sharifah F Syed-Omar, **Jenny G Low**, Helen M Oh, Yuan Wei, Professor Dale Fisher, Sasheela SL Ponnampalavanar, Limin Wijaya, Linda K Lee, Eng-Eong Ooi, Adeeba Kamarulzaman, Lucy C Lum, Paul A Tambyah, Yee-Sin Leo
3	Randomized, Double-Blind, Placebo-Controlled, Dose Exploratory Phase 2a Clinical Study to Evaluate the Antiviral Activity, Pharmacokinetics, Safety and Tolerability of Celgosivr in Adults with Confirmed Dengue Fever (NMRC/TCR/005), $1,493,981, 02/12-04/13, PI	The Current Status of Dengue Drug Therapeutics Research and Development *Journal of Infectious Diseases (editorial)* 2017 Mar 1; 215(Suppl 2): S96–S102 **Jenny GH Low**, Eng Eong Ooi and Subhash G Vasudean Optimizing celgosivir therapy in mouse models of dengue virus infection of serotypes 1 and 2: the search for a window for potential therapeutic efficacy. *Antiviral Res 2016 Jan 13;127:10-19. Epub 2016 Jan 13.* Satoru Watanabe, Kitti Wing Ki Chan, Geoffrey Dow, Eng Eong Ooi, **Jenny G. Low**, and Subhash G. Vasudevan Efficacy and safety of celgosivir in patients with dengue fever (CELADEN): a phase 1b, randomised, double-blind, placebo-controlled, proof-of-concept trial. *Lancet Infect Dis. 2014 Aug;14(8):706-15.* **Low JG**, Sung C, Wijaya L, Wei Y, Rathore AP, Watanabe S, Tan BH, Toh L, Chua LT, Hou Y, Chow A, Howe S, Chan WK, Tan KH, Chung JS, Cherng BP, Lye DC, Tambayah PA, Ng LC, Connolly J, Hibberd ML, Leo YS, Cheung YB, Ooi EE, Vasudevan SG. Dose- and schedule-dependent protective efficacy of celgosivir in a lethal mouse model for dengue virus infection informs dosing regimen for a proof of concept clinical trial. *Antiviral Res. 2012 Oct;96 (1):32-5.* Watanabe S, Rathore AP, Sung C, Lu F, Khoo YM, Connolly J, **Low J**, Ooi EE, Lee HS, Vasudevan SG Celgosivir treatment misfolds dengue virus NS1 protein, induces cellular pro-survival genes and protects against lethal challenge mouse model. *Antiviral Res; 92(3):453-60*Rathore AP, Paradkar PN, Watanabe S, Tan KH, Sung C, Connolly JE, **Low J**, Ooi EE, Vasudevan SG.
4	Antibiotics for Klebsiella Liver Abscess Study (A-KLASS). Singapore ID Initiatives. SIDI/2013/006. $199,120.00. 30 Aug 2013- 31 Dec 2017. Co-I	On going recruitment
5	A multicenter open-label randomized controlled phase 2B trial comparing Vancomycin versus Daptomycin for the treatment of Methicillin Resistant Staphylococcus aureus bacteremia due to isolates with high vancomycin minimum inhibitory concentrations.	Vancomycin versus daptomycin for the treatment of methicillin-resistant Staphylococcus aureus bacteremia due to isolates with high vancomycin minimum inhibitory concentrations: study protocol for a phase IIB randomized controlled trial. *Trials. 2014 Jun 19;15:233.* Kalimuddin S, Phillips R, Gandhi M, de Souza NN, **Low JG**, Archuleta S, Lye D, Tan TT.

	Singapore ID Initiatives. SIDI/2013/007. $404,209.00. 01 Oct 2013- Nov 2015. Co-I	
6	A Phase 1 study to Determine the safety and efficacy of the PIKA rabies vaccine using the PIKA Adjuvant. YiSheng Biopharma sponsor, $384,000.00. Nov 2014- Aug 2015. Co-I	An accelerated Rabies Vaccine Schedule Based on Toll-like Receptor 3 (TLR3) agonist PIKA Adjuvant Augments Rabies Virus Specific Antibody and T cell response in Healthy Adult Volunteers Vaccine. 2017 Feb 22;35(8):1175-1183. Limin Wijaya, Christine Y.L. Tham, Yvonne F.Z. Chan, Abigail W.L. Wong, L.T. Li, Lin-Fa Wang, Antonio Bertoletti, **Jenny G. Low**
7	Phase 2 single-centre, open labelled, randomized study in healthy naïve adult subjects aged 21 to 65 years for inactivated purified rabies vaccine MOHIAFCat1-11005. S$722,700.00. 19 Feb 2016- 28 Feb 2018	Completed. MS preparation.
8	A Phase II, Double-Blind, Controlled Trial to Assess the Safety and Immunogenicity of Different Schedules of Takeda's Tetravalent Dengue Vaccine Candidate (TDV) in Healthy Subjects Aged Between 2 and <18 Years and Living in Dengue Endemic Countries in Asia and Latin America. $1,746,248.80, 2015-2018. Site-PI	Follow up period
9	Ketotifen as a Treatment for Vascular Leakage during Dengue Fever (Ketoden) NMRC CTGL13feb010. $1,597,942.62. July 2014- July 2016	On going recruitment

Phase III clinical efficacy vaccine trials

Over the past 15 years, PATH has conducted more than 30 Phase III and/or IV clinical trials, all in low- and middle-income countries, in support of licensure of vaccines against influenza, Japanese encephalitis, malaria, meningitis A, polio, and rotavirus. This experience has included establishing and managing clinical trial sites in Africa and Asia, including investments in clinical and laboratory facilities, as well as training in Good Clinical Practice (GCP), communications, and financial management, among other skills. **Table 5** is an illustrative list highlighting PATH's diverse late-stage clinical trial experience.

Table 5. Select PATH Phase III/IV Clinical Vaccine Trials – All Countries

Short Title	Indication	Phase	Number of Subjects	Population	Countries	Clinical Trial Registry Number
Efficacy of GSK Biologicals' Candidate Malaria Vaccine 257049	Malaria	III	15459	Infants, Toddlers	Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, Tanzania	NCT00866619
Immunogenicity and Safety of Beijing TiantanBio Liquid Bivalent Oral Poliomyelitis Vaccine in Comparison to a WHO Prequalified Comparator	Polio	III	750	Infants	Kenya	NCT02434770
Efficacy Trial to Evaluate the Protective Efficacy of Three Doses of Oral Rotavirus Vaccine (ORV) 116E (ROTAVAC)	Rotavirus	III	6800	Infants	India	NCT01305109
Efficacy and Safety of Live-Attenuated Influenza Vaccine (LAIV)	Influenza	III	1761	Children	Bangladesh	NCT01797029
Clinical Efficacy of Trivalent Live-Attenuated Influenza Vaccine (LAIV) Among Children in Senegal	Influenza	III	1761	Children	Senegal	NCT01854632
Efficacy of Serum Institute of India's Rotavirus Vaccine (ROTASIIL)	Rotavirus	III	7500	Infants	India	NCT02133690
Safety of meningococcal A conjugate vaccine	Meningitis	III	6000	Adults, Children, Toddlers	Mali	PACTR 2010030001913177
Antibody persistence after MenAfriVac®	Meningitis	III/IV	950	Children	Ghana	ISRCTN No. 10763234
Long-term follow-up of children who participated in PsA-TT-007 in Mali	Meningitis	III/IV	825	Children	Mali	ISRCTN No. 37623829

Long-term immunogenicity of JE vaccine and response to a booster	Japanese Encephalitis	III/IV	561	Children	Bangladesh	NCT02514746
Japanese Encephalitis Vaccine administered with Measles-Mumps-Rubella Vaccine	Japanese Encephalitis	III/IV	628	Infants	Philippines	NCT02880865

Technology transfer

PATH facilitates technology transfer to developing-country manufacturers and provides ongoing support to help those manufacturers develop, introduce, and scale-up vaccine products that will be accessible and affordable for use in low-income countries. **Table 6** is an illustrative list of successful technology transfer activities.

Table 6. Select PATH Vaccine Technology Transfer Experience

Product	Technology Transferred	Source	Recipient	PATH Role
MenAfriVac®	Conjugation method	US Food and Drug Administration, Center for Biologics Evaluation and Research	Serum Institute of India Ltd. (SIIL)	Identified source and recipient, provided technical support as a core partner in the Meningitis Vaccine Project.
Polyvalent bovine rotavirus vaccine	Wuhan (China), SIIL (India), Shanta (India) and Butantan (Brazil) licensed the live attenuated bovine-human reassortant rotavirus vaccine (BRV) from the US NIH, and PATH provided enabling technology for the vaccine development program	US National Institutes of Health	Wuhan, SIIL, Shanta, Butantan	Provided funding, reagents and technical assistance.
Pneumococcal whole cell vaccine	Master cell bank and associated processes, methods and assays	Children's Hospital Boston and PATH	PT Bio Farma (Indonesia)	PATH collaborated with CHB on the preclinical development of this vaccine candidate and led the conduct of early stage clinical trials to demonstrate its potential, then transferred the technology for the vaccine candidate to PT Bio Farma for manufacturing and further development.
Non-replicating rotavirus vaccine (NRRV)	Materials and know how for the purification and characterization of NRRV proteins at commercial scale	US National Institutes of Health	SK Chemicals (South Korea)	PATH developed the vaccine candidate and completed both preclinical and early stage clinical trials for this vaccine candidate, then transferred the technology for the vaccine candidate to SK Chemicals for manufacturing and further development. PATH is also providing funding to SK. (Press release attached)

Bringing vaccines through a regulatory pathway over the past 10 years

Profectus. Since the Profectus Vaccines Group was spun out of Wyeth Vaccines Research in 2008, the regulatory activities have been restricted to those required to initiate and conduct the phase 1 clinical trials outlined in **Table 2**. However, as described above, the senior leadership of Profectus has extensive major pharma experience and contributed to the approval of several vaccines, including a series of paediatric combination vaccines, Prevnar®, Prevnar 13®, FluMist®, and Rotashield®.

PATH has partners closely with manufacturers, including both multinational pharmaceutical companies and emerging-market manufacturers, to achieve licensure of 6 products in Africa and Asia. PATH also supports manufacturers in achieving WHO prequalification for their vaccines, including supporting the first vaccines from China and India to be WHO prequalified. In the field of malaria, our partnership with GSK has resulted in the first malaria vaccine candidate to be assessed by a stringent regulatory agency, which received a positive scientific opinion from the European Medicines Agency (EMA) under the agency's Article 58 procedure. **Table 7** is a list of products on which PATH has partnered closely with manufacturers to achieve licensure over the past 10 years:

Table 7. PATH Vaccine Regulatory Approval Experience

Product	Indication	Manufacturer	Primary regulatory authority	Alternative pathway (if applicable)	WHO PQ	Additional regulatory authorities
MenAfriVac®	Meningitis A	Serum Institute of India, Ltd.	Drugs Controller General of India (DCGI)/ Central Drugs Standard Control Organization (CDSCO)	N/A	June 2010	Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Côte d'Ivoire, Ethiopia, Ghana, Mali, Niger, Nigeria, Togo
MenAfriVac® 5 micrograms	Meningitis A (pediatric)	Serum Institute of India, Ltd.	DCGI/CDSCO	N/A	Dec. 2014	Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Côte d'Ivoire, Ethiopia, Ghana, Mali, Niger, Nigeria, Togo
RTS,S (Mosquirix™)	Malaria (*P. falciparum*)	GSK	European Medicines Agency	Article 58	Pending	
Rotavac®	Rotavirus	Bharat Biotech	DCGI/CDSCO	N/A	PQ submission pending review	
SA 14-14-2	Japanese encephalitis	Chengdu Institute of Biological Products	China Food and Drug Administration	N/A	August 2013	Cambodia, the Democratic People's Republic of Korea (North Korea), India, Laos, Nepal, Sri Lanka
Rotasiil®	Rotavirus	Serum Institute of India, Ltd.	DCGI/CDSCO	N/A	PQ submission pending review	

Infrastructures and facilities in-house,

Profectus Research and Development Laboratories: Profectus Biosciences currently leases over 40,000 square feet of laboratory and office space in Building 205 on the IRG campus in Pearl River, NY. The facility is state-of-the-art, newly renovated with secure key-card access, central air, a newly installed purified deionized (DI) water system, centrally distributed CO_2, and uninterrupted power provided by a 600-gallon diesel generator. Air within the laboratories is single pass and all laboratories, with one exception, are under negative pressure with respect to common and office areas. The Compliant Laboratory, used to rescue viral vectors under conditions suitable for initiating cGMP manufacture, is under positive pressure relative to the hallway. Laboratories include a Bioreactor suite; a Common Equipment area; a laboratory dedicated to Molecular Biology/Virology; a Flow Cytometry/ Sequencing Room; a laboratory dedicated to Immunology; a laboratory dedicated to clinical assays; a self-contained keypad restricted access Compliant Rescue Laboratory; a walk-in 4°C cold room; a glass wash, autoclave, and drying oven area, and; other support areas such as Millipore Elix 20 deionized water room, storage, utility, and IT rooms.

UTMB GNL Facilities: Dr. Thomas Geisbert has dedicated BSL-2 laboratory space in the GNL totaling over 1,500 square feet. He also has access to the BSL-4 facilities in the GNL and the adjacent Robert E. Shope, MD Laboratory that together offer 14,330 square feet of BSL-4 space. The facility is unique in that it is the only currently operational BSL-4 facility in the United States that is located on an academic campus. This unique location benefits the proposed studies in several ways. Regarding physical resources, the GNL has many specialized assets that are essential for the proposed work. In addition, being located on an academic campus, Dr. Geisbert is a faculty member in the Department of Microbiology and Immunology and currently supports four graduate students in his laboratory. Faculty from several academic Departments at UTMB are located within the GNL. This academic environment fosters creativity and collaboration. In particular, having many investigators with diverse areas of expertise housed within the GNL is particularly advantageous in promoting collaborative efforts that are invaluable to the success of many research projects. It is anticipated that this environment with a cadre of faculty with areas of expertise including molecular biology, immunology, pathology and pathogenesis, structural biology, genomics, and many other disciplines will be highly beneficial to this proposal.

Dr. Geisbert's BSL-2 laboratory and the BSL-4 facilities at UTMB contain sufficient cell culture incubators, laminar flow hoods, chemical hoods, centrifuges, and other equipment required to conduct the proposed research. Clinical pathology instruments including hematology, clinical chemistry, and blood coagulation analyzers are available in both the BSL-2 laboratories and in the BSL-4 facilities at UTMB. In addition, multiplex machines, microscopes, and an array of other equipment for monitoring and processing samples are available. Dedicated BSL-2 and BSL-4 animal rooms contain appropriate rodent and/or NHP cages necessary for the proposed work. Dedicated BSL-4 necropsy rooms contain downdraft tables and specialized equipment for performing necropsies. In addition to standard light and fluorescence microscopy, the GNL offers a range of specialized imaging equipment, including combined confocal and multiphoton microscopy imaging systems at the BSL-2 level for molecular imaging of thick specimens and intravital microscopy. An IVIS 200 system for *in vivo* small animal molecular optical imaging, a microPET/CT whole animal imaging system, digital X-ray, and ultrasound equipment are available in the GNL BSL-4 laboratory. The GNL also has a full complement of gel imaging capabilities as well as a traditional x-ray film processor. In addition, the campus has *in situ* confocal microscopy and endoscopic optical coherence tomography equipment.

The **UTMB Animal Resource Center** (ARC) is responsible for housing and care to all animals on campus. The ARC encompasses 89,694 square feet in nine facilities across the UTMB campus. UTMB is a registered Research Facility under the Animal Welfare Act. It has a current Letter of Assurance on file with the Office for Protection from Research Risks. The Geisbert laboratory and the ARC have a documented ability to conduct work in accordance with guidelines for *Biosafety in Microbiological and Biomedical Laboratories* (Fifth Edition, December 2009) and the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (Revised 2015). The Department of Defense, CDC, and USDA have approved our laboratories for work with select agents and AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care International) has certified our animal facilities (Accreditation #000870). UTMB follows the:

- *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (2015 reprint)
- *Guide for the Care and Use of Laboratory Animals*, Eighth Edition (2011)
- US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- USDA-administered Animal Welfare Act (Public Law [PL] 89-544 as amended by PL 91-579 [1970], PL 94-279 [1976], and PL 99-198 [1985]) and published in 9 CFR Parts 1-3 on Final Rules regarding Animal Welfare
- Health Research Extension Act of 1985 (PL 99-158) regarding "Animals in Research"; and
- PL 103-43 regarding the "Plan for Use of Animals in Research" (1993).

UTMB's ARC follows all standards for AAALAC and Institutional Animal Care and Use Committee compliance. Depending on space availability, animals may be housed in ARC facilities with animal care provided by the ARC staff including two full-time veterinarians. These facilities are capable of housing NHPs, rats, mice, hamsters and guinea pigs under BSL2/3 biohazard containment, and encompass 72,847 square feet. All facilities feature full-service animal care procedural and maintenance facilities, including laminar flow isolator caging systems, sterilization equipment, procedure rooms, cage-washing, and autoclaving.

USU-HMJF Facilities. Uniformed Services University (USU) is the medical school at which approximately half of the physicians in the Armed Services receive their graduate training. Research at USU is supported primarily by extramural grants, as in other medical schools. The PI is a tenured Professor in the Department of Microbiology and Immunology, which includes 12 full-time Faculty members. The overall focus of the Department is mechanisms of infectious diseases and the host response/immunology. A major focus of the PI's Department is virology and viral Immunology, and the PI has many talented and productive colleagues with whom to interact, in addition to his multiple international collaborative research activities. Faculty interests and active research programs at USU are diverse, with many nationally- and internationally-known investigators, including the PI's chair, Dr. Alison O'Brien, who was the 2008-9 President of the American Society for Microbiology (ASM). The PI has had and is currently involved in active collaborations within the University, in areas of viral immunology and vaccine and antiviral therapeutics and animal model development (with Dr. Joseph Mattapallil and Dr. Brian Schaefer). USU is also physically located directly across from the main NIH campus in Bethesda, and within a short distance from Frederick, MD, and the PI maintains a very active collaboration with NCI, NIH, investigators (Dr. Dimiter Dimitrov and his group) which has been in place for over 27 years since the PIs postdoctoral work began at NIH in 1989. The overall broad scientific environment at both USU and the NIH is highly conducive to productive collaborations. The PI has often uses these resources to his advantage, both for his research objectives and interests, but also in his role as the Director of the Emerging Infectious Diseases Graduate Program (for the Ph.D.) because he has activity recruited adjunct faculty appointments within the EID graduate program for both on and off-campus scientists interested in participating in graduate education and graduate student training. Dr. Broder submits such appointments requests through the Office of Dean of the Medical School (USU). The USU/EID program can accept 5 fully supported student positions per academic year, for 3 years, at which time the student's mentor begins support, and there is no tuition or fees associated with the EID program. The PI has been well-supported by both the Department of Microbiology and Immunology and the USU Office of Research. Overall, the available technical resources (and University support for continually improving technical resources) is exceptional.

Laboratory: The PI's laboratories are divided into 3 rooms totaling 1,440 sf, and are equipped with eight CO2 incubators for tissue culture, 4 inverted and 1 bright field microscopes, high speed and ultracentrifuges, four biological safety cabinets, 2 -20°C, 3 -80°C freezers, 4 liquid nitrogen freezers 6 refrigerator/freezers, 4 PCR machines, 2 ELISA plate reader, and various small equipment items (gel electrophoresis, circulating adjustable water baths, heat blocks). 2 complete GE-ATKA low pressure chromatography systems, with integrated UV detectors, fraction collectors, and pump systems, and gradient fractionator apparatus.

Animal: Animals if applicable are maintained in the University's laboratory animal facilities under the supervision of a full-time veterinarian. These facilities are a modern AAALC accredited, central animal tract of about 50,000 sq. ft. The animal care and use program is managed by the Department of Laboratory Animal Medicine which is directed by a veterinarian who is an ACLAM Diplomat and staffed with one other veterinarian, a graduate animal husbandryman, and about 30 technicians.

Computers: A pentium computer, scanners and two laser jet printers in the PI's office and 9 pentium computers in the laboratories/offices are connected to a departmental server and hardwired to the central VAXC computer.

Office: The PI has an office separate from, but across from the laboratory. Three full-time secretaries are available to provide support within the department.

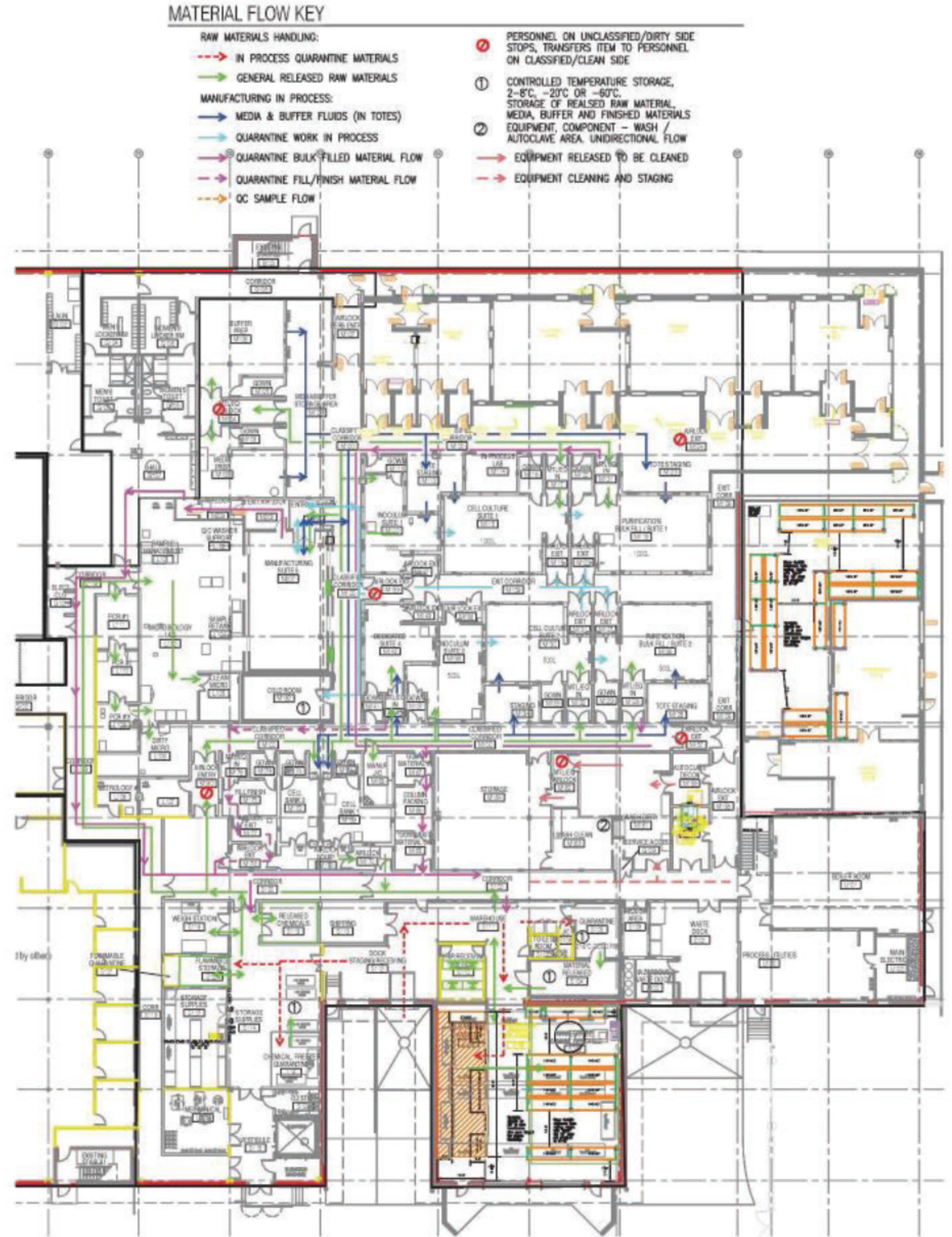
Catalent Facilities. Catalent is a contract manufacturer of Master Cell Banks and Bulk Drug Substance (BDS) which as monoclonal, fusion and recombinant mammalian derived proteins for clinical Phase I and II use. Pharmaceutical manufacturing activities of the site. GMP compliance begins with the Master Cell Bank production. BDS is manufactured following ICH Q7, Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients and EU GMP Annex 2, Manufacture of Biological Active Substances and Medicinal Products for Human Use Suitable for Phase I/II Clinical Studies. Catalent is committed to the manufacturing, processing, packaging and holding of Bulk Drug Substances in full compliance with cGMPs to assure that they consistently meet the safety, identity, strength, quality, and purity characteristics they are purported to meet. All procedures are clearly defined and are checked systematically. Protocols are created for equipment qualifications, assay qualifications, stability, and experimental design, where appropriate. Specifications are maintained for all raw materials and GMP product. The measures of Catalent's systems are implemented by a corporate Quality Manual, a GMP Site Master File (SMF), a validation Master Plan (VMP), Standard Operating Procedures (SOPs), Batch Production Records (BPRs), Job Aids (JA), protocols, specifications, and forms.

Floor Diagram: The GMP Manufacturing layout is shown in below.

cGMP Manufacturing System, process flow and description: Suites for two (2) separate manufacturing trains are provide; one for 500L and the other for 1000L of cell culture volume. Each train is comprised of three (3) separate rooms with separate air handling; an inoculation suite, a cell culture suite, and a purification suite. An additional suite will be fitted to accommodate a future line to manufacture smaller volumes of cell culture broth up to 50 L of volume. Manufacturing operations utilize single use technology where possible. All primary doors separating the labs and manufacturing from the remainder of the facility and the office areas utilize card readers for access control as defined by Catalent.

Inoculation Suites: Each inoculation suite is configured to allow for scale-up of cell mass from a frozen vial to approximately 20% of the production scale bioreactor volume (100 L or 250L) for a 500 L or 1000 L Single Use Bioreactors (SUB), respectively. Each outfitted inoculation suite is setup with a biosafety cabinet, incubator, wave bioreactor(s), appropriately sized SUB, and all supporting equipment to allow scale-up from amp thaw to 100 L or 250 L (as applicable). These vessels may also be used as production vessels as business requires. Drains from the inoculation suites are tied to a process drain system. Any biocontaminated waste is collected and inactivated within the suite prior to release.

Cell Culture Suites: The 500 L and 1000 L cell culture suites are provided with a SUB capable of operating at the respective volume. These production bioreactors are inoculated by the final seed bioreactor in the respective inoculation suite by passing tubing through a wall pass-through and aseptically fusing the tubing at the other end. The production bioreactors are fed with initial media, feed media, and bicarbonate solution (for pH control) from corridor alcoves next to the suites. The bioreactor skids are equipped with peristaltic pumps to provide controlled feed of the aforementioned solutions. Load cells measure the weight of the contents of the bioreactors. Near each of the production single-use bioreactors, there are utility panels with process compressed air, instrument air, carbon dioxide, and oxygen. Two (2) additional utility panels in each suite are positioned to support product recovery and viral inactivation unit operations. Process compressed air, instrument air, chilled water (supply and return) and temperature controlled water (supply and return) are provided at these panels. At the end of a production run, samples are drawn from the bioreactor for quality assays and manufacture of End of Production Cells (EPC), as required. Processing takes place through the use of closed bag/tubing systems and transferred from room to room through tube pass-throughs. All pre-viral purification steps are also conducted in these suite with a complete segregation to minimize contamination.



Purification Suites: The purification processes are unit operation driven. Depending upon the process, a sequence of operational steps consisting of column chromatography and tangential flow filtration (TFF) is normally required. The purification suites are therefore flexibly configured. The cell culture and purification suites are physically isolated to ensure separation of equipment and materials pre and post-viral inactivation. Procedural controls are utilized to ensure personnel fully re-gown before entering the purification suites after working in the inoculation or cell culture suites. Utility panels are strategically positioned within the purification suites and are adjacent to the corridor alcove for buffer delivery. There are three (3) utility panels in each of the purification suites to support the various unit operations. Process compressed air, instrument air, and chilled water are provided at each panel. One of the panels also contains temperature controlled water (supply and return) for re-warming of materials.

Cold Storage Room: A 2-8°C cold storage room is located within the manufacturing space for storage of in-process raw materials, buffers, and drug substances waiting for final bulk filtration/sampling.

Bulk Filtration: Concentrated active drug in appropriate buffer system in bag(s) is brought to the bulk filtration suite. Under a HEPA-filtered laminar air flow hood, the concentrate is filtered with 0.2 µm filters into either a bulk container or smaller containers. The product is appropriately sampled and remaining filled container(s) are taken to the product warehouse for quarantine storage at the appropriate temperature. Process compressed air is provided in this processing area.

Cell Banking/EPC: The cell banking/EPC laboratories are provided with carbon dioxide. Two (2) airlocks at the exit of the labs are provided with liquid nitrogen service to fill liquid nitrogen Dewars and a controlled rate freezer. Proper ventilation and oxygen monitoring is provided in these rooms. Drains from the lab are tied to the process drain system. Any biocontaminated waste is collected and inactivated within the suite or transferred to the waste autoclave area for treatment prior to release.

Media Preparation Suite: A weighing and dispensing booth, located in the warehouse, is utilized to create batch quantities of components for addition to the mixing bag systems prior to entry into the manufacturing area. Raw materials are brought into the suite through a material air lock after wipe down. There are four (4) media preparation bag systems: one (1) at 650 L and three (3) mixtainers, one (1) at 200 L, one (1) at 100 L, and one (1) at 50 L. Only one (1) Lev mixer is provided, so one media preparation can be done at a time using the disposable bag mixing systems. Smaller scale mixing equipment is shared with the buffer preparation suite. A utility panel with instrument air and process air is installed between the two preparation units. A point of use heat exchanger is provided to cool USP Water-for-Injection (WFI) for use in the preparation of media. The heat exchanger is a U-tube shell and tube heat exchanger with double tube sheet construction. Tubes and bonnet is fully drainable and constructed of polished, 316L stainless steel. Flow through the sub-loop and the heat exchanger is hot, except when cold WFI is requested. The heat exchanger is cooled with the HVAC chilled water system. The heat exchanger is insulated and has white PVC jacketing. The heat exchanger is located in the interstitial space above the room. A single user drop point is provided near the utility panel. Media prepared in the media preparation suite is transferred and filtered into bag systems in mobile plastic containers in the corridor alcove adjacent to the suite.

Buffer Preparation Suite: The buffer preparation suite operates similarly to media preparation suite described in the previous section. A weighing and dispensing booth, located in the warehouse, is utilized to control these operations. Two (2) buffer preparation disposable bag mixing tanks systems are provided: one (1) at 1000 L and one (1) at 500 L capacity. Two (2) Lev mixers are provided. Therefore, two buffer preparations can take place at a given time. Smaller scale mixing equipment is shared with the media preparation suite. Two (2) utility panels with instrument air, and process compressed air, are installed. As with media preparation, prepared buffers are transferred via peristaltic pump through inline filters to bag systems in mobile plastic containers located in the corridor alcove adjacent to the suite. The same heat exchanger that supports media prep is used for the buffer prep area. Two (2) user drop points off of the heat exchanger sub-loop are provided near the utility panels. These user points can also be used by manufacturing personnel to fill bags with WFI.

Wash Area: The wash area is divided into two (2) distinct functions: rinsing/wash area and the cleaning and sterilization of small components (e.g. stir rods, flasks). Soiled mixing systems and smaller components enter the area at a common point and are staged in different areas. When needed, mixing systems rinsing is done with USP purified water through a hose or a spray nozzle. Compressed air is used to blow excess liquid from the unit. Cleaned mixing systems are staged on the opposite side of the cleaning station and conveyed to the media or buffer preparation alcove for filling, or to a suite for use. Bags are placed into mixing systems within the Media Prep (MP) or Buffer Prep (BP) areas. Small components are staged on a rack for cleaning. A pass-through parts washer is provided. It is supplied with USP purified water for initial rinse and cleaning, and USP Water-for-Injection for final rinse. Parts are removed from the washer on the clean side and staged. Those parts that require autoclaving are loaded into a pass-through autoclave. The autoclave has an integral pure steam generator. The generator is fed with purified water. A steam quality sampling point is included in the design.

Standby Power Supply: The purpose of the Standby Diesel Generator system is to provide partial facility AC power to mitigate short outages, and provide power to bring systems and processes to a safe shutdown condition. The standby diesel generator output is connected to an automatic transfer switch that signals the generator to start, and transfers the standby loads to the generator after the generator has started.

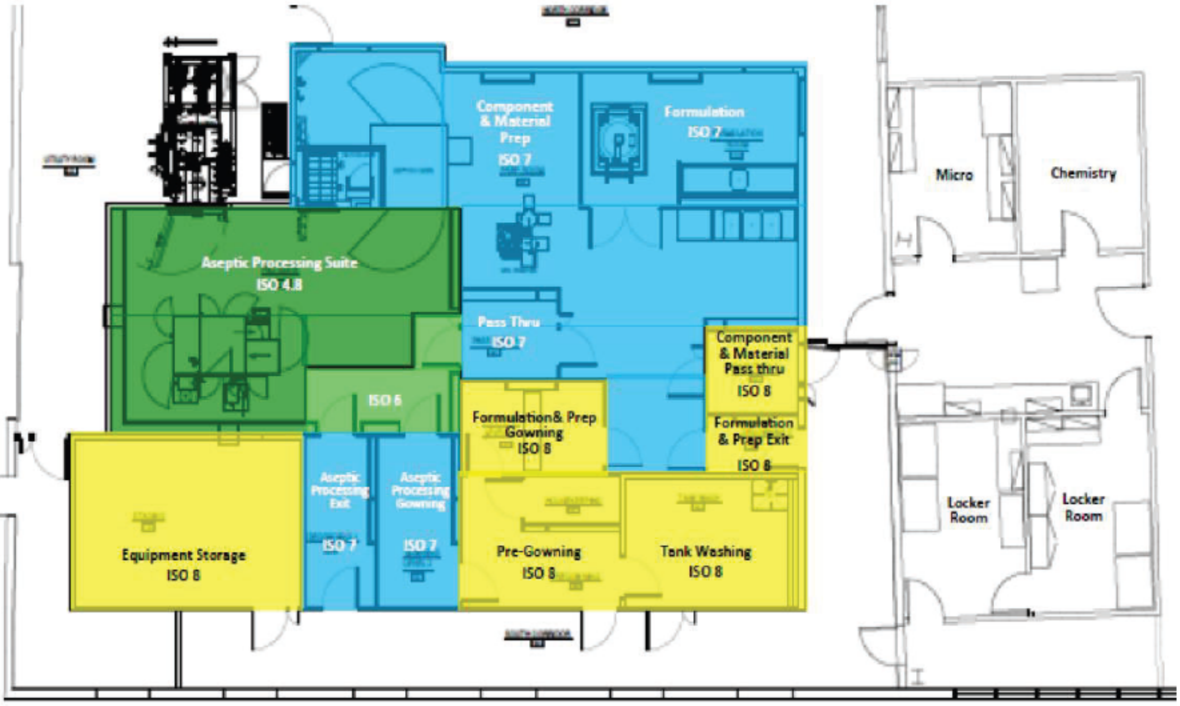
UPS Power Supply: The UPS system provides immediate / no break AC power for short periods of time. It is used to supply power to critical control systems. The UPS is used for equipment inside the data center, Catalent process control equipment, and existing building services equipment. In addition, stand- alone UPS's are installed on critical production and analytical equipment as appropriate

Heating, Ventilation, and Air Conditioning Systems: The HVAC systems associated with the facility is designed in accordance with the International Building Code, NFPA requirements, and ASHRAE standards. Room design conditions i.e. air change rates, temperatures, etc. are those typically found within cGMP facilities with similar operations and in accordance with ISO guidelines where specified in the Design Criteria for the Catalent Madison Project. Air Handlers and HVAC equipment is monitored and controlled via the Building Automation System (BAS) system. Each air handler is fitted with a dedicated controller for its respective unit. The AHU controller is networked on a BAS control area LAN and accessed via the BAS web portal. Field instruments and control devices are hardwired utilizing discrete signals directly to the HVAC controllers. Environmental monitoring of cGMP areas is accomplished through the Process Control System and Environmental Monitoring System (PCS) specifically designed to monitor temperature, humidity and differential pressure (DP) parameters within the production areas. Differential Pressure sensors have their impulse tubing routed to static pressure probes located in the ceilings of the rooms that are monitored for DP. A common area is used as the reference for each static measurement. The HVAC system also includes chilled water plant steam, hot water for reheat and heat reclaim system.

Building Automation System (BAS): Building Automation System (BAS) services the cGMP and R&D areas. This system monitors and controls utility, mechanical, electrical, HVAC and facility-related support systems not directly involved with production systems. The primary function of the BAS is to control cGMP & R&D noncritical equipment and systems. In addition, monitoring of R&D equipment and systems is performed by the BAS.

GRAM Facilities. GRAM is a new CMO for Profectus since the previous facility at Catalent to manufacture PBS's protein vaccine with Aluminium Phosphate has been shut down. GRAM has qualified the unique aseptic compounding which is a critical process in making protein vaccine with aluminium. Profectus QA and Outsourcing has reviewed the Media Fill Qualification Summary Report and verified that the established processes at GRAM will be applicable in manufacturing HeV-sG vaccines. GRAM is qualified to fill up to 144,000 vials at 3,000 vial/hour for 48 hours.

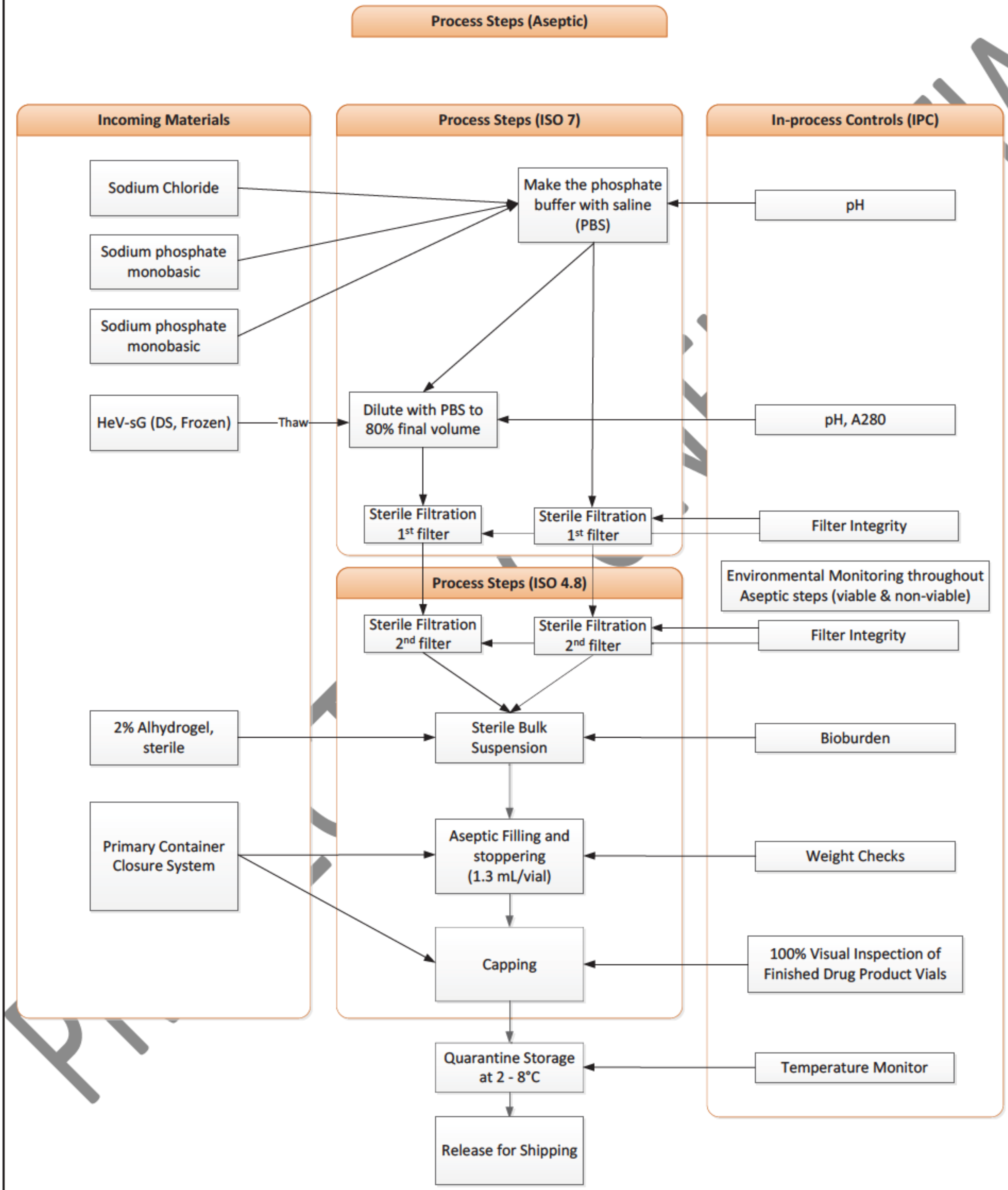
Grand River Aseptic Manufacturing (GRAM) is a full-service parenteral contract manufacturing organization supporting new product development and cGMP manufacturing. GRAM is approved by FDA to manufacture 4 commercial drug products since 2013. Current portfolio is 57% biologics and 43% small molecules with 80% being sterilized by aseptic filtration and 20% by terminal sterilization. GRAM is approved by the FDA for aseptic manufacturing with and without terminal sterilization. GRAM manufactures drug products in vials and syringes to support clinical trial from Phase 1 to Phase 3.



Manufacturing areas

GRAM is located in downtown Grand Rapids, Michigan. The manufacturing space is 11,400 ft^2 within a facility of 28,000 ft^2. In addition to the manufacturing area, other areas provides supporting functions such as analytical and microbiological testing, formulation development, process development/validation, labelling/packaging, warehouse, and storage

Operation of the manufacture Process: GRAM is equipped with a USP compliant Water for Injection system, a fully automatic filling machine inside an isolator, a vial washer, and a de-pyrogenation oven. The manufacturing areas have designated areas that are compliant to ISO standards; ISO 4.8 (Grade A under EU GMP Annex 1) for the aseptic filling suite, ISO 7 for the preparation areas, and ISO 8 for the general areas as schematic presented in various colors above. Drug products are manufactured on a campaign basis according to applicable cGMP guidelines. The manufacturing area is dedicated to production of only one product at any one time, with complete cleaning and changeover between products. Warehouse personnel identify and label all incoming raw materials and packaging materials according to established standard procedures. Samples for QC testing are selected by the QC Department. Materials are stored in a logical quarantine area until all required acceptance activities have been completed. Data related to the status, lot numbers, material description, and location and the lot of the materials to be used are handled according to established standard procedures. The concept of the facility includes high level containment control and aseptic manufacturing by the use of isolation systems and automated manufacturing equipment. Flows for materials and personnel are restrictedly designed to prevent cross-contamination between suites and to prevent the introduction of any adventitious agents into the processes. The HVAC (Heating, Ventilation and Air Conditioning) system servicing the facility are designed to provide proper control and segregation of the environments. All the environmental classified rooms related to the sterile manufacturing areas are at a decreasing nominal pressure differential starting from the highest classified rooms down to the lower grades. In sterile area parameters such as overpressure, temperature, humidity, non-viable particulate levels are continuously monitored and recorded. Alert and action limits are described in a master program that ensures maintenance of proper temperature, humidity and ventilation. All process equipment is qualified and validated accordingly and routine maintenance is scheduled according to established standard procedures. Written procedures are designed to prevent microbiological and particle contamination of products. A routine environmental control of viable and not viable particles is performed in the sterile area and the relevant trend is periodically examined. The sterilization processes are validated and documented via media fill every 6 months in a rotational basis. All manufacturing operations are performed in accordance with Standard Operating Procedures and process or solution batch records. The manufacture process of HeV-sG-V Drug product with in-process control is presented below.



<u>Duke-NUS Facilities.</u> SingHealth Investigational Medicine Unit, Singapore General Hospital, Block 7 Level 7, Outram Road, Singapore 169608. & Duke-NUS Medical School, 8 College Road, Singapore 169857.

Manufacturing and scale-up for industrial suppl

Profectus Manufacturing and Scale-Up Experience: Profectus performs GMP, GLP, and GCP activities through outsourcing, and has developed and implemented the key quality system policies and procedures needed to support the manufacture, testing, and conduct of human clinical trials utilizing GMP, GLP and GCP contract service providers. These standard operating procedures (SOPs) include systems for quality policy and quality roles and responsibilities, document control, quality agreements, batch record review and product disposition, deviations/investigations, employee GMP, GLP and GCP training, stability programs, contractor audits and qualification programs, outsourcing, and managing/investigating clinical trial product complaints.

Prospective contract service providers are evaluated using established Standard Operating Procedures (SOPs) for qualifying vendors. Profectus utilizes qualifying audits as a mechanism for initially qualifying cGMP, GLP, and clinical (GCP) contract service providers for use in drug and vaccine development programs, and performs routine, directed or other audits to measure and monitor on-going GMP, GLP, and GCP compliance. In addition, it is the policy of Profectus to execute Quality Agreements with all GMP, GLP, and GCP contract service providers prior to the release of clinical trial materials (CTMs), the performance of non-clinical GLP studies at contractor sites, or the conduct of clinical trials by Clinical Research Organizations (CROs). Profectus reviews and approves all master and executed batch records, including quality control testing and stability documentation in support of the manufacture of CTMs. All investigations are thoroughly reviewed and approved by Profectus QA. Service contracts are managed through an interdisciplinary Contract Management Team led by the Executive Director of Outsourcing, or designee. The Contract Management Team is comprised of the appropriate individuals from both Profectus and the contract service provider. The Contract Management Team holds regularly scheduled meetings with agendas, minutes and time constrained action items. This approach has supported the successful GMP manufacture of 5 pDNA vaccines (pDNA-HIVgag/pol, pDNA-HIVenv/nef-tat-vif, 2 Lots of pDNA-huIL-12, and pDNA-huIL-15), and 4 rVSV vaccines [rVSV-HIVgag, rVSV-HIVenv, rVSV-EBOV(Zaire), and rVSV-MARV(Angola)].

Profectus has audited, qualified and contracted with Fisher BioServices (FBS) for GMP storage and cold chain management for distribution of clinical supplies. A Quality Agreement is also in place. FBS has more than 22 years providing worldwide collection, storage and distribution of biological materials with 26 facilities in the US, 2 in Europe, 1 in Asia, and over 240 employees. Profectus currently has contracts with FBS for GMP storage at +4°C, -20°C, and -80°C. E. coli cell banks and various CTM produced under multiple US government contracts are presently in storage at FBS, and CDC and USDA permits are in place to allow storage of VSV vectors at the appropriate FBS facilities.

Profectus has audited and contracted with three Charles River Laboratory (CRL) facilities for GMP and GLP testing. The CRL group is a widely recognized and respected GLP and GMP contract testing organization with facilities worldwide. Profectus has audited and qualified the facilities we propose to use for this contract effort and have a Quality Agreement in place to cover all CRL facilities.

- CRL-Montreal, Canada provides IND enabling GLP toxicology testing. This group has already performed two successful toxicology studies for Profectus using pDNA and VSV vectors. Rabbits, which is the animal species proposed for toxicology testing under this contract, were used for both previous IND-enabling toxicology studies. CRL also has the capability to perform GLP toxicology studies in other species, including mice, rats, and various monkeys, should it be required. CRL-Montreal has been granted the Health Canada and Canada Food Inspection Agency permits that are required to conduct toxicology studies in their facility with VSV, a BL-2 class organism.
- CRL-Wilmington, MA provides GLP safety testing for both research and product release. This site provides murine neuro-virulence testing by intra-thalamic inoculation of VSV vectors; in vivo adventitious agent testing in embryonated eggs, suckling mice and guinea pigs for MVS and CTM drug substance (DS); and General Safety Testing in guinea pigs which is required for release of each lot of CTM. Protocols and final reports for each of these tests are reviewed and approved by Profectus QA. CDC and USDA permits are in place to allow CRL-Wilmington to handle VSV vectors.
- CRL-Malvern, PA performs in vitro adventitious agent testing (direct and indirect Mycoplasma and Tissue Culture Safety Test) for MVS and CTM DS as well as nucleic acid sequencing of MVS and CTM DS. These tests are required for release of MVS and CTM. Protocols and final reports for each of these tests are reviewed and approved by Profectus QA. CDC and USDA permits are in place to allow CRL-Malvern to handle VSV vectors.

The two individuals within Profectus BioSciences with primary responsibility for outsourcing are Tracy Chen, Ph.D., Senior Director of Operations and Outsourcing, and Marc Tremblay, Director, Quality Assurance. Each has over two decades of experience in their respective roles and expertise in their areas covering all phases of pharmaceutical and vaccine Development.

PATH partners with emerging-market manufacturers around the world to develop, introduce, and scale-up vaccine products that will be accessible and affordable for use in low-income countries. Our initiatives currently support producers in India, Brazil, China, Indonesia, Serbia, Brazil and Vietnam, on vaccines targeting a range of infections, including influenza, Japanese encephalitis, rotavirus, pneumococcus, yellow fever, measles, rubella, human papillomavirus, polio, and Group B Streptococcus (GBS).

We provide technical expertise, project management, financial support, and assistance in complying with national and global regulatory bodies for vaccine development and introduction. This includes assisting manufacturers in achieving WHO prequalification, a critical step in expanding vaccine access. Our support helps manufacturers improve safety and quality system compliance, conduct clinical trials according to international standards, and achieve efficiencies that lower costs, improve yields, and enable scale-up to meet national, regional, and global needs.

Examples of PATH's work to strengthen manufacturing capacity and scale up the supply of life-saving vaccines include:

- Helping a manufacturer in Vietnam (Institute of Vaccines and Medical Biologicals; IVAC) ready its influenza vaccine production facility and advance pandemic and seasonal vaccine candidates in clinical studies.
- Supporting the production of cell-culture-based influenza vaccines at a state-owned vaccine and biological production company in Vietnam (VABIOTECH) by helping to obtain licenses, conducting technical training, and purchasing equipment and supplies.
- Providing technical and financial assistance to help a Chinese manufacturer (Chengdu Institute of Biological Products Co., Ltd.) build and validate a new facility to meet the international manufacturing standards required for WHO prequalification of its Japanese encephalitis vaccine, which was the first Chinese vaccine ever to achieve WHO prequalification.
- Supporting technology transfer, process development and validation, and manufacturing scale-up of a meningitis A vaccine developed by an Indian manufacturer (Serum Institute of India, Ltd.) specifically for use in sub-Saharan Africa.
- Partnering with the only vaccine manufacturer in South Africa (The Biovac Institute) to develop a low-cost polyvalent GBS conjugate vaccine that will significantly reduce neonatal mortality caused by GBS in Africa and other low-income regions of the world.

The training and technical support that we provide to emerging-market vaccine manufacturers is helping to speed and strengthen their ability to sustainably supply critical vaccines for the future.

Vaccine trials and associated results published by the applicants' PI in the last 5 years
Please list clinical trials, with trial ID hyperlink and associated results published by PI in the last 5 years
PBS list of relevant publications

1. Xu R., F. Nasar, S. Megati, A. Luckay, M. Lee, S.A. Udem, **J.H. Eldridge**, M.A. Egan, E. Emini, E. and D. Clarke. 2009. Prime-Boost Vaccination with Recombinant Mumps Virus and Recombinant Vesicular Stomatitis Virus Vectors Elicits an Enhanced Human Immunodeficiency Virus Type 1 Gag-Specific Cellular Immune Response in Rhesus Macaques. J. Virol., 83:9813-9823.
2. Mire, C.E., D. Matassov, J.B. Geisbert, T.E. Latham, K.N. Agans, R. Xu, A. Ota-Setlik, M.A. Egan, K.A. Fenton, D.K. Clarke, **J.H. Eldridge**, and T.W. Geisbert 2015. Single-dose Attenuated VesiculoVax Vaccines Protect Primates against Ebola Makona Virus. Nature 520:688-691.
3. Matassov, D., C. Mire, T. Latham, J.B. Geisbert, R. Xu, A. Ota-Seklik, K.N. Agans, D.J. Kobs, M.Q.S. Weding, A. Burnaugh, T.L. Rudge, C.L. Sabourin, M.A. Egan, D.K. Clarke, T.W. Geisbert, and **J.H. Eldridge**. 2016. Single Dose Tri-Valent VessiculoVax Vaccine Protects Macaques from Lethal Ebolavirus and Marburgvirus Challenge. Sci. Transl. Med. (submitted).
4. Hay, C.M., S.S. Li, M. Elizaga, N. Kochar, G.J. Wilson, K. Cohen, B. Sanchez, S.C. De Rosa, R. Xu, A. Ota-Setlik, D. Morris, G. Finak, M. Allen, H.V. Tieu, I. Frank, M.E. Sobieszczyk, D. Hannaman[1], R. Gottardo, P.B. Gilbert, G.D. Tomaras[1], M.A. Egan, **J.H. Eldridge**, M.J. McElrath, N. Frahm, and the NIAID HIV Vaccine Trials Network. 2016. An IL-12 pDNA Enhanced HIV-1 Multiantigen pDNA Vaccine Delivered with Electroporation and with an HIV-1 rVSV Boost is Safe and Immunogenic. (in preparation).
5. Vernacchio, L., H. Bernstein, S. Pelton, C. Allen, K. MacDonald, J. Dunn, D.D. Duncan, G. Tsao, V. Laposta, **J.H. Eldridge**, S. Laussucq, D. Ambrosino, and D.C. Molrine. 2002. Effect of monophosphoryl lipid A (MPL) on T-helper cells when administered as an adjuvant pneumococcal-CRM197 conjugate vaccine in healthy toddlers. Vaccine 20:3558-3667.
6. Pride, M., P. Seubert, M. Grundman, M. Hagen, **J.H. Eldridge**, and R.S. Black. 2008. Progress in the active immunotherapeutic approach to Alzheimer's disease: Clinical investigations into AN1792-associated meningoencephalitis. Neurodegenerative Dis. 5:194-196.
7. Spearman, P., S. Kalams, M. Elizaga, B. Metch, Y-L. Chiu, M. Allan, K.J. Weinhold, G. Ferrari, S.D. Parker, M.J. McElrath, S.E. Frey, J.D. Fuchs, M.C. Keefer, M.D. Lubeck, M. Egan, R. Braun, **J.H. Eldridge**, L. Corey, and the NIAID HIV Vaccine Trials Network. 2008. Safety and immunogenicity of a CTL multi-epitope peptide vaccine for HIV with or without GM-CSF in a phase I trial. Vaccine 27: 243-249.
8. Forrest, B.D., M.W. Pride, A.J. Dunning, M.R.Z. Capeding, T. Chotpitayasunondh, J.S. Tam, R. Rappaport, **J.H. Eldridge**, and W.C. Gruber. 2008. Correlation of cellular immune responses with protection against culture-confirmed influenza in young children. Clin. Vaccine Immunol. 15: 1042-1053.

UTMB list of relevant publications

1. Mire, CE, Geisbert, TW. Evaluation of medical countermeasures against ebolaviruses in nonhuman primate models. Methods Mol Biol. 2017;1628:293-307. doi: 10.1007/978-1-4939-7116-9_24.
2. Geisbert TW. First Ebola virus vaccine to protect human beings? Lancet. 2017 Feb 4;389(10068):479-480. doi: 10.1016/S0140-6736(16)32618-6. Epub 2016 Dec 23.

3. Mire CE, Geisbert TW, Feldmann H, Marzi A. Ebola virus vaccines - reality or fiction? Expert Review of Vaccines. 2016; 15(11):1421-1430. NIHMSID: NIHMS832351, PMID: 27078187, PMCID: PMC5145306
4. Whitt MA, Geisbert TW, Mire CE. Single-vector, single-injection recombinant vesicular stomatitis virus vaccines against high-containment viruses. Methods in Molecular Biology. 2016; 1403:295-311. PMID: 27076138
5. Matassov D, Marzi A, Latham T, Xu R, Ota-Setlik A, Feldmann F, Geisbert JB, Mire CE, Hamm S, Nowak B, Egan MA, Geisbert TW, Eldridge JH, Feldmann H, Clarke DK. Vaccination with a highly attenuated recombinant vesicular stomatitis virus vector protects against challenge with a lethal dose of Ebola virus. The Journal of Infectious Disease. 2015; 212 Suppl 2:S443-51. PMID: 26109675, PMCID: PMC4564554
6. Mire CE, Geisbert JB, Versteeg KM, Mamaeva N, Agans KN, Geisbert TW, Connor JH. A Single-vector, single-injection trivalent filovirus vaccine: proof of concept study in outbred guinea pigs. The Journal of Infectious Disease. 2015; 212 Suppl 2:S384-8. PMID: 25957964, PMCID: PMC4564539
7. Meyer M, Garron T, Lubaki NM, Mire CE, Fenton KA, Klages C, Olinger GG, Geisbert TW, Collins PL, Bukreyev A. Aerosolized Ebola vaccine protects primates and elicits lung-resident T cell responses. The Journal of Clinical Investigation. 2015; 125(8):3241-55. PMID: 26168222, PMCID: PMC4563760
8. Mire CE, Matassov D, Geisbert JB, Latham TE, Agans KN, Xu R, Ota-Setlik A, Egan MA, Fenton KA, Clarke DK, Eldridge JH, Geisbert TW. Single-dose attenuated Vesiculovax vaccines protect primates against Ebola Makona virus. Nature. 2015; 520(7549):688-91. NIHMSID: NIHMS677263, PMID: 25853476, PMCID: PMC4629916
9. Safronetz D, Mire C, Rosenke K, Feldmann F, Haddock E, Geisbert T, Feldmann H. A recombinant vesicular stomatitis virus-based Lassa fever vaccine protects guinea pigs and macaques against challenge with geographically and genetically distinct Lassa viruses. PLoS Neglected Tropical Diseases. 2015; 9(4):e0003736. PMID: 25884628, PMCID: PMC4401668
10. de Wit E, Marzi A, Bushmaker T, Brining D, Scott D, Richt JA, Geisbert TW, Feldmann H. Safety of recombinant VSV-Ebola virus vaccine vector in pigs. Emerging Infectious Disease. 2015; 21(4):702-4. PMID: 25811738, PMCID: PMC4378486
11. Geisbert TW. Emergency treatment for exposure to Ebola virus: the need to fast-track promising vaccines. JAMA. 2015; 313(12):1221-2. PMID: 25742306
12. Marzi A, Feldmann F, Geisbert TW, Feldmann H, Safronetz D. Vesicular stomatitis virus-based vaccines against Lassa and Ebola viruses. Emerging Infectious Disease. 2015; 21(2):305-7. PMID: 25625358, PMCID: PMC4313664
13. Mire CE, Geisbert JB, Agans KN, Satterfield BA, Versteeg KM, Fritz EA, Feldmann H, Hensley LE, Geisbert TW. Durability of a vesicular stomatitis virus-based Marburg virus vaccine in nonhuman primates. PLoS One. 2014; 9(4):e94355. PMID: 24759889, PMCID: PMC3997383
14. Mire CE, Geisbert JB, Marzi A, Agans KN, Feldmann H, Geisbert TW. Vesicular stomatitis virus-based vaccines protect nonhuman primates against Bundibugyo ebolavirus. PLoS Neglected Tropical Diseases. 2013; 7(12):e2600. PMID: 24367715, PMCID: PMC3868506
15. Mire CE, Versteeg KM, Cross RW, Agans KN, Fenton KA, Whitt MA, Geisbert TW. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virology journal. 2013; 10:353. PMID: 24330654, PMCID: PMC3878732

PATH list of relevant publications

1. Zaman K., A.M. Naser, M. Power, M. Yaich, L. Zhang, A.S. Ginsburg, S.P. Luby, M. Rahman, S. Hills, M. Bhardwaj, **J. Flores**. Lot-to-lot consistency of live attenuated SA 14-14-2 Japanese encephalitis vaccine manufactured in a Good Manufacturing Practices facility and non-inferiority with respect to an earlier product. Vaccine. 2014 Oct 21;32(46):6061-6. doi: 10.1016/j.vaccine.2014.09.012. Epub 2014 Sep 18.
2. Rudenko L., I. Kiseleva, M. Stukova, M. Erofeeva, A. Naykhin, S. Donina, N. Larionova, M. Pisareva, V. Krivitskaya, **J. Flores**. Clinical testing of pre-pandemic live attenuated A/H5N2 influenza candidate vaccine in adult volunteers: Results from a placebo-controlled, randomized double-blind phase I study.. Vaccine. 2015 Sep 22;33(39):5110-7. doi: 10.1016/j.vaccine.2015.08.019. Epub 2015 Aug.

USU-HMJF list of relevant publications

1. Bonaparte, M. I., A. S. Dimitrov, K. N. Bossart, G. Crameri, B. A. Mungall, K. A. Bishop, V. Choudhry, D. S. Dimitrov, L.-F. Wang, B. T. Eaton, and **C.C. Broder**. Ephrin-B2 Ligand is a Functional Receptor for Hendra Virus and Nipah Virus. Proc Natl Acad Sci U S A. 102(30):10652-7. 2005. (***from the cover***)
2. Mungall, B.A., Middleton, D, Crameri, G, Bingham, J, Halpin, K, Russell, G, Green, D, McEachern, J, Pritchard, L.I, Eaton, B.T., Wang, LF, Bossart, K.N., **Broder, C.C.** A feline model of acute Nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. J. Virol. 80(24):12293-302. 2006.
3. McEachern, J.A., Bingham, J., Crameri, G., Green, D.J., Hancock, T.J., Middleton, D., Feng, Y.R., **Broder, C.C.**, Wang, L.F. and Bossart, K.N. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Virology, 26(31):3842-52. 2008.

4. Bossart, K.N., Zhu Z., Middleton, D., Klippel, J., Crameri, G., Bingham, J., McEachern, J.A., Green, D., Hancock, T.J., Chan, Y.P., Hickey, A.C., Dimitrov, D.S., Wang, L.F., **Broder, C.C***. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. Plos Pathogens. Oct. 5(10) 2009. PMCID: PMC2765826.
5. Pallister J, Middleton D, Wang LF, Klein R, Haining J, Robinson R, Yamada M, White J, Payne J, Feng YR, Chan YP, **Broder CC**. A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. Vaccine. 2011, 29(34):5623-30. PMCID: PMC3153950
6. Bossart, KN, Geisbert, TW, Feldmann, H, Zhu, Z, Feldmann, F, Geisbert, JB, Yan, L, Feng, Y-R, Brining, D, Scott, D, Wang, Y, Dimitrov, AS, Callison, J, Chan, Y-P, Hickey, AC, Dimitrov, DS, **Broder, CC***, and Rockx, B. A neutralizing human monoclonal antibody protects African Green monkeys from Hendra virus challenge. Sci. Transl. Med. 3, 105ra103. 2011. *corresponding author. (***from the cover***). PMCID: PMC3313625.
7. Bossart KN, Rockx B, Feldmann F, Brining D, Scott D, Lacasse R, Geisbert JB, Feng YR, Chan YP, Hickey AC, **Broder CC**, Feldmann H, Geisbert TW. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med. 4(146):146ra107. 2012. (***from the cover***) PMCID: PMC3516289.
8. Mire CE, Geisbert JB, Agans KN, Feng YR, Fenton KA, Bossart KN, Yan L, Chan YP, **Broder CC**, Geisbert TW. A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. J Virol. 2014. 88(9):4624-31. PMCID: PMC 24522928.
9. Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, Frazer L, Huang JA, Edwards N, Wareing M, Elhay M, Hashmi Z, Bingham J, Yamada M, Johnson D, White J, Foord A, Heine HG, Marsh GA, **Broder CC**, Wang LF. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis. 2014 Mar;20(3). PMCID: PMC3944873.
10. Geisbert TW, Mire CE, Geisbert JB, Chan YP, Agans KN, Feldmann F, Fenton KA, Zhu Z, Dimitrov DS, Scott DP, Bossart KN, Feldmann H, **Broder CC***. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med. 2014, (***from the cover***) 6(242):242ra82. PMCID: PMC4467163.

Duke-NUS list of relevant publications. Attached as **Table 4** (above)

2.3. Stakeholder engagement strategy

Development of the vaccine will take place under the regulatory oversight of US FDA, and the consortium will seek scientific advice from US FDA and other relevant regulators to ensure that the development plan is tailored to meet the requirements for stockpiling, emergency use, and eventual licensure as appropriate. More information on this is included in section 4.6.

The consortium will work with multiple partners in countries where we conduct clinical trials, including researchers, regulators, and other decision-makers. We will collaborate with in-country researchers in developing the vaccines and building capacity through investments in clinical trial and laboratory facilities and training. As the studies progress, we will convene local trial groups and other experts in countries where the clinical development is taking place to confidentially share progress and preliminary findings, build evidence for regulatory and policy discussions, and plan for upcoming activities. We also will partner with the local trial groups in the dissemination of results once the in-country trials have concluded, including publication and presentation for scientific audiences, as well as local dissemination activities in the communities where the trials are conducted.

We also will work with key decision-makers in the countries where we are conducting clinical trials, including regulators and ethics committees, to ensure that they have access to the information necessary to make evidence-based decisions around the testing and potential use of vaccines.

We recognize that engagement of regulators and decision-makers at the global level (e.g., WHO) and in countries at high risk of Lassa feverwill be important to ensure that they have the information needed to make decisions about deployment of the vaccine in the event of an outbreak. It is our understanding that CEPI intends to facilitate discussions to build consensus around emergency use protocols and other key issues in advance of an outbreak to facilitate rapid deployment of stockpiled vaccines when an outbreak is identified. Our consortium is eager to engage in and support these discussions as appropriate.

The consortium also will engage with WHO's R&D Blueprint for Action to Prevent Epidemics and other global coordinating mechanisms that are working to establish norms and standards and ensure streamlined approaches to development of vaccines for use during outbreaks and epidemics. The focus of this engagement will be to ensure that our efforts are consistent with global approaches (i.e., target product profiles, roadmaps, clinical endpoints, etc.) and policies. We also anticipate bringing the results of our clinical trials to the attention of the WHO Product Development for Vaccines Advisory Committee (PDVAC) and Strategic Advisory Group of Experts (SAGE) and other advisory bodies to inform policy around Lassa fever vaccines.

3. Project background

3.1. The proposed vaccine candidate

Vaccine Candidate: A recombinant subunit vaccine derived from the ectodomain of HeV attachment G glycoprotein (HeV-sG) in formulation with Alhydrogel™ for intra-muscular injection.

3.2. Scientific rationale

Scientific Rationale: As noted above, the HeV-sG subunit vaccine is based on the currently licensed HeV-sG vaccine for veterinary use (Equivac® HeV, Zoetis), which has been administered to more than 300,000 horses in Australia (see https://www.health4horses.com.au/Documents/H4H_Factsheet_V5_WEB2.pdf. In addition, accidental administration of the veterinary vaccine suggests robust immunogenicity in humans.

3.3. Pre-clinical evidence to date

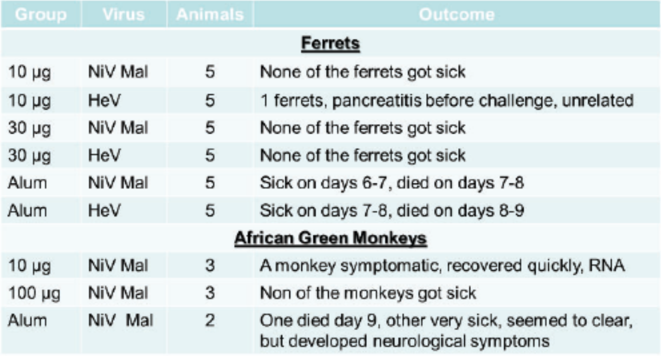
Preliminary Data

HeV-sG in animal vaccine studies. Several animal models have been used to study NiV and HeV disease, including: cats (12, 13), hamsters (16-18), ferrets (14, 19), horses, and AGMs (10, 20). To date, HeV-sG subunit vaccine in co-formulation with the adjuvants Alhydrogel™ and CpG ODM 2007 has been tested in four animal species: cats, ferrets, AGMs, and horses. Following 2 doses of vaccine separated by 21 days, all immunized animals were protected from lethal challenge with either HeV or NiV (**Table 8**). Immunization not only protected against mortality, but also prevented all clinical symptoms and resulted in no virus genetic material being detectable in any tissues harvested from the immunized cats, ferrets, and AGMs.

Table 8: Summary of HeV-sG vaccine challenge protection studies

model / virus	**HeV-sG dose / adjuvant**	**# animals challenged**	**# animals protected**
Cat / NiV **(12)**	100 µg / Montanide - Quil A – DEAE dextran	2	2
	Adjuvant only (Control)	2	0
Cat / NiV **(13)**	50 µg / CpG ODN 2007 - Alum	2	2
	25 µg / CpG ODN 2007 - Alum	2	2
	5 µg / CpG ODN 2007 - Alum	2	2
	Adjuvant only (Control)	2	0
Ferret /HeV (14)	100 µg / CpG ODN 2007 - Alum	2	2
	20 µg / CpG ODN 2007 - Alum	2	2
	4 µg / CpG ODN 2007 - Alum	2	2
	Adjuvant only (Control)	2	0
AGM / NiV (15)	100 µg / CpG ODN 2006 - Alum	3	3
	50 µg / CpG ODN 2006 - Alum	3	3
	10 µg / CpG ODN 2006 - Alum	3	3
	Adjuvant only (Control)	1	0
Horse / HeV	All immunized animals survived.		

Alhydrogel (Alum) as single adjuvant. The preliminary data presented in **Table 5** were generated using HeV-sG in co-formulation with both Alhydrogel and CpG ODN 2006 adjuvants. To simplify vaccine development, subsequent studies were performed using Alhydrogel™ only. Two studies in ferrets and one in AGM confirmed that HeV-sG formulated with Alhydrogel™ alone provides a fully protective vaccine. Each study had a low dose group (10 µg HeV-sG per dose), a high dose group (30 µg for ferrets and 100 µg for AGMs per dose), and a control group dosed with Alhydrogel™ only. The ratio of HeV-sG /Alum was kept at 1/25 as in the preliminary studies. The results from these studies are summarized in **Table 9**. In brief, all vaccinated animals were protected from lethal challenge with either NiV or HeV. Thus, Alhydrogel™ alone is an effective adjuvant for HeV-sG.



Group	Virus	Animals	Outcome
			Ferrets
10 µg	NiV Mal	5	None of the ferrets got sick
10 µg	HeV	5	1 ferrets, pancreatitis before challenge, unrelated
30 µg	NiV Mal	5	None of the ferrets got sick
30 µg	HeV	5	None of the ferrets got sick
Alum	NiV Mal	5	Sick on days 6-7, died on days 7-8
Alum	HeV	5	Sick on days 7-8, died on days 8-9
			African Green Monkeys
10 µg	NiV Mal	3	A monkey symptomatic, recovered quickly, RNA
100 µg	NiV Mal	3	Non of the monkeys got sick
Alum	NiV Mal	2	One died day 9, other very sick, seemed to clear, but developed neurological symptoms

Table 9. Results from the HeV-sG/Alum vaccine formulation efficacy test against NiV challenge in ferrets and AGMs and against HeV challenge in ferrets. All vaccinated animals survived challenge, but one monkey in the low dose group became symptomatic and was positive of viral RNA. The same monkey had the lowest serum IgG anti-NiV titer, suggesting a correlation betweenthe serum IgG titer and protection.

HeV-sG/Alum formulation. To this point in development, all the animal studies utilized a HeV-sG/Alum ratio 1/25. Thus, if a 100µg dose HeV-sG of this formulation were to be tested in the clinic, it would contain 2.5 mg of Al^{3+}, which is twice the maximum dose recommended by the FDA for single site administration. To determine if a lower amount of alum could be used, absorption/desorption studies were performed at various HeV-sG/Alum ratios. These studies demonstrated that HeV-sG is fully absorbed to Alhydrogel™ at a ratio as low as 1/7. Therefore, a ratio of 1/10 HeV-sG/Alhydrogel™ provides 70% absorption saturation and a 100µg dose of HeV-sG will contain 1 mg Al^{3+}, which is 80% of the maximum allowed Al^{3+} dose per shot. Therefore, a rabbit immunogenicity study was performed that directly compared the immunogenicity of a 30µg dose of HeV-sG when formulated with Alhydrogel™ at ratios of 1/10 vs 1/25. As shown in **Figure 1**, changing the HeV-sG/Alum ratio from 1/25 to 1/10 did not alter HeV-sG immunogenicity, and both formulations provided exceptionally good responses. Thus, a 1/10 HeV-sG/Alhydrogel™ formulation was selected for development and clinical evaluation.

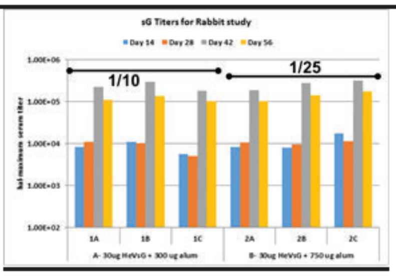


Figure 1. Immunogenicity study to test HeV-sG/Alum ratio effect. Two groups of three rabbits were immunized with formulation at 1/10 (Vac 1) and at 1/25 (Vac 2). Sera were tittered for anti-HeV-sG antibodies. Vac 1 and Vac 2 showed indistinguishable immune responses, measured in serum titers.

Preliminary stability data for the HeV-sG/Alum formulation. To support development, a 32-week stability study of the HeV-sG/Alhydrogel™ formulation used in the above studies was conducted. As seen in **Figure 2**, unformulated HeV-sG is stable at 4⁰C for 32 weeks, but undergoes degradation if stored at 21⁰C or 37⁰C for this period. In contrast, Alhydrogel™ formulated HeV-sG remains 100% bound and intact at either 4⁰C or 21⁰C for 32 weeks. Thus, HeV-sG/Alhydrogel™ provides a product with projected multi-year stability at refrigerator temperature, that can withstand extended handling at room temperature, and that will likely withstand multi-day handling at 37⁰C.

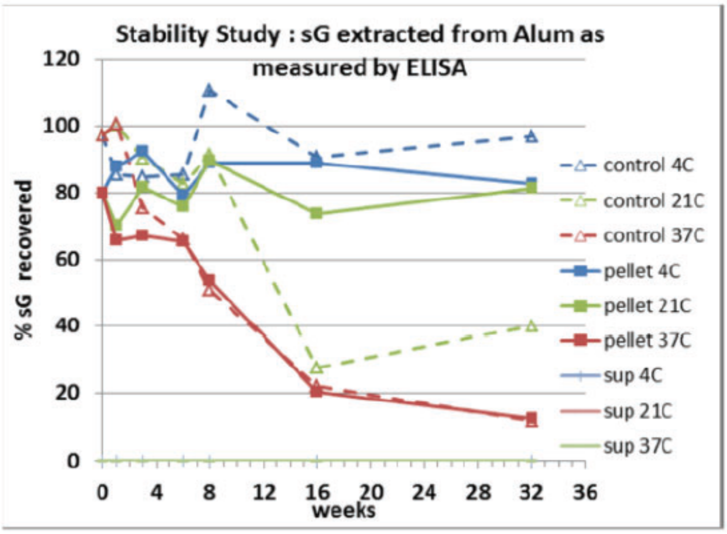


Figure 2. HeV-sG/Alhydrogel™ vaccine is stable for 32 weeks when stored at 4⁰C and 21⁰C. Formulated and non-formulated HeV-sG was stored at 4ᵒC, 21ᵒC, and 37ᵒC for 32 weeks. Samples were examined at weeks 1, 3, 6, 8, 16, and 32 to determine HeV-sG integrity, quantity, and percent bound to the Alhydrogel™ by quantitative ELISA that discriminates between intact and degraded HeV-sG. Control = non-formulated HeV-sG, Sup = supernatant of centrifuged HeV-sG/Alhydrogel™, Pellet = HeV-sG extracted from the pellet of centrifuged HeV-sG/Alhydrogel™.

Cell line creation and MCB manufacture. A HEK-293 Research Cell Bank (RCB) expressing HeV-sG protein (HeV-sG-HEK-293) was prepared by Profectus, using multiple transductions of parent HEK-293F cells with a recombinant MoMuLV carrying the HeV-sG gene, followed by two consecutive rounds of limited dilution cloning. The productivity of the RCB was greater than 1 gm/L HeV-sG when grown in shake flasks using a preliminary optimized feeding schedule. The RCB was used as a seed to manufacture a HeV-sG-HEK-293 master cell bank (MCB) at CRL – lot #5407580MCB2, December 31, 2013, 325 vials, 1.16×10^7 viable cells/vial. The MCB has passed all testing and has been released by Profectus QA.

Expression and purification of HeV-sG. Under Profectus oversight, our CMO, Catalent, has developed a scalable upstream process (USP) suitable for cGMP protein production, in which the concentration of HeV-sG in the supernatant exceeds 0.6 g/L, which was judged adequate for further development. A scalable downstream purification process (DSP) suitable for cGMP manufacture of HeV-sG has also been developed at Catalent. The process consists of: 1) Media clarification by depth filtration (3M Zeta Plus 60SP02A); 2) Triton-X 100 added to 0.1% v/v and incubated for one hour (for viral inactivation) followed by pH reduction to 5.0; 3) Capto MMC mixed-mode hydrophobic cation exchange chromatography with elution over a 20 column volume gradient starting with 100% 25mM MES, pH 6.0 and moving to 25mM MES, pH 6.0, 0.5M NaCl followed by increase in pH to 8.0; 4) intermediate purification using CaptoAdhere mixed-mode hydrophobic anion exchange chromatography with elution over a 20 column volume gradient starting with 100% 50mM Tris, pH 8.0 and moving to 50mM Tris, pH 8.0, 1M NaCl, followed by adding an equal volume 2M ammonium sulfate, pH 7.5; 5) polishing purification using Phenyl HP hydrophobic interaction chromatography with elution over a 32 column volume gradient starting with 80% 50mM Tris, 1M ammonium sulfate, pH 7.5 and moving to 50mM Tris, pH 7.5; 6) an additional polishing chromatography by diafiltration with 7 volumes 50mM Tris, pH 7.5 followed by Sartobind Q membrane in flow-through mode with 50mM Tris, pH 7.5; 7), and viral filtration by using a Virosart 150 CPV capsule filter. Finally, the HeV-sG solution was dialyzed against PBS (10 mM sodium phosphate, 150mM NaCl, pH 7.0), concentrated to 9.84 mg/mL using tangential flow filtration with a 50 kD MWCO PES membrane, and sterile filtered through a 0.2-µm PES filter. This cGMP-like purification process was used to purify HeV-sG from the supernatant of a 10-L bioreactor run to obtain 2.5 grams HeV-sG, 2x125 mL in PETG bottles at 9.84 mg/mL in PBS. This non-GMP DS was used to formulate the toxicology lot of HeV-sG/Alhydrogel™ vaccine.

Vaccine formulation, toxicology lot manufacture. The toxicology lot of HeV-sG/Alhydrogel™, labelled as "0.1 mg/mL HeV-sG, 1.0 mg/mL Al^{3+} in Aluminum Hydroxide Suspension for Injection, (Toxicology Supplies), Lot PUP05401A" from 19 July 2016 was prepared at Catalent Pharma. 624 of the 649 filled vials were made available for stability testing, the GLP toxicology study, and animal efficacy testing studies. A 36-page batch record details each step in the preparation process that basically consists of mixing 10.2 mL HeV-sG DS with 100 mL Alhydrogel™ in 890 mL PBS with continuous stirring.

GLP Tox Study. A 4-cycle (1 dose every 3 weeks) toxicity study in rabbits administered HeV-sG/Alum by intramuscular injection initiated October 2016 at CRL (**Table 10**). Three groups of 20 New Zealand White Rabbits, aged from 3 to 5 months and from 2.0 to 4.0 kg each are being used in the study. The control group was dosed with saline, the low dose group was injected with the maximum intended human dose, and the high dose group was injected with 3X the maximum intended human dose. The un-audited toxicology report contains no significant observations, and the final audited report will be available on or about 01-Aug-2017.

Table 10. A 4-cycle Study in Rabbits Administered HeV-sG/Alhydrogel™ by Intramuscular Injection

Group No.	Test Item[a]	Dose Level (mg/day)	Dose Concentration (mg/mL)	Dose Volume (mL)	No. of Animals			
					Main Study[b]		Recovery Study[c]	
					Males	Females	Males	Females
1	Saline Control	0	0	3.0	5	5	5	5
2	HeV-sG-V	0.1	0.1	1.0	5	5	5	5
3	HeV-sG-V	0.3	0.1	3.0	5	5	5	5

[a] Dosing will be performed once every three weeks (= 1 cycle) for 4 cycles

[b] Main study animals will undergo necropsy 3 days following the last dose administration.

[c] Recovery study animals will undergo necropsy 28 days following the last dose administration.

CRL is an accredited CMO for carrying out GLP toxicity studies and we do not expect procedural complications with the study. Antibody titers from blood samples will be determined under well documented practices at Profectus.

Animal efficacy confirmation studies. Two AGM challenge studies has been conducted to confirm the HeV-sG/Alhydrogel™ vaccine produced under GMP-like conditions will protect against lethal challenge with NiV and HeV. Twelve AGMs were used in each study. In the first study, groups of AGMs were vaccinated on experimental days 0 and 28 with 100 µg (group 1, N=6) or 300 µg (Group 2, N=3) of the same preparation of HeV-sG/Alhydrogel™ used in the GLP toxicology study, and a control group of 3 AGMs received Alhydrogel™ suspension only. On experimental day 56, all AGMs in study 1 were challenged with a lethal dose of NiV (1998-99 Malaysian outbreak strain). Within 13 days of challenge, all control AGMs had succumbed to lethal NiV disease, while all vaccinated animals remained completely healthy. Study 2 was carried out identically to the first, except that the challenge consisted of HeV (1994 Australian outbreak strain). As in the first study, all vaccinated AGMs remained healthy, while 2 of the 3 control animals died of haemorrhagic disease.

3.4. Clinical evidence to date

The HeV-sG/Alhydrogel™ vaccine candidate has not yet entered clinical evaluation. However, the human immune response to accidental inoculation (N=2) with the veterinary vaccine has been shown to be potent and to cross-neutralize both NiV and HeV (I. Smith and C. Broder, unpublished).

3.5. Assays, animal models and supporting relevant data developed

The assays to be employed to support clinical evaluation are described under **Task 2** in **Section 4.2 Objectives** (below). Characterization of the HeV-sG/Alhydrogel™ vaccine candidate and the animal models providing the data that support advancement of the vaccine into clinical evaluation are described in **Section 3.3 Pre-clinical evidence to date** (above).

3.6. Evidence from other pathogens

The HeV-sG/Alhydrogel™ vaccine candidate is a subunit vaccine specific to NiV and HeV. However, subunit vaccines have been successfully developed for a number of viral pathogens, including HBV, HPV, influenza.

3.7. Intellectual property

Profectus BioSciences, Inc. has executed a term sheet and is finalizing the license necessary to manufacture, use, sell and sublicense its subunit Nipah/Hendra soluble G vaccine. Profectus's Nipah/Hendra soluble G vaccine does not come within the scope of any valid third party issued patent claims. Therefore, Profectus has freedom to operate.

4. Research and Technical Objectives – Statement of work

4.1. Project governance/management and applicant's and partner's roles and responsibilities in the project

Table 12 provides a listing of the 12 Primary and 5 Optional Tasks that make up the work proposed under this application, and the Cooperating Partner(s) or contracted organization(s) responsible for completion of the Task. Each of these Tasks is described in detail in section 4.2 Objectives, including Task-specific Deliverables and Go/No Go criteria. Each **Task** will be managed through the establishment of an interdisciplinary Task Management Team comprised of the appropriate individuals from both Profectus and the collaborating partner or contract service provider. The Task Management Teams will hold regularly scheduled meetings with agendas, minutes and time constrained action items. The overall timing of the **Tasks** and their relationship for decision making is illustrated in the **Detailed Milestone Plan**.

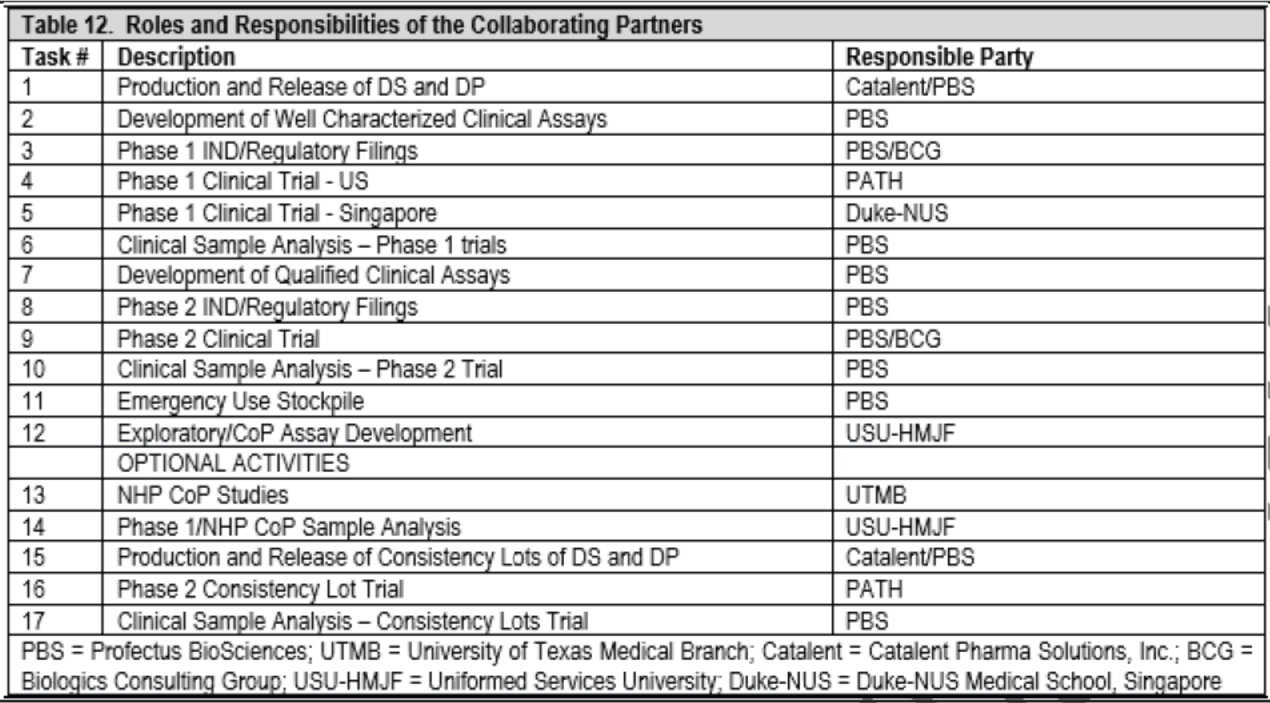
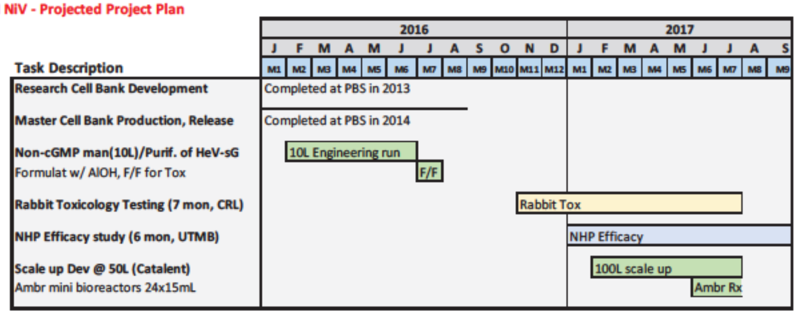


Table 12. Roles and Responsibilities of the Collaborating Partners

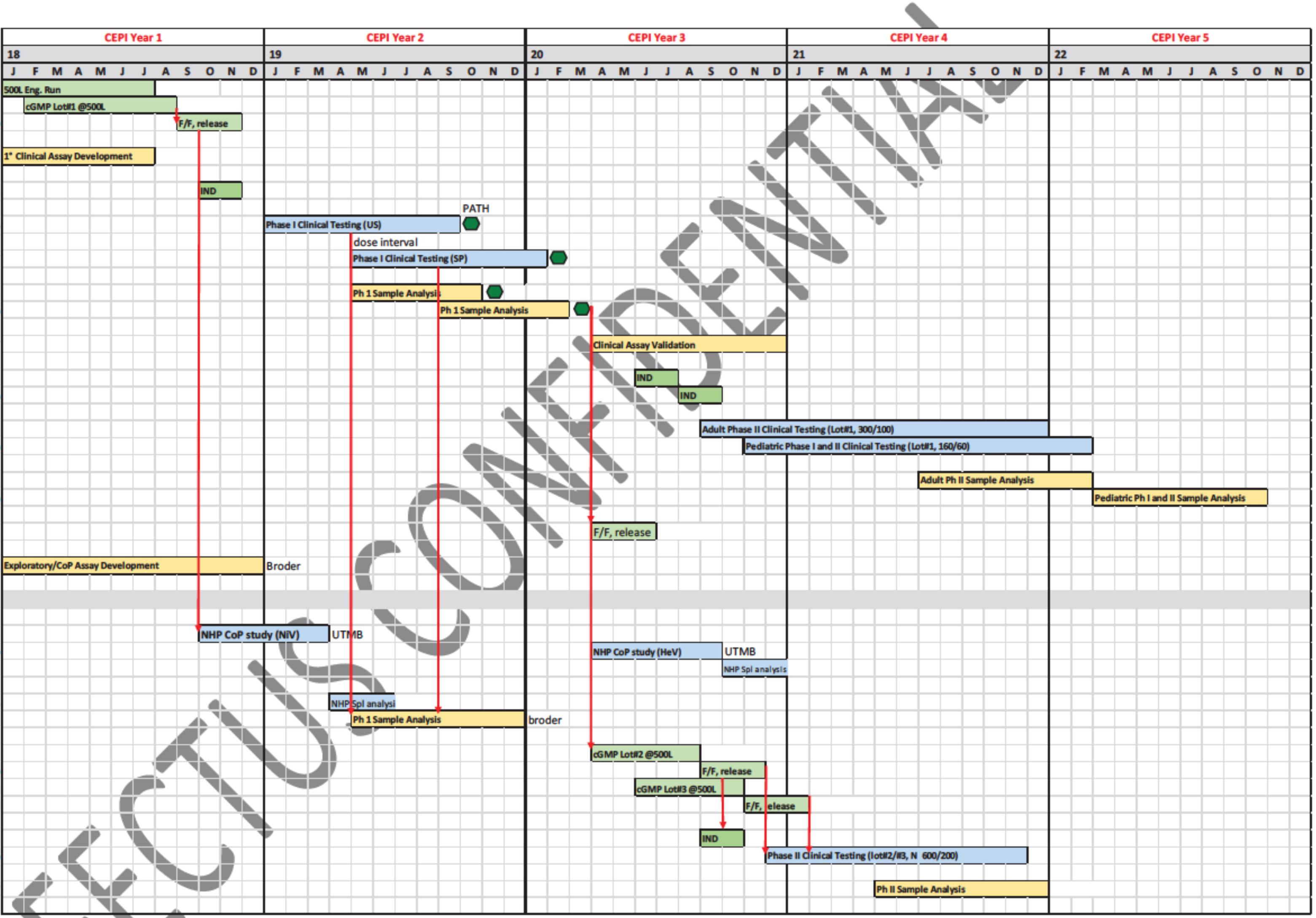
Task #	Description	Responsible Party
1	Production and Release of DS and DP	Catalent/PBS
2	Development of Well Characterized Clinical Assays	PBS
3	Phase 1 IND/Regulatory Filings	PBS/BCG
4	Phase 1 Clinical Trial - US	PATH
5	Phase 1 Clinical Trial - Singapore	Duke-NUS
6	Clinical Sample Analysis – Phase 1 trials	PBS
7	Development of Qualified Clinical Assays	PBS
8	Phase 2 IND/Regulatory Filings	PBS
9	Phase 2 Clinical Trial	PBS/BCG
10	Clinical Sample Analysis – Phase 2 Trial	PBS
11	Emergency Use Stockpile	PBS
12	Exploratory/CoP Assay Development	USU-HMJF
	OPTIONAL ACTIVITIES	
13	NHP CoP Studies	UTMB
14	Phase 1/NHP CoP Sample Analysis	USU-HMJF
15	Production and Release of Consistency Lots of DS and DP	Catalent/PBS
16	Phase 2 Consistency Lot Trial	PATH
17	Clinical Sample Analysis – Consistency Lots Trial	PBS

PBS = Profectus BioSciences; UTMB = University of Texas Medical Branch; Catalent = Catalent Pharma Solutions, Inc.; BCG = Biologics Consulting Group; USU-HMJF = Uniformed Services University; Duke-NUS = Duke-NUS Medical School, Singapore

CEPI NiV - Projected Project Plan



Task	Task Description	
1	non-cGMP Man/Purif/Release @500L (Catalent)	1
	cGMP Man/Purif/Release DS Lot#1 @500L (Catelent)	1a
	F/F/Release PhI CTM (3,000 vials @100mcg/vial, Catalent)	1b
	s	
2	Clinical Assay Dev/Characterization (7mon, PBS)	2
3	Prep, submit IND (2 mon, PBS/BCG)	3
4	Ph I Clininical Trial USA, N 78 (9 mon, PATH)	6
5	Ph I Clininical Trial Singapore N 78 (9 mon, Duke/NUS)	7
6	Ph I Clininical Sample testing (USA Trial, PBS)	6a
	Ph I Clininical Sample testing (Singapore Trial, PBS)	6b
7	Clinical Assay Qualification (9mon, PBS)	7
8	IND Amendment to support Adult Ph II (PBS/BCG)	8a
	IND Amendment to support Pediatric Ph I and II (PBS/BCG)	8b
9	Adult Ph II Clininical Trial Singapore (16 mon, PATH)	9a
	Pediatric Ph I and II Clininical Trial Singapore (16 mon, PATH)	9b
10	Adult Ph II Clininical Sample Testing (8 mon, PBS)	10a
	Pediatric Ph I and II Clininical Sample Testing (8 mon, PBS)	10b
11	Emergenccy Use Stockpile (50,000 vials, GRAM)	11
12	Exploratory/CoP Assay Development (12 mon, USU)	12
	ACTIVITIES TO SUPPORT LICENSURE	
13	NHP NiV CoP Study (6 mon, UTMB)	13a
	NHP HeV CoP Study (6 mon, UTMB)	13b
	NHP Sample analysis (3 mon, USU)	13c
14	NHP Sample analysis (3 mon, USU)	14
	PhI Sample analysis (8 mon, USU)	14a
15	cGMP Man/Purif/Release DS Lot#2 @500L (Catelent)	15a
	F/F/Release Ph II CTM (50,000 vials, GRAM)	15b
	cGMP Man/Purif/Release DS Lot#3 @500L (Catelent)	15c
	F/F/Release Ph II CTM (50,000 vials, GRAM)	15d
16	IND Amendment to support Ph II lot consistency (PBS/BCG)	16a
	Ph II Clininical Trial Lot#2/#3 Singapore (12 mon, PATH)	16b
17	Ph II Clinical Sample testing USA (PBS)	17



4.2. Objectives

This application proposes to advance the HeV-sG/Alhydrogel™ vaccine through Phase 2 clinical development and to produce and stockpile sufficient vaccine stocks to support emergency use and for potential licensure studies. To achieve these goals, the specific tasks outlined in **Table 5** will be undertaken. The proposed timeline for these activities is outlined in the attached **Detailed Milestone Plan**.

What follows is a brief description of each proposed task:

Task 1: **Production and Release of HeV-sG/Alhydrogel DS and DP**: GMP production of HeV-sG Bulk Drug Substance (BDS) will be performed at Catalent Pharma Solutions, Madison, WI. Now that the engineering run is complete, the MPR and associated GMP manufacturing methods have been finalized. Manufacturing runs will be produced under cGMP conditions at 1,000L working volume. Profectus will provide Catalent with a set of product specifications, or acceptance criteria, in writing, before GMP manufacturing commences. Based on the yields obtained in pilot-scale runs, it is projected the 1,000L GMP run will provide approximately 120 gm of DS. Since Catalent does not have an automated fill/finish facility (the toxicology lot was filled by hand in their cGMP suite), fill/finish of the Bulk Drug Product (BDP) will be performed at Grand River Aseptic Manufacturing, Inc, Grand Rapids, MI per fill requirements in terms of vial size and volume provided by Profectus. Two fill/finish runs are anticipated. The first run will supply 1,000 vials at 100µg/dose to support a phase 1 dose escalation study (10.0µg, 33.0µg, and 100.0µg) to establish safety and the optimally immunogenic dose. The second filling run will provide approximately 50,000 vials at the optimal clinical dose to support phase 2 testing and the stockpile of vaccine for emergency use. The finished vials will be stored at Fisher BioServices. Final release testing for the GMP material will be consistent with the General Biological Products Standards (21CFR610.11). Master Batch Records and all testing documentation will be provided to Profectus for QA review and final release. A complete listing of the testing and release criteria is provided in **Section 4.7 Chemistry, Manufacturing & Control (CMC) Development.**

> **Deliverables and Go/No Go Criteria:** The deliverables for this work segment will be fully characterized Lots of HeV-sG/Alhydrogel™ vaccine produced under full GMP compliance. Failure of any one of the components to pass release testing would result in an inability to proceed to clinical evaluation.

Task 2: **Development of Well Characterized Clinical Assays**: To support phase I clinical evaluation of the HeV-sG/Alhydrogel™ vaccine, Profectus will develop four well characterized assays to measure HeV and NiV GP immune responses in vaccine recipients. The assays will measure serum HeV and NiV GP-specific IgG antibody by direct ELISA, and serum HeV and NiV neutralizing antibody by plaque reduction neutralization of a green fluorescent protein (GFP)-expressing rVSV pseudo-typed with HeV or NiV GP. Each will be based on assays which PBS has developed and characterized for use in clinical trials of the mono-valent and tri-valent rVSVN4CT1-vectored filovirus vaccines. The general process for the transition of research assays to well characterized assays for use in support of phase 1 clinical testing is outlined below:

1. Research assay SOPs will be converted to DRAFT Profectus Test Methods. These Test Method documents will be the change control documents which outline all aspects of the assays, including:
    i. Scope
    ii. Roles and responsibilities
    iii. Safety
    iv. Abbreviations and definitions
    v. Reagents, materials and equipment
    vi. Procedures
    vii. References
2. In addition, for each DRAFT Profectus Test Method, the conduct or preparation of all supporting assay activities and reagents will be documented in DRAFT Profectus "Lab Method" documents. As an example:
    i. Preparation of clinical reagent lots
    ii. Procedures for assigning biological units to antibody reference standards
    iii. Preparation of Dulbecco's Phosphate Buffer Saline with 0.1% Tween-20
3. With DRAFT Test and Lab Method documents in place, the assays will be conducted over numerous days by several operators to confirm assay precision and reproducibility and the results of this testing compiled into "Characterization Reports".
    i. To demonstrate reproducibility, the assay will be conducted twice on three separate occasions using the same source material. Reproducibility will be based on a measure of the coefficient of variance (CV) for the assay read-out. CVs of <20% will be sufficient for demonstration of reproducibility.
    ii. For precision evaluations, positive control samples (reference standard and R&D antiserum for example) will be used as "samples". For each sample, a combination of different plates, test days and different operators will be set up to evaluate precision. The assays will be performed three times by one operator, and a second operator performed the same assay three times.

Profectus QA will review and approve the assay Characterization Reports. With the completion of the assay Characterization Report, the DRAFT Test and Lab Methods will also be reviewed and approved as FINAL Test and Lab Methods by PBS QA.

Deliverables and Go/No Go Criteria: The deliverables for this work segment will be FINAL Test and Lab Methods. Failure to qualify any of the assays would necessitate redevelopment and possibly delay the phase 1 clinical program.

Task 3: Phase 1 IND/Regulatory Filings: Over the past 4 years Profectus has prepared 8 IND license applications (BB-IND-14557, BB-IND-14665, BB-IND-14770, BB-IND-14976, BB-IND-15459, BB-IND-15462, BB-IND-16670, and BB-IND-16783) which have all been successful. The writing and assembly of the application for the US trial, the planning and execution of the communications and meetings with the FDA will be led by Dr. Susan Sciotto-Brown, Senior Director of Regulatory Affairs at Profectus. Electronic submission of the IND will be performed under subcontract through the electronic portal maintained by Biologics Consulting Group, Rockville, MD. The exact design of the trial will be determined by the guidance supplied by the FDA in response to a pre-IND briefing package. However, it is anticipated this will be a randomized, double-blind, placebo-controlled, truncated dose escalation trial evaluating vaccine doses 10.0µg, 33.0µg, and 100.0µg administered by IM injection in 3 groups of 26 healthy adult subjects (20 active/6 placebo). Subjects will be administered two doses of vaccine separated by 56 days.

A second phase 1 trial is proposed at the clinical trials center of the Duke-NUS Medical School in Sigapore, an area where NiV is endemic. The Profectus Regulatory Affairs group will interface with the Duke-NUS collaborators in the preparation of the necessary local regulatory filings. Although the exact design of this trial will be determined in discussions with the local regulatory agency, it is anticipated this trial will be a randomized, double-blind, placebo-controlled trial using the 33.0µg dose to evaluate vaccine dose interval (28, 56, and 120 days) in 3 groups of 26 healthy adult subjects (20 active/6 placebo).

Deliverable and Go/No Go Criteria: The deliverables for this work segment will be the safe to proceed letters from the US FDA and the relevant Singapore regulatory agency. The risk in preparation of these applications is mitigated by the experience and demonstrated success of the Profectus team in preparing IND license applications.

Task 4: Phase 1 Clinical Trial - US: Two partners have been identified to conduct phase 1 clinical evaluation of the HeV-sG/Alhydrogel™ vaccine. The first is PATH, Seattle WA and Washington, DC, who will use their clinical trials infrastructure to conduct a Phase I trial to test the safety and immunogenicity of the HeV-sG/Alhydrogel™ vaccine in normal healthy adults in the US. Although final design will be established in consultation with US FDA, it is anticipated this will be a randomized, double-blind, placebo-controlled, truncated dose escalation trial evaluating vaccine doses of 20 µg, 50 µg, and 100µg HeV-sG protein in a 1/10 wt/wt formulation with Alhydrogel™ adjuvant administered by IM injection in 3 groups of 25 healthy adult subjects (20 vaccine5 placebo). Subjects in this 3 groups will be administered two doses of vaccine separated by 56 days. A fourth group of subjects (20 vaccine/5 placebo) will receive a single dose of HeV-sG at the highest tolerated level. The primary objective of the study is to assess vaccine safety and tolerability by close clinical and laboratory follow-up of subjects after each vaccination; the secondary objective will be evaluation of the vaccine immunogenicity.

Deliverables and Go/No Go Criteria: The deliverables will be interim and final clinical trials reports demonstrating that the HeV-sG/Alhydrogel™vaccine is safe, well tolerated and immunogenic. This will support the initiation of **Task 5** (Phase I clinical testing in Singapore), and **Task 8** (Phase 2 clinical testing). Any results which suggest the vaccine is not safe, well tolerated or immunogenic will trigger a review to determine if it is judged prudent to discontinue development of the vaccine.

Task 5: Phase 1 Clinical Trial - Singapore: The second clinical trial partner is Dr. Linfa (Lin-Fa) Wang, Ph.D. FTSE, Professor & Director, Programme in Emerging Infectious Disease, Duke-NUS Medical School who has independent co-funding to conduct a phase 1 trial in Singapore where NiV is endemic. Drs. Broder and Dimitrov have had a longstanding collaboration with Dr. Wang to study Henipaviruses and develop an effective vaccine. The design of the Phase 1 clinical trial will be determined in collaboration with clinical site investigators, the funders of the trial, the Profectus development team, and the local regulatory authorities. Trial initiation is planned to follow demonstration in the PATH trial of vaccine safety at the dose level selected from the phase 1 study in the US This dose level when administered twice by IM injection to normal healthy adults at 91, 181, and 366 day intervals The primary endpoint will be safety with secondary endpoints consisting of immunogenicity defined by ELISA and neutralizing anti-NiV and anti-HeV antibody responses.

Deliverables and Go/No Go Criteria: The deliverables will be interim and final clinical trials reports demonstrating that the HeV-sG/Alhydrogel™vaccine is safe, well tolerated and immunogenic. This will support the initiation of **Task 8** (Phase 2 clinical testing). Any results which suggest the vaccine is not safe, well tolerated or immunogenic will trigger a review to determine if it is judged prudent to discontinue development of the vaccine.

Task 6: Clinical Sample Analysis – Phase 1 trials: In support of the phase 1 clinical evaluation of the HeV-sG/Alhydrogel™ in the US and Singapore, Profectus will perform the well characterized assays to measure immune responses to NiV and HeV in vaccine recipients. The assays will measure serum IgG antibody by direct ELISA, and serum neutralizing antibody by plaque reduction neutralization of GFP-expressing rVSV pseudo-typed with the relevant GPs.

Deliverables and Go/No Go Criteria: The deliverables for this work segment will be interim and final clinical trial assay reports. The failure of these assays to remain in control would necessitate redevelopment and trigger a review to determine if further vaccine development is justified.

Task 7: **Development of Qualified Clinical Assays**: A key task will be to establish qualified assays, standard operating procedures (SOPs) and acceptance criteria that will establish the identity, structural integrity, purity of HeV-sG consistent with the General Biological Products Standards (21CFR610.11). Currently, we have SDS-PAGE analysis and binding to anti-G MAb, m102.4, ELISA developed under the preclinical development as product specific release assays. Other product specific assays may be added as deemed necessary by the Product Development Team. Microbial sterility, bioburden, endotoxin and other standard release assays that are used to characterize protein based products to meet 21CFR610.11 will also be used. Profectus will import an authentic henipavirus infection, replication and reporter system based on the non-pathogenic CedPV that is being developed by Dr. Broder's laboratory to determine serum neutralization titers (Task 12 below). Profectus will optimize and qualify the assays to be suitable for GLP practices. We will also evaluate whether immunogenicity in rabbits will provide a suitable potency assay as specified in the General Biological Products Standards. Induction of cross-reactive NiV serum neutralizing titers >160 after immunization will confirm the suitability of rabbits to evaluate the potency of the product.

Task 8: **Phase 2 IND/Regulatory Filings**: Phase 2 testing of the HeV-sG/Alhydrogel™ vaccine for expanded safety and immunogenicity will be conducted by PATH in multiple clinical sites, likely located in multiple countries. Therefore, the regulatory filings to support Phase 2 testing will be prepared by a working team with members from both the PATH and Profectus Regulatory Affairs groups.

Deliverables and Go/No Go Criteria: The deliverables for this work segment will be the safe to proceed letters from the US FDA and the relevant foreign regulatory agencies. The risk in preparation of these applications is mitigated by the experience and demonstrated success of the Profectus and PATH Regulatory Affairs groups in preparing the relevant applications.

Task 9: **Phase 2 Adult and Phase 1/2 Child Clinical Trials**:

Adults - It is planned that phase 2 testing of the HeV-sG/Alhydrogel™ vaccine for expanded safety and immunogenicity will be conducted by PATH, and that it will examine the dose level and dosing interval identified as best supporting rapid and sustained protective immunity. Although it is difficult to accurately predict the required phase 2 trial size until the phase 1 trial safety and immunogenicity profiles are known, some assumptions can be made for planning purposes. It is anticipated that a phase 2 trial of approximately 400 (320 active/80 placebo) will be adequate to establish human safety and immunogenicity data bases, which when viewed in the context of the completed and ongoing AGM protection studies, will be adequate to support clinical efficacy (phase 3) evaluation in an outbreak, and potential emergency use with appropriate authorization. This trial will be conducted at established trial sites with strong infrastructure for the collection of adverse events, to best support emergency use authorization in the event of an outbreak.

Deliverables and Go/No Go Criteria: The deliverables will be interim and final clinical trials reports demonstrating that the HeV-sG/Alhydrogel™vaccine is safe, well tolerated and immunogenic in Phase 2 testing. This will support the use of this vaccine with emergency use authorization and trigger **Task 10** (Emergency use stockpile). Any results which suggest the vaccine is not safe, well tolerated or immunogenic will trigger a review to determine if it is judged prudent to stockpile or discontinue development of this vaccine.

Children - PATH will conduct a Phase 1/2 clinical trial to test the safety and immunogenicity of the HeV-sG vaccine in healthy 3-17 year old children in Bangladesh, a country where several outbreaks of Nipah infection have occurred. This will be a randomized, double-blind, placebo-controlled, descending age study to evaluate the optimal vaccine dose/schedule determined from the Phase 2 trial in adults. The trial will first enrol thirty 8-17 year old subjects who will receive vaccine (20), or placebo (10). Once the safety data is analysed by the safety team (including independent experts), a second group of thirty 3-7 year old children will receive the vaccine (n=20) or placebo (n=10). Absence of safety concerns in these two group will lead to starting a third cohort of 160 subjects (120 vaccine, 40 placebo recipients) who will receive the same vaccine regimen. The primary objective of the study is to assess vaccine safety and tolerability by close clinical and laboratory follow-up of subjects after each vaccination; the secondary objective will be evaluation of the vaccine immunogenicity in this age group.

Deliverables and Go/No Go Criteria: The deliverables will be interim and final clinical trials reports demonstrating that the HeV-sG/Alhydrogel™vaccine is safe, well tolerated and immunogenic in 3-17 year old children. This will support the use of this vaccine with emergency use authorization in this age group (Emergency use stockpile). Any results which suggest the vaccine is not safe, well tolerated or immunogenic will trigger a review to determine if it is judged prudent to use the vaccine in this age group.

Task 10: **Clinical Sample Analysis – Phase 2 trial**: In support of the phase 2 clinical evaluation of the HeV-sG/Alhydrogel™, Profectus will perform qualified assays to measure immune responses to NiV and HeV in vaccine recipients. The assays will measure serum IgG antibody by direct ELISA, and serum neutralizing antibody by plaque reduction neutralization of GFP-expressing rVSV pseudo-typed with the relevant GPs.

Deliverables and Go/No Go Criteria: The deliverables for this work segment will be interim and final clinical trial assay reports. The failure of these assays to remain in control would necessitate redevelopment and trigger a review to determine if further vaccine development is justified.

Task 11: **Emergency Use Stockpile**: As outlined above, a single 1,000L run of HeV-sG is projected to yield more than sufficient vaccine to allow the stockpiling of 50,000 doses for investigational use during an outbreak, and potentially emergency use with appropriate authorization. In addition, preliminary data indicate that multi-year stability of the Alhydrogel™ formulated vaccine can be anticipated. Thus, it is proposed a portion of the BDS be formulated and used to fill 50,000 doses of vaccine at the dose level determined to be optimal in the phase 1 dose-finding trial conducted by PATH. Further, it is proposed this be carried out as soon as practical after the phase 1 results are known.

This vaccine would be eligible for two of FDA's expedited development and review programs, Fast Track and Priority Review. Fast Track is a process designed to facilitate the development of products that address serious conditions and fill unmet medical need by providing enhanced Agency support during product development. Priority Review designation allows for accelerated BLA review and is based on a product's potential to provide a significant improvement in the prevention of a disease relative to standard of care.

<u>**Deliverables and Go/No Go Criteria:**</u> The deliverables for this work segment will be 50,000 doses of HeV-sG/Alhydrogel™ vaccine produced under full GMP compliance and released for clinical use. Failure of the vaccine to pass release testing would result in an inability to proceed to stockpile.

<u>Task 12</u>: Exploratory/CoP Assay Development: Although assays are available to support clinical evaluation, it is believed that a more appropriate assay to establish a CoP should be investigated. The assay will be enabled by the recent identification of a third henipavirus, Cedar virus (CedPV), that was isolated from pooled bat urine collected in Queensland, Australia[1]. CedPV is closely related to HeV and NiV, genetically and antigenically. A notable distinction between CedPV and HeV/NiV lies in the phosphoprotein gene (P), which contains the open reading frames for the V and W proteins in HeV/NiV. The CedPV P gene does not encode the V and W proteins[2], which contribute to HeV and NiV pathogenicity[3-5], and is an important factor underlying its failure to cause disease in experimental infections of mice, ferrets or guinea pigs[1]. Despite the pathogenic differences and low level of protein sequence homologies of CedPV G to HeV and NiV G (29% and 30%, respectively), CedPV infection, like both HeV and NiV, is supported by the cell surface protein ephrin-B2 as an entry receptor[1]. The Broder lab has been studying the functional characteristics and cellular receptor use profile of CedPV and its interactions with host cells of varied species. A reverse genetics system has recently been established for CedPV and this platform represents a new system which can be exploited in the present application that can be used safely under BSL-2 containment.

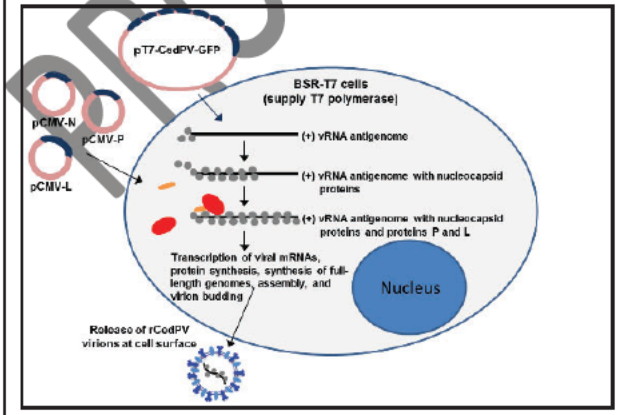
<u>*Innovation:*</u> The difficulties faced in the quantitation and assessment of vaccine-induced neutralizing antibody responses to NiV/HeV are significant owing to the requirements of BSL-4 containment for use of infectious virus. The innovation here are two-fold: **First**, we propose here a cell-based SNT that will make use of an <u>authentic</u> henipavirus infection and replication reporter system (a replication competent, reporter gene-encoding CedPV) which has never been done before. Second, the recombinant CedPV based platform requires only BSL-2 containment, relieving the cost and technical challenges of the high-containment environment when 100's to 1000's of samples will need to be tested.

<u>*Approach: Develop, characterize and adapt a rCedPV luciferase reporter virus for use in SNT*</u>.

Here, we will develop the very first cell-based, authentic henipavirus infection, replication and reporter system based on the non-pathogenic CedPV. We will rescue a second CedPV reporter virus encoding the *Photinus pyralis* luciferase gene[6] (rCedPV-Luc) and characterize and compare it to wild-type and rCedPV-GFP viruses using cell-based infection tropism and growth kinetics assays. The novelty of our approach is that the virus reporter system is an authentic henipavirus and one that may be used in the BSL-2 setting.

<u>*Experimental Methods and Design:*</u>

<u>Generation of plasmids and rescue of recombinant viruses:</u> We have already constructed a recombinant CedPV antigenome clone by synthesis of several large segments of the genome into pOLTV5[7] between a T7 RNA promoter and hepatitis delta virus (HDV) ribozyme. The pOLTV5 vector was similarly used for cloning and expression of recombinant HeV[8] and methods similar to those used in the rescue of recombinant NiV[9]. The first reporter virus chosen was using Turbo-GFP, and the GFP gene was modified to be flanked by the CedPV intragenic P stop and M start sequences conforming to the "rule of six". As with recombinant HeV[8] the GFP gene was inserted between the P and M genes to maintain the levels of N and P transcripts. Helper plasmids for the N, P, and L genes from CedPV were also prepared (pCMV). To generate recombinant CedPV, pCMV CedPV N, pCMV CedPV P, pCMV CedPV L, and pOLTV5 full-length rCedPV-GFP anti-genome plasmid were mixed with Lipofectamine LTX (Invitrogen) in OptiMEM, and used to transfect 5x10^5 BSR-T7 cells (**Figure 1**).



<u>**Figure 1**. Plasmid transfection and rescue of recombinant CedPV.</u> Full length genome clone together with the N, P, and L helper plasmids are transfected into T7-polymerase expressing BSR-T7 cells. Rescued virus is recovered from cell culture by the collection of both supernatant and freeze-thawed cells and amplified on Vero cell cultures.

At day 4, GFP was observed in BSR-T7 cells transfected with the rCedPV-GFP antigenome and N, P, and L helper plasmids. Non-adherent BSR-T7 cells and culture supernatant were collected and freeze-thawed 3-times. Vero cells at a density of 1x10^6 cells/well in 6-well plates were inoculated with the freeze-thaw supernatant. Maximal GFP signal and syncytia were observed after 6 days, supernatant was clarified by centrifugation and 300µL of supernatant was passaged to fresh 1x10^6 Vero cells in 6-well plates. Within 24h, GFP signal and syncytia were observed. At 3 days, maximal GFP signal was observed and supernatant was passaged on fresh Vero cells (75cm2 flasks) for amplification of rCedPV-GFP. **Figure 2** shows rescue results with the pOLTV5 full-length rCedPV-GFP anti-genome plasmid. We also plan to increase the efficiency of CedPV rescue, and the pOLTV5-CedPV vector has been modified to express a 5' HDV ribozyme site anterior to the CedPV 3' leader sequence, which has been demonstrated to increase the rescue efficiency of paramyxoviruses by reverse genetics[10]. With this modified anti-genome two additional recombinant viruses will be made; a CedPV void of any reporter gene but retaining the introduced restriction site (rCedPV+6) which will be our wild-type CedPV, and luciferase encoding virus (rCedPV-Luc). **Figure 3** shows the planned viral genomes and the current GFP-encoding genome of rCedPV. The rCedPV-GFP virus has been amplified on Vero cells, and typically 8 T-75cm2 flasks, after 4 days in culture, the final viral stock, after purification and resuspension, had a titer of $1x10^8$ pfu/mL in ~3ml; and is stored aliquoted at-80ºC.

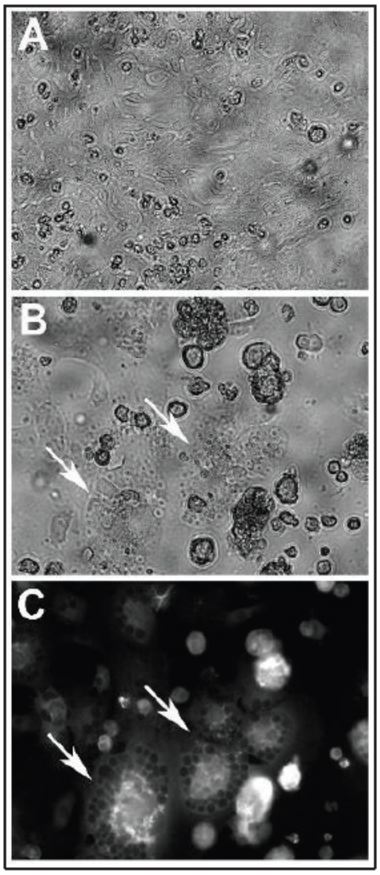


Figure 2. Rescued rCedPV-GFP from the plasmid transfected BSR-T7 cells at day 10. A: an unsuccessful well with no rescue, bright field. B: the successful rescue well showing syncytia, bright field. C: the same field as in B using fluorescence. White arrows are highlighting syncytia, the darker circles within the syncytia are nuclei.

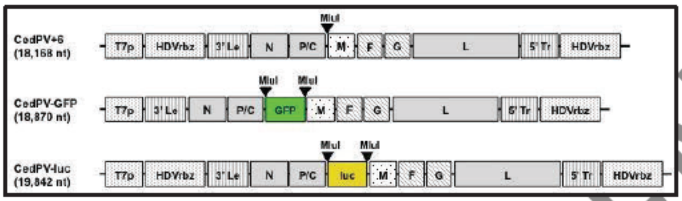
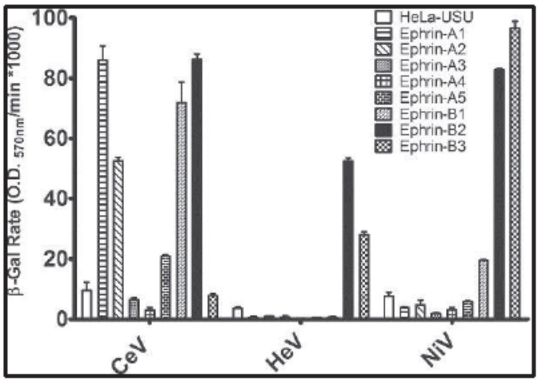


Figure 3. Anti-genome clones in-hand. All genomes adhere to the rule of 6. T7p: promtor; HDVrbz: hepatitis D virus ribozyme; Le: leader; Tr: trailer.

We have determined that CedPV exhibits a remarkably broader ephrin receptor usage profile, and cells bearing CedPV G and F can also mediate fusion with cells expressing ephrin-B1 and the glycosylphosphatidylinositol (GPI) anchored ephrin-A proteins; ephrin-A1, A2 and A5. Shown in **Figure 4** is the CedPV F and G cell fusion obtained with all B and A class ephrins. CedPV cell fusion also correlates with CedPV G glycoprotein binding to ephrins and this data has been confirmed by surface plasmon resonance experiments with soluble ephrins and CedPV soluble G. (C. Navaratnarajah, R. Cattaneo, and C. Broder, data not shown).

Figure 4. CedPV (CeV) glycoprotein-mediated fusion with a panel of A-type and B-type ephrin-expressing cells. Target cells were prepared by transfecting a panel of ephrin expression plasmids into HeLa USU cells infected with vCB21R(Laz-reporter vaccinia virus) and allowed to fuse with CedPV F+G expressing HeLa USU effector cells infected with vTF7.3 (T7-polymerase reporter vaccine virus. Fusion is quantitated as the rate of β-galactosidase activity[11]. CedPV mediated fusion with cells expressing ephrins-A1, -A2, -A5, -B1, and -B2.



Generation of rCedPV Chimeras: The Broder lab was the first to show the unique functional similarities between NiV and HeV G and F glycoproteins by demonstrating that they are the only paramyxoviruses that can bi-directionally functionally complement heterotypically[12] perhaps owing to their aa identity (~83% and 89% for G and F). Our new data with CedPV has provided a breakthrough in finally distinguishing between a functional vs. non-functional F-G interaction. The CedPV-F is only 42% and 43% identical with HeV-F and NiV-F respectively; and CedPV-G is 29% and 30% identical with HeV-G and NiV-G. Performing F and G coexpression and fusion assays using combinations of CedPV, HeV and NiV (**Figure 5**) has provided some very important observations. Ephrin-B2 and -B3 negative HeLa-USU cells are negative for CedPV fusion, as are ephrin-B3 bearing Hela-B3 cells. Fusion does occur with 293T and ephrin-B2 bearing HeLa-B2 cells. Thus, CedPV-F has heterotypic function with both HeV-G and NiV-G, but CedPV-G has heterotypic function with only HeV-F and not NiV-F and correlates with ephrin use. Of significance, these data support the hypothesis that we will be able to construct and generate novel rCedPV isolates using our reverse genetics system, whereby the F and G genes within the rCedPV genome maybe removed and replaced by with NiV F and G or HeV F and G. These new reporter gene encoding **authentic henipaviruses** that may be used under BSL-2 containment and they will be the ideal tools in establishing a robust SNT assay to assess virus neutralizing antibody responses in HeV-sG vaccinated subjects. Experiments on the generation and manipulation of the non-pathogenic rCedPVs, including the proposed development of novel chimeric viruses using the NiV and HeV F and G glycoproteins, have been approved by the Institutional Biosafety Committee (IBC). The chimeric viruses will in fact be loss-of-function recombinant viruses because of the reduced ephrin receptor use profiles between CedPV and either NiV or HeV.

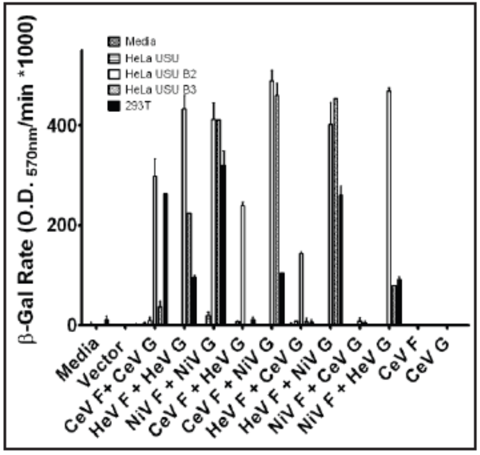


Figure 5. CedPV mediated cell fusion and heterotypic mixing with HeV and NiV. Various target cell populations are shown in the legend.

Characterization of the recombinant CedPV viruses: We expect the reporter gene encoding virus will not be hindered in any way based on the amplification results and titer of rCedPV-GFP, but we will analyze the replication kinetics of rCedPV-Luc and also compare those results to wild-type rCedPV (no reporter gene). Each virus will be used in experiments to determine receptor tropism and compared to the CedPV cell fusion assays using CedPV F and G expression as done with HeV and NiV[11-14].

Expected Results and Data Analysis: We are preparing the CedPV+6 and CedPV-Luc anti-genome plasmids and will proceed with the rescue of each of these new viruses. The rescue and amplification of the rCedPV+6 and the rCedPV-Luc viruses will be carried out as detailed above and recombinant virus stocks prepared. We expect that all recombinant viruses will have an infection profile identical to the cell-cell fusion assays using recombinant expressed F and G from CedPV, NiV and HeV. Because we have rescued the GFP-encoding CedPV, we do not anticipate any unexpected limitations in recusing the rCedPV chimeras encoding NiV or HeV F and G. Based on the replication results with rCedPV-GFP, we expect that large amounts of reporter virus stocks can be prepared, providing a consistent source of material for conducting the number of SNT assays expected to be carried out.

Deliverables and Go/No Go Criteria: The deliverables for this work segment will be FINAL Test and Lab Methods. Failure to characterize this assay would remove it from consideration as the best measure of immunity.

Optional Work Proposed Under the Product Development Strategy

It is the understanding of Profectus that the development gap CEPI envisions filling extends only to the point of establishing an investigational vaccine emergency use stockpile, and that studies directed toward licensure are viewed as out of scope. However, the collaborating partners propose that studies beyond those minimally needed to enable emergency use will enhance the likelihood that a pharmaceutical partner will step forward to license the vaccine and ensure stable supply. Therefore, we propose optional work to support licensure, as follows.

Task 13: Animal Rule Studies to Establish a Correlate of Protection Against Lethal NiV and HeV Challenge: These studies will seek to establish an immune response in the AGM, the most relevant model of human NiV and HeV disease. The exact design of these studies will be decided in consultation with the US FDA, however, the following design has been used to successfully determine a preliminary correlate of immunity for Marburg virus. In brief, Groups of 10 AGMs will be administered a single IM dose of various dose levels of HeV-sG/Alhydrogel™, ranging from a high of 100.0 µg to a low of 1.0 µg **(Table 13)**. A control group of 10 AGMs will receive saline control. On day 28 all AGMs will be bled to obtain samples of sera, and on day 36 following immunization they will be administered a lethal challenge of NiV. This study is designed to induce serologic responses that span the range from non-protective through protective. By determining the ELISA, neutralization, and fine-specificity responses outlined above, it will be possible to determine which immune response(s) and the level(s) that correlate with protection from challenge. An identical study using HeV lethal challenge will be conducted to establish the immunologic correlate(s) of protection against this virus. Once the most useful correlate of protection is established it will be possible to design assays that bridge/normalize the AGM protective response to the same response in human recipients of the HeV-sG vaccine.

Table 13. HeV-sG/Alhydrogel™ Dose-down Study in AGMs

Group	N	Vaccine Dose	Vaccination	Bleed	Challenge
1	10	100.0 µg HeV-sG/Alhydrogel™	Day 0	Day 28	Day 36
2	10	33.3 µg HeV-sG/Alhydrogel™			
3	10	10.0 µg HeV-sG/Alhydrogel™			
4	10	3.3 µg HeV-sG/Alhydrogel™			
5	10	1.0 µg HeV-sG/Alhydrogel™			
6	10	Control (saline)			
Total	60				
Notes: All immunizations delivered IM in the deltoid as a single 1.0mL injection.					

Task 14: Phase 1/NHP CoP Sample Analysis: It is anticipated that the CedPV-based neutralization assay developed under **Task 12** will provide the most appropriate measure of the protective immune response against NiV and HeV. To establish this, it is proposed the Broder laboratory uses this assay to quantify the neutralizing antibody responses in subjects vaccinated in the phase 1 dose escalation study conducted under **Task 4**, and the NHP responses in the dose-down CoP study conducted under **Task 13**. In this way it will be possible to bridge the human neutralizing antibody response to the protective response in NHP, and establish a human CoP.

Deliverables and Go/No Go Criteria: The deliverable for this work segment will be a final Study Report documenting the the human CoP against NiV and HeV morbidity and mortality.

Task 15: Production and Release of Consistency Lots of DS and DP: Vaccine licensure, whether using the standard or animal rule route to licensure, requires the demonstration that the vaccine product can be consistently manufactured to the established release specifications, and that various Lots of vaccine induce an equivalent immune response in the target population. Generally, three consecutive Lots of DP produced using the final process, at or near final scale, with at least two Lots produced in the final production facility, are judged necessary. Thus, it is proposed that successful completion and CEPI review of the phase 1 clinical trials trigger the manufacture and release of two additional consecutive consistency Lots of HeV-sG/Alhydrogel™ DP.

Deliverables and Go/No Go Criteria: The deliverables for this work segment will be two additional Lots of HeV-sG/Alhydrogel™ vaccine produced under full GMP compliance and released for clinical use. Failure of the vaccine to pass release testing would result in an inability to proceed to the consistency Lots trial under **Task 16**.

Task 16: Phase 2 Consistency Lot Trial: The demonstration of Lot-to-Lot immunogenic consistency generally requires testing of three consecutive Lots, produced as described above, each be tested in approximately 400 subjects (300 active/ 100 placebo). Therefore, a Lot consistency trial in 800 subjects (600 active/ 200 placebo) with equal distribution of the two-additional consistency Lots would be performed by PATH. Ideally, these studies would be conducted in areas of NiV and HeV endemicity, to evaluate immunogenicity in the population(s) most likely to require an outbreak response.

Deliverables and Go/No Go Criteria: The deliverables will be interim and final clinical trials reports demonstrating that the HeV-sG/Alhydrogel™vaccine is safe, well tolerated, and provides Lot-to-Lot immunogenic consistency. This will support the use of this vaccine with emergency use authorization and support licensure under the "animal rule". Any results which suggest the vaccine is not safe, well tolerated or consistently immunogenic will trigger a review to determine if it is judged prudent to continue development of this vaccine.

Task 17: Clinical Sample Analysis – Consistency Lots Trial: In support of the consistency Lots trial of the HeV-sG/Alhydrogel™ vaccine, Profectus will perform validated assays to measure immune responses to NiV and HeV in vaccine recipients. The assays will measure serum IgG antibody by direct ELISA, and serum neutralizing antibody by plaque reduction neutralization of GFP-expressing rVSV pseudo-typed with the relevant GPs. It is anticipated that the CedPV-based neutralization assay developed under **Task 12** will be available for use, successfully validated, and used in support of this trial.

Deliverables and Go/No Go Criteria: The deliverables for this work segment will be interim and final clinical trial assay reports.

4.3. Endpoints

The endpoints for the proposed work are provided as the deliverables for each of the **17 Tasks** detailed in **Section 4.2 Objectives** (above).

4.4. Non/pre-clinical product development approach

Key immunogenicity/efficacy data in any eventual animal model used

Establish an Immunologic CoP in a Relevant Animal Model and Bridging the Correlate to the Human Response. Optional Work Proposed Under the Product Development Strategy in **Section 4.2** (above) outlines the tasks required to establish a human CoP for licensure under the "animal rule"

Dose-ranging/rationale for doses for clinical testing .

The vaccine doses and adjuvant formulation selected for phase 1 clinical testing are based upon the pre-clinical animal studies outlined in **Section 3.3 Pre-clinical evidence to date** (above).

4.4.1. Assay development

The development and characterization of the ELISA and Neutralization assays proposed under this application are described under **Tasks 2, 7, and 12** in **Section 4.2 Objectives** (above).

4.4.2. Toxicology

GLP Tox Study. A 4-cycle (1 dose every 3 weeks) toxicity study in rabbits administered HeV-sG/Alum by intramuscular injection initiated October 2016 at CRL. Three groups of 20 New Zealand White Rabbits, aged from 3 to 5 months and from 2.0 to 4.0 kg each are being used in the study. The control group was dosed with saline, the low dose group was injected with the maximum intended human dose, and the high dose group was injected with 3X the maximum intended human dose. The study design is presented in **Table 14** below:

Table 14. A 4-cycle Study in Rabbits Administered HeV-sG/Alhydrogel™ by Intramuscular Injection

Group No.	Test Item[a]	Dose Level (mg/day)	Dose Concentration (mg/mL)	Dose Volume (mL)	No. of Animals			
					Main Study[b]		Recovery Study[c]	
					Males	Females	Males	Females
1	Saline Control	0	0	3.0	5	5	5	5
2	HeV-sG-V	0.1	0.1	1.0	5	5	5	5
3	HeV-sG-V	0.3	0.1	3.0	5	5	5	5

[a] Dosing will be performed once every three weeks (= 1 cycle) for 4 cycles

[b] Main study animals will undergo necropsy 3 days following the last dose administration.

[c] Recovery study animals will undergo necropsy 28 days following the last dose administration.

CRL is an accredited CMO for carrying out GLP toxicity studies and we do not expect procedural complications with the study. Antibody titers from blood samples will be determined under well documented practices at Profectus.

4.5. Clinical Development Plan CDP

The Clinical Development Plan (CDP) for this vaccine includes a “first-in-human” safety trials in adults (18-50 years old), a phase 2 dose ranging study in adults, and a phase 1/2 study in children (3-17 years old). In the event of an outbreak after the completion of the phase 2 studies, additional pharmacovigilance and an effectiveness trial would be proposed. Additional studies to examine production lot consistency, duration of protection, and safety in older persons may be necessary. The regulatory strategy for the vaccine will be based on successful application of the “Animal Rule” requirements, namely the identification of a clear immune correlate measure of protection in appropriate animal models and identifying a dose of vaccine in humans that induces a “protective” level of immunity. Successful identification of an appropriate dose and regimen would support the stockpiling of the vaccine and potential emergency use. Additional safety and effectiveness studies may need to be conducted with the stockpile material depending on the observations of the phase 1/2 studies.

Clinical objectives

Describe the clinical objectives of the project by including quantifiable criteria (e.g., “go/no-go” decision criteria), goals and time-lines for the program. Please link/match these aspects with the milestone plan

The baseline approach we are proposing to CEPI includes four clinical trials, described briefly below. The objectives, endpoints, rationale, design, and other aspects of these trials is described in more detail in the abbreviated protocol synopses included at the bottom of this section.

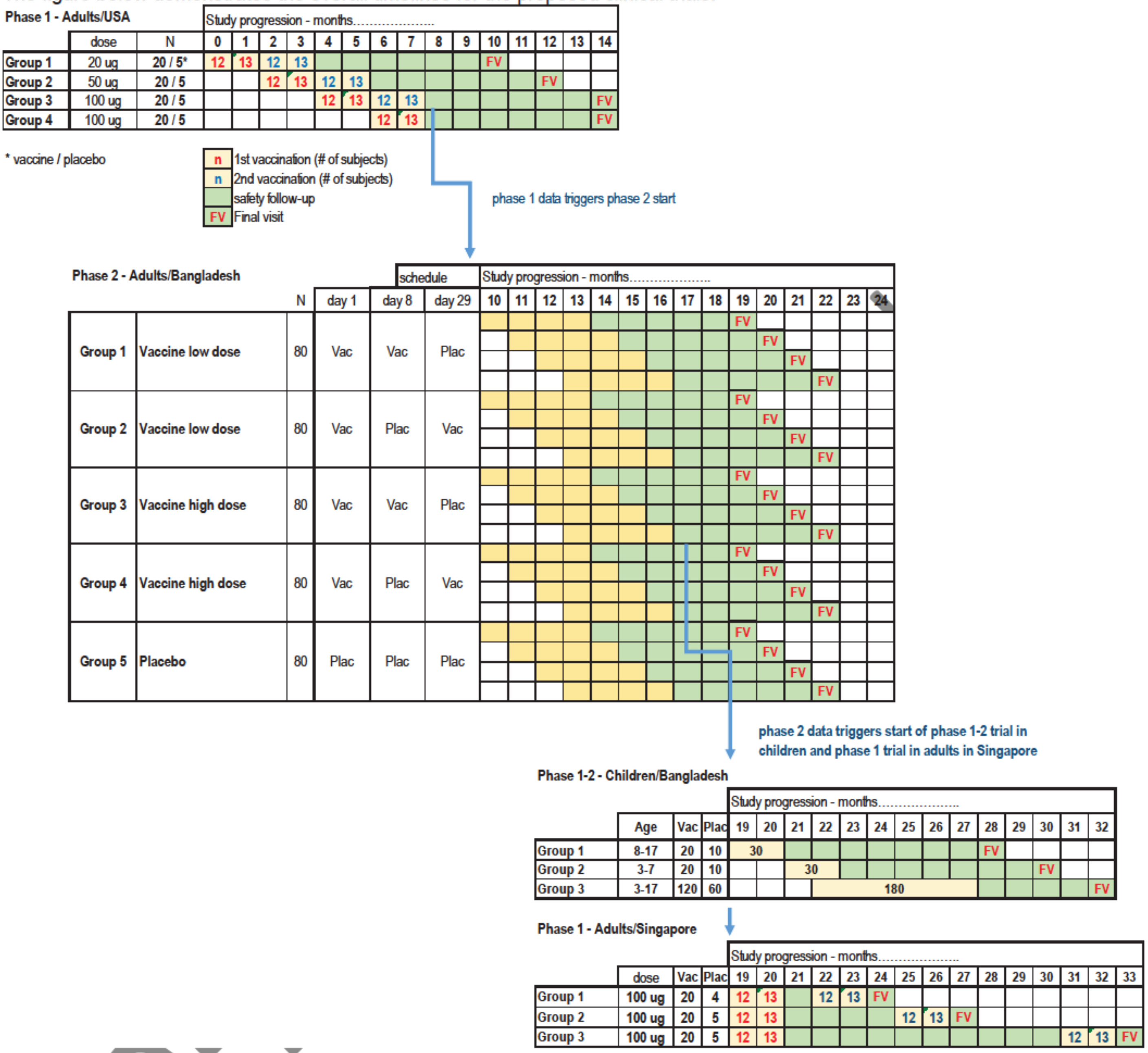
A Phase 1 randomized, placebo-controlled, double blind study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in healthy US adults. The first study of the vaccine will evaluate the vaccine’s safety in a limited number of 18-50 years old subjects under a typical double-blinded randomized, placebo-control dose-escalation design. The study will be conducted at an experienced clinical research unit in the US, under an IND to be sponsored by Profectus and will focus on closely investigating post-vaccination safety parameters, including solicited local (pain, tenderness, erythema, induration) and systemic (fever, malaise/fatigue, myalgia, arthralgia, headache, nausea, vomiting) and systemic reactogenicity, vaccine-related adverse experiences (AEs) and serious AEs (SAEs). The trial will be conducted in a dose escalation design in which the subjects will receive two doses of 20ug (group 1), 50ug (group 2), or 100 ug (group 3) of vaccine 28 days apart. Immunogenicity will be tested at baseline, 3 weeks after each vaccination and at a late time point (90 days post final vaccination). An additional cohort of 25 subjects (20 vaccinees, 5 placebos) will receive a single dose of 100 ug of vaccine (group 4). This first phase 1 study will support the conduct of a phase 2 study to expand the safety database and refine the selection of dose/schedule, providing that the vaccine is safe and tolerable and that at least one of the groups under test shows an immune response rate of 50% on the assay identified as potential “Correlate of Protection” in the preclinical studies.

A Phase 2 randomized, placebo-controlled, double blind, dose ranging study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in Bangladeshi adults. If the immunogenicity bar set up for the phase 1 trial is met, the two highest tolerated doses observed in that study (noted as “low” and “high” hereon) will be tested at two different time intervals between the first and second vaccination, 7 days and 28 days. The optimal dose/schedule will be selected from this study. Five groups of healthy 18-50 year old adults will receive: Group 1: 2 injections of “low dose” vaccine on days 1 and 8, and placebo on day 28; Group 2: 2 injections of “low dose” vaccine on days 1 and 28 and placebo on day 8; Group 3: 2 injections of “high dose” vaccine on days 1 and 8 and placebo on day 28; Group 4: 2 injections of “high dose” vaccine on days 1 and 22 and placebo on day 28; Group 5: placebo on days 1, 8 and 28. The study will be conducted in 2-3 clinical sites in, Bangladesh. Statistical analysis to select the optimal dose/schedule will be conducted by a “Selection Design” approach.

A Phase 1 randomized, placebo-controlled, double-blind dose interval study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in Singaporean adults. The safety and immunogenicity of the vaccine will also be assessed in a limited number of 18-45 year old subjects in Singapore in a study leading to identify the optimal interval between the priming dose of vaccine and a second, “booster” dose. The highest tolerated vaccine dose identified in the phase 1 will be used. The study will be conducted as a double-blinded randomized, placebo-control design. The general schema of the study is shown below. In summary, the trial will randomize subjects in three groups to receive: group (1) vaccine on days 1 and 91; group (2) vaccine on days 1 and 181; group (3) vaccine on days 1 and 366. Immunological testing will be conducted 28 after the day 1 vaccination, on the day of the second vaccination and 5 and 28 days later.

A Phase 1-2 randomized, placebo-controlled, double blind study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in healthy 3-17 year old children in Bangladesh. Once safety data are available from the phase 1 and phase 2 studies in adults and the vaccine is shown to be safe and immunogenic, a phase 1-2 clinical trial will be conducted in younger populations (ages 3-17, inclusive) to support delivery of the vaccine to individuals of that age range in the event of an outbreak. The study will start out as a descending age trial in which initially children 8-17 years old will receive two doses of vaccine at the dose level and schedule selected from the studies in adults, or placebo (group 1), followed for a cohort (group 2) of 3-7 year old children to begin enrolling when and if the safety in the group 1 cohort is demonstrated. Following demonstration of safety in group 2, 60 additional children 3-17 years old will be vaccinated along with 30 placebo recipients randomized at the same time. The study will be conducted at an experienced clinical research unit in a country where Nipah outbreaks have occurred (e.g., Bangladesh) and will focus on closely investigating post-vaccination safety parameters, including solicited reactogenicity (typical local and systemic reactions to parenteral vaccines), vaccine-related adverse experiences (AEs) and serious AEs (SAEs).

The figure below demonstrates the overall timelines for the proposed clinical trials.



Phase 1 - Adults/USA

	dose	N	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group 1	20 ug	20 / 5*	12	13	12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV				
Group 2	50 ug	20 / 5			12	13	12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV		
Group 3	100 ug	20 / 5					12	13	12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV
Group 4	100 ug	20 / 5							12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV

Study progression - months....................

* vaccine / placebo

n 1st vaccination (# of subjects)
n 2nd vaccination (# of subjects)
🟩 safety follow-up
FV Final visit

phase 1 data triggers phase 2 start

Phase 2 - Adults/Bangladesh

		N	day 1	day 8	day 29	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
			schedule			Study progression - months....................														
Group 1	Vaccine low dose	80	Vac	Vac	Plac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 2	Vaccine low dose	80	Vac	Plac	Vac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 3	Vaccine high dose	80	Vac	Vac	Plac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 4	Vaccine high dose	80	Vac	Plac	Vac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 5	Placebo	80	Plac	Plac	Plac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		

phase 2 data triggers start of phase 1-2 trial in children and phase 1 trial in adults in Singapore

Phase 1-2 - Children/Bangladesh

	Age	Vac	Plac	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Group 1	8-17	20	10	30		🟩	🟩	🟩	🟩	🟩	🟩	🟩	FV				
Group 2	3-7	20	10			30		🟩	🟩	🟩	🟩	🟩	🟩	🟩	FV		
Group 3	3-17	120	60				180						🟩	🟩	🟩	🟩	FV

Study progression - months....................

Phase 1 - Adults/Singapore

	dose	Vac	Plac	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Group 1	100 ug	20	4	12	13	🟩	12	13	FV									
Group 2	100 ug	20	5	12	13	🟩	🟩	🟩	🟩	12	13	FV						
Group 3	100 ug	20	5	12	13	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	12	13	FV

Study progression - months....................

Clinical trial planning

Phase 1 (adults): The phase 1 study will be conducted in the US, under FDA IND. It will include 100 healthy US adults (18-50 years old, inclusive, at time of first vaccination).

Phase 2 (adults): The phase 2 study is planned to be conducted in an area where outbreaks of Nipah virus have occurred (e.g., Bangladesh). The study will include 400 healthy adults (18-50 years old, inclusive, at time of first vaccination). The study will be conducted under FDA IND, and will have the approval of the local regulatory authority. We will utilize 2-3 sites to ensure sufficient availability of study subjects.

Phase 1/2 (children): The Phase 1/2 study in children is planned to be conducted in Bangladesh. The study will be conducted under FDA IND, and will have the approval of the local regulatory authority.

Clinical trial execution

Please describe the intended strategy and rationale for clinical trial sites, and possible establishment of clinical trial sites; planning for clinical trial execution.

Phase 1 (adults): Given that HeV-sG has never been tested in humans it will be important to select centers with high level of experience and associated with top medical expertise, diagnostic, and treatment facilities. The phase 1 study will be conducted in the US, under FDA IND, at one of the academic sites sponsored by the NIH (DMID Vaccine Evaluation Units) and with which PATH has long-standing collaboration (e.g., U. Maryland Center for Vaccine Development, Children's Hospital Medical Center, Cincinnati) or at Johns Hopkins University Center for Immunization Research. PATH has successfully conducted two to four phase 1 or phase 2 clinical trials at those sites, including in patient studies (for live vaccine and challenge studies). In most cases PATH sponsored the regulatory submissions.

The Phase 1 study in Singapore will be conducted at the Duke-NUS Medical School, a center with proven experience in the conduct of clinical trials, under the direction of Prof. Linfa Wang.

Phase 2 (adults): The phase 2 study is planned to be conducted in areas in which Nipah virus outbreaks have occurred, most likely in Bangladesh or in India. PATH has conducted several phase 1-2 trials in Bangladesh for childhood diarrhea (live shigella, killed ETEC, live rotavirus) and JEV vaccines, and recently a phase 3 rotavirus vaccine trial in collaboration of the BMGF's PROVIDE consortium. Alternatively, the studies could be conducted in India, where PATH has conducted multi-site efficacy trials of rotavirus vaccines leading to the licensure of two products. Although the regulatory sponsorship for the trials in India and Bangladesh was the responsibility of the local partners (Serum Institute of India and Bharat manufacturers in India and the icddrb (International Center for Diarrheal Diseases Research in Bangladesh) PATH was directly involved in implementation and monitoring of the studies, as well as in the preparation of the regulatory submission (IND-equivalent, CSRs, BLAs and manuscripts).

The phase 2 will require the engagement of a minimum of two sites in Bangladesh, or three sites If the study were to be implemented in India. SOPs and site/CRO selection tools are available at PATH for final evaluation and selection of the appropriate clinical trial sites for the phase 2 study. Preselected clinical sites will be audited unless they are currently participating in PATH studies, in which case documentation is available about site performance and quality.

PATH will prepare all trial-specific documents and will use its SOPs for the implementation of the study. Selected sites and CROs (for data management, randomization, monitoring and statistical support, immunological laboratories) will be directly contracted by PATH, who will prepare the sites for the trial and monitor day to day activities, including auditing of facilities, data and procedures.

Phase 1/2 (children): The Phase 1 study in children will be conducted at an experienced clinical research unit in Bangladesh. Most of the vaccine trials that PATH has conducted in India and Bangladesh (with minor exceptions) have included children (6 weeks to 10 years old), including testing of a live shigella vaccine, inactivated oral ETEC, several live rotaviruses and a JEV live virus vaccine candidate. The conduct of these studies has been impeccable (from the standpoint of GCP and protocol adherence) and has led to important steps on the qualification of those vaccines (e.g., the JEV Chengdu vaccine trial in Bangladesh was essential for WHO prequalification). Working closely with the leadership of icddrb, PATH has been able to facilitate the efficient review of the studies within the center as well as with the Bangladesh MOH.

Site selection and trial implementation will follow the PATH SOPs referenced above. If possible, PATH will seek to use one of the sites selected to conduct the Phase 2 study.

Target study populations

An outbreak of Nipah virus infection will be a major public health emergency that would likely affect individuals of all ages, primarily active adults and school age older toddlers and children, reason why we propose to conduct the initial phase 1 and phase 2 testing of the HeV-sG in those populations. The participants will be selected among healthy individuals, to provide for an optimal evaluation of its potential, and diminish the chance of observing SAEs or severe AEs due to underlying disease.

For the Phase 1 and 2 components of the CDP that will be supported by CEPI, we will limit the studies to healthy adults and children 3-17 years of age. These studies will provide a safety database potentially sufficient to deploy vaccine under the "Animal Rule," providing that the immunogenicity results support that the vaccine is capable of inducing an immune response qualitatively and quantitatively similar to that observed to prevent infection from challenge in the animal models. Additional clinical studies that we are considering, beyond the completion of this grant, include a "lot consistency" study to test the equivalence of the immunogenicity of three different lots of the vaccine.

If and when HeV-sG is submitted and approved under the "Animal Rule," and if an outbreak of Nipah virus occurs, we will conduct a pharmacoivigilance / effectiveness studies to more directly evaluate the vaccine. The use of the vaccines in the elderly, pregnant women, and individuals with underlying disease will be fostered by post-licensure studies (post-deployment, in case of emergency use).

Candidate vaccine control strategy

The Clinical Development Plan (CDP) for this vaccine includes a "first-in-human" safety trials in adults (18-50 years old), a phase 2 dose ranging study in adults, and a phase 1/2 study in children (3-17 years old). In the event of an outbreak after the completion of the phase 2 studies, additional pharmacovigilance and an effectiveness trial would be proposed. Additional studies to examine production lot consistency, duration of protection, and safety in older persons may be necessary. The regulatory strategy for the vaccine will be based on successful application of the "Animal Rule" requirements, namely the identification of a clear immune correlate measure of protection in appropriate animal models and identifying a dose of vaccine in humans that induces a "protective" level of immunity. Successful identification of an appropriate dose and regimen would support the stockpiling of the vaccine and potential emergency use. Additional safety and effectiveness studies may need to be conducted with the stockpile material depending on the observations of the phase 1/2 studies.

Clinical objectives

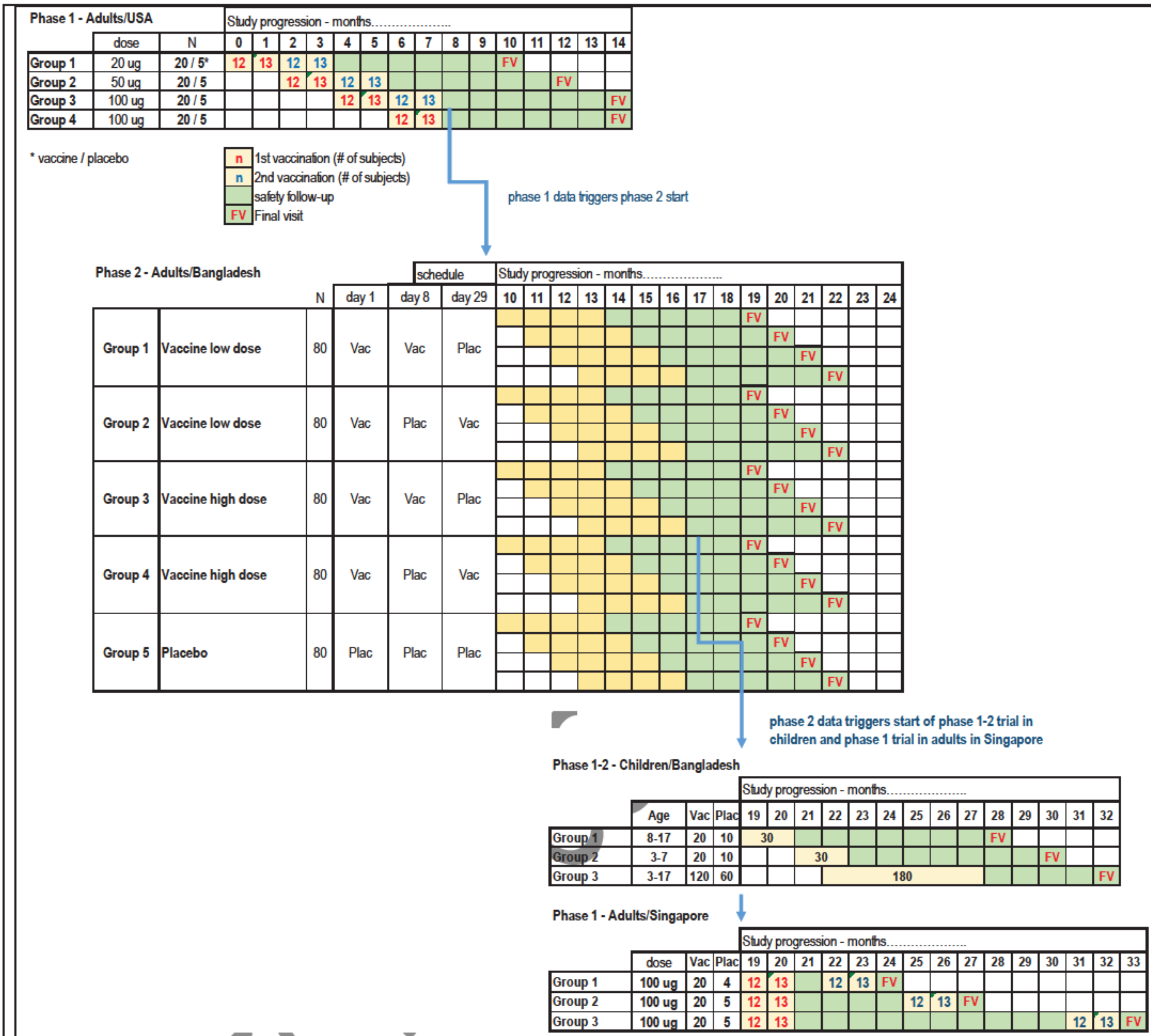
The baseline approach we are proposing to CEPI includes four clinical trials, described briefly below. The objectives, endpoints, rationale, design, and other aspects of these trials is described in more detail in the abbreviated protocol synopses included at the bottom of this section.

A Phase 1 randomized, placebo-controlled, double blind study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in healthy US adults. The first study of the vaccine will evaluate the vaccine's safety in a limited number of 18-50 years old subjects under a typical double-blinded randomized, placebo-control dose-escalation design. The study will be conducted at an experienced clinical research unit in the US, under an IND to be sponsored by Profectus and will focus on closely investigating post-vaccination safety parameters, including solicited local (pain, tenderness, erythema, induration) and systemic (fever, malaise/fatigue, myalgia, arthralgia, headache, nausea, vomiting) and systemic reactogenicity, vaccine-related adverse experiences (AEs) and serious AEs (SAEs). The trial will be conducted in a dose escalation design in which the subjects will receive two doses of 20ug (group 1), 50ug (group 2), or 100 ug (group 3) of vaccine 28 days apart. Immunogenicity will be tested at baseline, 3 weeks after each vaccination and at a late time point (90 days post final vaccination). An additional cohort of 25 subjects (20 vaccinees, 5 placebos) will receive a single dose of 100 ug of vaccine (group 4). This first phase 1 study will support the conduct of a phase 2 study to expand the safety database and refine the selection of dose/schedule, providing that the vaccine is safe and tolerable and that at least one of the groups under test shows an immune response rate of 50% on the assay identified as potential "Correlate of Protection" in the preclinical studies.

A Phase 2 randomized, placebo-controlled, double blind, dose ranging study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in Bangladeshi adults. If the immunogenicity bar set up for the phase 1 trial is met, the two highest tolerated doses observed in that study (noted as "low" and "high" hereon) will be tested at two different time intervals between the first and second vaccination, 7 days and 28 days. The optimal dose/schedule will be selected from this study. Five groups of healthy 18-50 year old adults will receive: Group 1: 2 injections of "low dose" vaccine on days 1 and 8, and placebo on day 28; Group 2: 2 injections of "low dose" vaccine on days 1 and 28 and placebo on day 8; Group 3: 2 injections of "high dose" vaccine on days 1 and 8 and placebo on day 28; Group 4: 2 injections of "high dose" vaccine on days 1 and 22 and placebo on day 28; Group 5: placebo on days 1, 8 and 28. The study will be conducted in 2-3 clinical sites in, Bangladesh. Statistical analysis to select the optimal dose/schedule will be conducted by a "Selection Design" approach.

A Phase 1 randomized, placebo-controlled, double-blind dose interval study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in Singaporean adults. The safety and immunogenicity of the vaccine will also be assessed in a limited number of 18-45 year old subjects in Singapore in a study leading to identify the optimal interval between the priming dose of vaccine and a second, "booster" dose. The highest tolerated vaccine dose identified in the phase 1 will be used. The study will be conducted as a double-blinded randomized, placebo-control design. The general schema of the study is shown below. In summary, the trial will randomize subjects in three groups to receive: group (1) vaccine on days 1 and 91; group (2) vaccine on days 1 and 181; group (3) vaccine on days 1 and 366. Immunological testing will be conducted 28 after the day 1 vaccination, on the day of the second vaccination and 5 and 28 days later.

A Phase 1-2 randomized, placebo-controlled, double blind study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in healthy 3-17 year old children in Bangladesh. Once safety data are available from the phase 1 and phase 2 studies in adults and the vaccine is shown to be safe and immunogenic, a phase 1-2 clinical trial will be conducted in younger populations (ages 3-17, inclusive) to support delivery of the vaccine to individuals of that age range in the event of an outbreak. The study will start out as a descending age trial in which initially children 8-17 years old will receive two doses of vaccine at the dose level and schedule selected from the studies in adults, or placebo (group 1), followed for a cohort (group 2) of 3-7 year old children to begin enrolling when and if the safety in the group 1 cohort is demonstrated. Following demonstration of safety in group 2, 60 additional children 3-17 years old will be vaccinated along with 30 placebo recipients randomized at the same time. The study will be conducted at an experienced clinical research unit in a country where Nipah outbreaks have occurred (e.g., Bangladesh) and will focus on closely investigating post-vaccination safety parameters, including solicited reactogenicity (typical local and systemic reactions to parenteral vaccines), vaccine-related adverse experiences (AEs) and serious AEs (SAEs). The figure below demonstrates the overall timelines for the proposed clinical trials.



Phase 1 - Adults/USA

	dose	N	Study progression - months……………….														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Group 1	20 ug	20 / 5*	12	13	12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV				
Group 2	50 ug	20 / 5			12	13	12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV		
Group 3	100 ug	20 / 5					12	13	12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV
Group 4	100 ug	20 / 5							12	13	🟩	🟩	🟩	🟩	🟩	🟩	FV

* vaccine / placebo

n	1st vaccination (# of subjects)
n	2nd vaccination (# of subjects)
🟩	safety follow-up
FV	Final visit

phase 1 data triggers phase 2 start

Phase 2 - Adults/Bangladesh

			schedule			Study progression - months……………….														
		N	day 1	day 8	day 29	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Group 1	Vaccine low dose	80	Vac	Vac	Plac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 2	Vaccine low dose	80	Vac	Plac	Vac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 3	Vaccine high dose	80	Vac	Vac	Plac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 4	Vaccine high dose	80	Vac	Plac	Vac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		
Group 5	Placebo	80	Plac	Plac	Plac	🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV					
							🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV				
								🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV			
									🟨	🟨	🟨	🟨	🟩	🟩	🟩	🟩	🟩	FV		

phase 2 data triggers start of phase 1-2 trial in children and phase 1 trial in adults in Singapore

Phase 1-2 - Children/Bangladesh

	Age	Vac	Plac	Study progression - months……………….													
				19	20	21	22	23	24	25	26	27	28	29	30	31	32
Group 1	8-17	20	10	30		🟩	🟩	🟩	🟩	🟩	🟩	🟩	FV				
Group 2	3-7	20	10			30		🟩	🟩	🟩	🟩	🟩	🟩	🟩	FV		
Group 3	3-17	120	60				180						🟩	🟩	🟩	🟩	FV

Phase 1 - Adults/Singapore

	dose	Vac	Plac	Study progression - months……………….														
				19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Group 1	100 ug	20	4	12	13	🟩	12	13	FV									
Group 2	100 ug	20	5	12	13	🟩	🟩	🟩	🟩	12	13	FV						
Group 3	100 ug	20	5	12	13	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	🟩	12	13	FV

Clinical trial planning

Describe the clinical trials planned for safety, immunogenicity and eventually efficacy, including assumptions related to intended location of studies, regulatory approval, target populations, and availability of study subjects.

Phase 1 (adults): The phase 1 study will be conducted in the US, under FDA IND. It will include 100 healthy US adults (18-50 years old, inclusive, at time of first vaccination).

Phase 2 (adults): The phase 2 study is planned to be conducted in an area where outbreaks of Nipah virus have occurred (e.g., Bangladesh). The study will include 400 healthy adults (18-50 years old, inclusive, at time of first vaccination). The study will be conducted under FDA IND, and will have the approval of the local regulatory authority. We will utilize 2-3 sites to ensure sufficient availability of study subjects.

Phase 1/2 (children): The Phase 1/2 study in children is planned to be conducted in Bangladesh. The study will be conducted under FDA IND, and will have the approval of the local regulatory authority.

Clinical trial execution

Please describe the intended strategy and rationale for clinical trial sites, and possible establishment of clinical trial sites; planning for clinical trial execution.

Phase 1 (adults): Given that HeV-sG has never been tested in humans it will be important to select centers with high level of experience and associated with top medical expertise, diagnostic, and treatment facilities. The phase 1 study will be conducted in the US, under FDA IND, at one of the academic sites sponsored by the NIH (DMID Vaccine Evaluation Units) and with which PATH has long-standing collaboration (e.g., U. Maryland Center for Vaccine Development, Children's Hospital Medical Center, Cincinnati) or at Johns Hopkins University Center for Immunization Research. PATH has successfully conducted two to four phase 1 or phase 2 clinical trials at those sites, including in patient studies (for live vaccine and challenge studies). In most cases PATH sponsored the regulatory submissions.

The Phase 1 study in Singapore will be conducted at the Duke-NUS Medical School, a center with proven experience in the conduct of clinical trials, under the direction of Prof. Linfa Wang.

Phase 2 (adults): The phase 2 study is planned to be conducted in areas in which Nipah virus outbreaks have occurred, most likely in Bangladesh or in India. PATH has conducted several phase 1-2 trials in Bangladesh for childhood diarrhea (live shigella, killed ETEC, live rotavirus) and JEV vaccines, and recently a phase 3 rotavirus vaccine trial in collaboration of the BMGF's PROVIDE consortium. Alternatively, the studies could be conducted in India, where PATH has conducted multi-site efficacy trials of rotavirus vaccines leading to the licensure of two products. Although the regulatory sponsorship for the trials in India and Bangladesh was the responsibility of the local partners (Serum Institute of India and Bharat manufacturers in India and the icddrb (International Center for Diarrheal Diseases Research in Bangladesh) PATH was directly involved in implementation and monitoring of the studies, as well as in the preparation of the regulatory submission (IND-equivalent, CSRs, BLAs and manuscripts).

The phase 2 will require the engagement of a minimum of two sites in Bangladesh, or three sites If the study were to be implemented in India. SOPs and site/CRO selection tools are available at PATH for final evaluation and selection of the appropriate clinical trial sites for the phase 2 study. Preselected clinical sites will be audited unless they are currently participating in PATH studies, in which case documentation is available about site performance and quality.

PATH will prepare all trial-specific documents and will use its SOPs for the implementation of the study. Selected sites and CROs (for data management, randomization, monitoring and statistical support, immunological laboratories) will be directly contracted by PATH, who will prepare the sites for the trial and monitor day to day activities, including auditing of facilities, data and procedures.

Phase 1/2 (children): The Phase 1 study in children will be conducted at an experienced clinical research unit in Bangladesh. Most of the vaccine trials that PATH has conducted in India and Bangladesh (with minor exceptions) have included children (6 weeks to 10 years old), including testing of a live shigella vaccine, inactivated oral ETEC, several live rotaviruses and a JEV live virus vaccine candidate. The conduct of these studies has been impeccable (from the standpoint of GCP and protocol adherence) and has led to important steps on the qualification of those vaccines (e.g., the JEV Chengdu vaccine trial in Bangladesh was essential for WHO prequalification). Working closely with the leadership of icddrb, PATH has been able to facilitate the efficient review of the studies within the center as well as with the Bangladesh MOH.

Site selection and trial implementation will follow the PATH SOPs referenced above. If possible, PATH will seek to use one of the sites selected to conduct the Phase 2 study.

Target study populations

Provide a high level summary of the targeted study population and include if and when you expect to have data on

- *Paediatric and elderly populations*
- *Pregnant/lactating women*
- *Populations with underlying diseases*

An outbreak of Nipah virus infection will be a major public health emergency that would likely affect individuals of all ages, primarily active adults and school age older toddlers and children, reason why we propose to conduct the initial phase 1 and phase 2 testing of the HeV-sG in those populations. The participants will be selected among healthy individuals, to provide for an optimal evaluation of its potential, and diminish the chance of observing SAEs or severe AEs due to underlying disease.

For the Phase 1 and 2 components of the CDP that will be supported by CEPI, we will limit the studies to healthy adults and children 3-17 years of age. These studies will provide a safety database potentially sufficient to deploy vaccine under the "Animal Rule," providing that the immunogenicity results support that the vaccine is capable of inducing an immune response qualitatively and quantitatively similar to that observed to prevent infection from challenge in the animal models. Additional clinical studies that we are considering, beyond the completion of this grant, include a "lot consistency" study to test the equivalence of the immunogenicity of three different lots of the vaccine.

If and when HeV-sG is submitted and approved under the "Animal Rule," and if an outbreak of Nipah virus occurs, we will conduct a pharmacoivigilance / effectiveness studies to more directly evaluate the vaccine. The use of the vaccines in the elderly, pregnant women, and individuals with underlying disease will be fostered by post-licensure studies (post-deployment, in case of emergency use).

Abbreviated protocol synopses

The proposal to CEPI includes four clinical trials, described in the protocol synopses below:

- A Phase 1 randomized, placebo-controlled, double blind study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in healthy US adults.

STUDY HYPOTHESES **Safety**: The HeV-sG vaccine will be well-tolerated and with an acceptable safety profile **Immunogenicity**: The HeV-sG vaccine is immunogenic and will induce neutralizing immune responses in 50% or more of participants in at least one of the study groups.
STUDY OBJECTIVES **Primary Objectives** **Safety**: to evaluate the safety and tolerability of the HeV-sG vaccine at three escalating dose levels in healthy adults

Immunogenicity: to evaluate the immunogenicity of two doses of the He-VsG vaccine at three different dose levels in healthy adults

Secondary Objectives

Safety: to evaluate the longer term safety (through 6 months after the last vaccination) of the HeV-sG vaccine at escalating dose levels.

Immunogenicity: to evaluate the immunogenicity of two doses of the HeV-sG vaccine six months after receipt of the last vaccination.

Exploratory Objectives

Immunogenicity: to develop additional immune assays to evaluate responses to the HeV-sG vaccine

STUDY ENDPOINTS

Primary Endpoints

Safety: 1) number of SAEs through 21 days after the last study injection; 2) number of AEs through 21 days after the last study injection; 3) number of Vaccine-induced local and systemic reactions (solicited reactions) during the 7 days after administration of each dose

Immunogenicity: 1) proportion of subjects with anti-HeV-sG seroresponses by ELISA (four-fold increase in antibody titers between baseline and 1-week and baseline and 3-weeks post-first study injection); 2) proportion of subjects with neutralizing antibody responses (4-fold increase in antibody titers between baseline and 1-week and baseline and 3-weeks post-second study injection)

Secondary Endpoints

Safety: 1) number of SAEs at any time during the study; 2) number of AEs at any time during the study

Immunogenicity: **1) g**eometric mean titers (GMT) (baseline and 1 week and baseline and 3 weeks post second study injection) in ELISA assays; 2) geometric mean titers (GMT) (baseline and 1 week and baseline and 3 weeks post second study injection) in Neutralization assays

STUDY RATIONALE

The subunit HeV-sG vaccine developed by Profectus be studied in a series of trials starting with this proposed phase 1, first in humans study. The trial focuses on vaccine safety, but will be able to provide early evidence of immunogenicity, in particular the ability of the vaccine to induce neutralization antibody, which has been identified as a potential correlate of protection in animal studies.

Subsequent to this study a phase 2 study in adults will be conducted to determine the appropriate dose and schedule. Given the unexpectedness of HeV, and NiV outbreaks, it will be virtually impossible to conduct a prospective double blind randomized efficacy trial. On the other hand, if the vaccine is demonstrated to induce the qualitative and quantitative responses associated with protection in non-human primates, it may be eligible for two FDAs expedited development and review programs, Fast Track and Priority Review. Application of the animal rule will be potentially feasible to accelerate introduction.

STUDY DESIGN

The trial will be a double-blind, randomized, placebo-controlled, dose-escalation study testing three different vaccine dose levels in separate cohorts, in which the second and third cohort will be enrolled based on assessment of safety and tolerability in the previous cohort. Each cohort will consist of 25 participants (20 vaccine recipients and 5 placebo recipients) per dose level, who will receive two intramuscular injections three weeks apart. The three dose levels of vaccine to be tested will be 20 ug, 50 ug and 100 ug. Progression from low- to high-dose will be dependent on a positive assessment of safety and tolerability during the week after the first injection in the adult low-dose cohort. A fourth cohort will be enrolled once safety data from the high dose cohort is available. Subjects in this group will receive a single dose of the vaccine high dose. Should all three doses be tolerated in this phase 1 study, a separate phase 2 will be conducted to further expand both safety and immunogenicity of the two higher dose levels in a Phase 2 study.

HeV-sG vaccine	n subjects		Vaccine dose	Vaccination schedule	
Phase 1 USA	Vaccine	Placebo		dose 1 (day)	dose 2 (day)
Group 1	20	5	20 ug	1	29
Group 2	20	5	50 ug	1	29
Group 3	20	5	100 ug	1	29
Group 4	20	5	100 ug	1	-

Progression from group 1 to groups 2 and 3 will require review of clinical and safety lab data through 7 days after the first dose at the lower dose level. Safety laboratory blood specimens will be drawn at baseline for all participants (screening) and 7 days after first study injection in Groups A, B and C. Blood samples for immunogenicity analysis will be obtained at baseline for and on Days 29, 36, 57 and 209.

IMMUNOLOGICAL ASSESSMENTS

- Anti-HeV-sG IgG antibodies by ELISA
- Neutralization activity against Nipah and Hendra virus using attenuated VSV-recombinants carrying the corresponding glycoproteins.

STATISTICAL CONSIDERATIONS

Safety: With 20 vaccine recipients per dose group, this study will provide a greater than 90% chance of observing an AE that has an 11% rate of occurrence.

Conversely, if no SAEs are observed among the 20 vaccine recipients per group, the study will be able to rule out SAEs occurring at a rate of approximately 14% based on the upper bound of the one-sided 95% confidence interval.

Immunogenicity: Based on the results in non-human primates receiving the HeV-sG vaccine, specific seroresponse rates are expected to be ≥80% for the optimal dose group. For comparisons between the optimal dose group and the combined placebo groups, this study will provide ≥90% power to detect ≥62 percentage point difference or 80% power to detect a ≥56 percentage point difference.

For the Geometric Mean Titer (GMT) endpoint, the $\log_{10}$ standard deviations will be descriptively estimated for each group. No comparison hypotheses will be applied.

- A Phase 2 randomized, placebo-controlled, double blind, dose ranging study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in Bangladeshi adults.

STUDY HYPOTHESES

Immunogenicity: The HeV-sG vaccine will be immunogenic in healthy Bangladeshi adults and will induce neutralizing antibody responses, the presumed correlate of protection, in >=50% (lower bound CI 20%) in at least one of the study groups.

Safety: The HeV-sG vaccine will be well-tolerated and with an acceptable safety profile

STUDY OBJECTIVES

Immunogenicity: to identify a vaccine dose / schedule that induces maximal immunogenicity of rapid onset. Towards that objective two vaccine injections will be administered on two different schedules and the responses after each vaccination evaluated at various timepoints.

Safety: to evaluate safety and tolerability of two HeV-sG vaccine injections at two different doses and two different schedules

Secondary Objectives

Immunogenicity: to evaluate the immunogenicity of two doses of HeV-sG vaccine 3 and 6 months after the last vaccination.

Safety: to evaluate vaccine safety through 6 months after the last vaccination

STUDY ENDPOINTS

Primary Endpoints

Immunogenicity: 1) Proportion of subjects exhibiting anti-HeV-sG neutralizing antibody responses (4-fold increase in antibody titers between baseline and 3-weeks post-second study injection); 2) Proportion of subjects with anti-HeV-sG seroresponses by ELISA (four-fold increase in antibody titers between baseline and 3-weeks post-second study injection)

Safety: 1) Number of vaccine related SAEs through the 6 months of the study; 2) Number of vaccine related vaccine related grade 3 AEs through the 6 months of the study; 3) Number of vaccine-induced local and systemic reactions (solicited reactions) during the 7 days after administration of each dose

Secondary Endpoints

Immunogenicity: 1) Proportion of subjects with anti-HeV-sG seroresponses by ELISA (four-fold increase in antibody titers between baseline and 3-weeks post-first study injection); 2) Proportion of subjects with neutralizing antibody responses (4-fold increase in antibody titers between baseline and 3-weeks post-first study injection); 3) Geometric mean titers (GMT) (baseline, 3 weeks post first and 3 weeks post second study injection) in ELISA assays; 4) Geometric mean titers (GMT) (baseline, 3 weeks post first and 3 weeks post second study injection) in neutralization assays

STUDY RATIONALE

The phase two trial will focus on the selection of an optimal safe regimen capable to induce neutralization responses in at least 50% of the subjects in one of the study groups (see study design below). The study will be conducted under a "selection design that would allow the identification of the optimal dose/schedule combination. It will be conducted in 2-3 sites in a country where outbreaks of Nipah virus occur with frequency (e.g., Bangladesh, India).

STUDY DESIGN

The trial will be a double-blind, randomized, placebo-controlled, dose/schedule selection study testing two different vaccine dose levels under two different schedules in separate cohorts which will be enrolled simultaneously. Each cohort will consist of 80 participants who will receive: Group 1: two intramuscular injections of the low dose of vaccine one week apart; Group 2: two intramuscular injections of the low dose of vaccine four weeks apart; Group 3: two intramuscular injections of the high dose of vaccine one week apart; Group 4: two intramuscular injections of the high dose of vaccine four weeks apart; Group 5: three intramuscular injections of placebo on days 1, 8 and 28. To maintain the blind, placebo injections will be given to subjects in groups 1-4 at either day 8 or day 28 (see schema below).

Phase 2	n subjects	Vaccine dose	Vaccination schedule		
			Day 1	Day 8	Day 28
Group 1	80	Low	Vac	Vac	Plac
Group 2	80	Low	Vac	Plac	Vac
Group 3	80	High	Vac	Vac	Plac
Group 4	80	High	Vac	Plac	Vac
Group 5	80	None	Plac	Plac	Plac

Vaccine dose will be selected from the safety data from the phase 1 trial. Blood samples for immunogenicity analysis will be obtained at baseline for all participants, and on Days 8, 22 and 43.

IMMUNOLOGICAL ASSESSMENTS

- Anti-HeV-sG IgG ELISA
- Neutralization activity against Nipah and Hendra virus using attenuated VSV-recombinants carrying the corresponding glycoproteins.

STATISTICAL CONSIDERATIONS

Immunogenicity: We will employ a selection design as a more efficient design for screening multiple regimens compared to traditional superiority or non-inferiority trials although the distinctions between these should be made clear. With 72 evaluable subjects per group, the selection design will provide >90% probability of choosing the optimal regimen if there is at least a 15 percentage point difference between the highest seroresponse rate and the 2nd highest seroresponse rate. For a two-sample comparison between the optimal group and the other combined vaccine groups, this study will provide ≥90% power to detect ≥ 21 percentage point difference (assuming 0.90* 80=72 evaluable subjects per group and 80% seroresponse in optimal group). A traditional design is powered to compare pairs of vaccine arms and hence requires a large total sample size to detect even moderate sized differences when there are many vaccines. In contrast, the selection design requires a smaller sample size since the trial is powered to rank vaccine arms against the placebo group and then select the top ranked vaccine. The selection design can be used appropriately to select among various doses of a vaccine product, various product combinations or various schedules. The procedure ranks the response rates of N treatment groups and selects the treatment with the largest observed response rate regardless of the magnitude of the difference between it and the other groups. Sample size calculations are driven by the smallest difference of interest between the best and next best treatment, and the minimum acceptable probability of correct selection. Simulations are used to calculate the sample size to ensure that if the true response rate of the best vaccine exceeds all others by the smallest possible difference, the probability of selecting that vaccine is sufficiently high.
For the Geometric Mean Titer (GMT) endpoint, $\log_{10}$ standard deviations will be descriptively estimated for each group. No comparison hypotheses will be applied.

Safety: The large sample size of this study (n=400 subjects / 800 vaccine exposures) allows a tighter bound on the rate of vaccine-related SAEs; e.g., if no SAEs are observed, the upper bound of an exact one-sided 95% confidence interval is 4% for each arm of 80, 2% for combined (low or high dose) arms of 160, and 1% for 320 vaccine recipients overall. Eighty vaccine recipients per group, or 160 recipients for combined groups will provide a greater than 90% chance of observing an AE that has a 3% and 1.5% chance of occurrence, respectively.

- A Phase 1/2 randomized, placebo-controlled, double blind study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in healthy 3-17 year old children in Bangladesh.

STUDY HYPOTHESES

Safety: The HeV-sG vaccine is safe and well tolerated in healthy 3-17 year old children in Bangladesh.
Immunogenicity: The HeV-sG vaccine is immunogenic in healthy 3-17 year old children in Bangladesh and will induce neutralizing immune responses in 50% or more of the participants.

STUDY OBJECTIVES

Primary Objectives:
Safety: To evaluate the safety and tolerability of two doses of HeV-sG vaccine in healthy Bangladeshi children
Immunogenicity: To evaluate the immunogenicity of two doses of the He-VsG three weeks after the second vaccination in healthy Bangladeshi children.
Secondary Objectives:
Safety: To evaluate the longer term safety (through 6 months after the last vaccination) of the HeV-sG vaccine
Immunogenicity: To evaluate the immunogenicity of the HeV-sG one week (or four weeks, depending on the schedule chosen) after receipt of the first vaccination.

STUDY ENDPOINTS

Primary Endpoints:

Safety: 1) Number of SAEs through 21 days after the last study injection; 2) Number of AEs through 21 days after the last study injection; 3) Number of vaccine-induced local and systemic reactions (solicited reactions) during the 7 days after administration of each dose

Immunogenicity: 1) Proportion of subjects with anti-HeV-sG seroresponses by ELISA (four-fold increase in antibody titers between baseline and 3-weeks post-second study injection); 2) Proportion of subjects with neutralizing antibody responses (four-fold increase in antibody titers between baseline and 3-weeks post-second study injection)

Secondary Endpoints:

Safety: **1)** Number of SAEs at any time during the study; 2) Number of AEs at any time during the study

Immunogenicity: 1) Proportion of subjects with anti-HeV-sG seroresponses by ELISA (four-fold increase in antibody titers between baseline and 3-weeks post-first study injection); 2) Proportion of subjects with neutralizing antibody responses (four-fold increase in antibody titers between baseline and 3-weeks post-first study injection); 3) Geometric mean titers (GMT) in ELISA assays at baseline and 3 weeks after second study injection; 4) Geometric mean titers (GMT) in neutralization assays at baseline and 3 weeks after second study injection

STUDY RATIONALE

It is imperative that the the Nipah-Hendra vaccine be tested in children, given that in the event of an outbreak they are likely to be the most vulnerable group. But such studies in children should only take place if the vaccine is safe and shows promise in the preceding adult studies. The Phase 1 study in children will be initiated as soon as the safety profile of the vaccine is supportive (over 300 adults would have been tested by then). The dose and schedule will be selected from the Phase 2 study results. The trial in children focuses on vaccine safety, but will also provide additional evidence of immunogenicity, in particular the ability of the vaccine to induce neutralization antibody. In order to proceed cautiously, the study will first enroll older (8-17 years old) children, and move down in age to 3-7 years old children only when the safety in the first group is confirmed.

STUDY DESIGN

The trial will be a double-blind, randomized, placebo-controlled, descending age study testing the dose and schedule of vaccine selected from the adult trials. The study will first enroll a cohort of 8-17 years old children and will move to a second cohort of 3-7 years old once the safety of the vaccine in the earlier age group is proven, including laboratory data on key parameters. If the safety profile of the vaccine is supportive a third cohort of children 3-17 years old will be included to complete a total of about 72 immunized children. Placebo recipients will be included in each cohort at a ratio of 1:2 (one placebo for two vaccine recipients) for a total of 36.

Each of the first two cohorts will include 30 children (20 vaccine and 10 placebo recipients). Once the safety of the first vaccine dose is confirmed in the first two cohorts, a third cohort of 180 3-17 years old children (120 vaccine and 60 placebo recipients) will be enrolled.

Phase 1 (children)	n subjects		subjects age range	Vaccine dose	Vaccination schedule	
	Vaccine	Placebo			dose 1 (day)	dose 2 (day)
Group 1	20	10	8-17 y/o	Selected from Phase 1	1	To be selected from adult studies
Group 2	20	10	3-8 y/o		1	
Group 3	120	60	3-17 y/o		1	

Blood samples for safety analysis will be obtained before (at screening) and 7 days after the vaccination in groups 1 and 2. Serum samples for immunogenicity analysis will be obtained at baseline, and 21 days after each vaccination for participants in groups 1 and 2. Progression to each new cohort will require review of clinical and safety laboratory data through 7 days after study injection in the previous cohort(s). These determinations will be made by the Safety Review Committee (SRC).

IMMUNOLOGY ASSESSMENTS

- Anti-HeV-sG IgG ELISA
- Neutralization activity against Nipah and Hendra virus using attenuated VSV-recombinants carrying the corresponding glycoproteins.

STATISTICAL CONSIDERATIONS

Safety: With a total of 160 vaccine recipients, this study will provide a greater than 90% chance of observing an AE that has a 1.5% or higher rate of occurrence.

Conversely, if no SAEs are observed among the 160 vaccine recipients, the study will be able to rule out SAEs occurring at a rate of approximately 2% based on the upper bound of the one-sided 95% confidence interval.

Immunogenicity: Based on the results in non-human primates receiving the HeV-sG vaccine, specific seroresponse rates are expected to be ≥80% for the optimal dose group. For comparisons between the vaccine and the placebo arms in groups 1 and 2, this study will provide ≥90% power to detect ≥ 48 percentage point difference. This takes into account a potential dropout rate of 10%.

For the Geometric Mean Titer (GMT) endpoint, the $\log_{10}$ standard deviations will be descriptively estimated for each group. No comparison hypotheses will be applied.

- A Phase 1 randomized, placebo-controlled, double-blind dose interval study to test the safety and immunogenicity of HeV-sG, a vaccine that targets Nipah and Hendra infections, in Singaporean adults.

STUDY HYPOTHESES

Safety: The HeV-sG vaccine will be well-tolerated and with an acceptable safety profile.

Immunogenicity: The HeV-sG vaccine is immunogenic and will induce neutralizing immune responses in 50% or more of participants in at least one of the study groups.

STUDY OBJECTIVES

Primary Objectives

Safety: to evaluate the safety and tolerability of the HeV-sG vaccine in healthy adults In Singapore.

Immunogenicity: to evaluate the immunogenicity of He-VsG vaccine, assess the effect of a second dose of vaccine and identify the optimal inter-dose interval.

Secondary Objectives

Safety: to evaluate the longer term vaccine safety (through 12 months from vaccination).

Immunogenicity: to evaluate the ability of HeV-sG vaccine to prime for immunological memory by assessing the ability of a second dose to boost the response at various time intervals.

STUDY ENDPOINTS

Primary Endpoints

Safety: 1) number of SAEs through 21 days after the last study injection; 2) number of AEs through 21 days after the last study injection; 3) number of Vaccine-induced local and systemic reactions (solicited reactions) during the 7 days after administration of each dose

Immunogenicity: 1) proportion of subjects with anti-HeV-sG seroresponses by ELISA (four-fold increase in antibody titers between baseline and 5 days, and baseline and 4-weeks post study injection); 2) proportion of subjects with neutralizing antibody responses (4-fold increase in antibody titers between baseline and 5 days, and baseline and 4-weeks post-second study injection)

Secondary Endpoints

Safety: 1) number of SAEs at any time during the study; 2) number of AEs at any time during the study

Immunogenicity: 1) geometric mean titers (GMT) at baseline, 5 days, and 4 weeks post first and 4 weeks post second study injection in ELISA assays; 2) geometric mean titers (GMT) at baseline, 5 days, and 4 weeks post first and 4 weeks post second study injection in Neutralization assays; 3) proportion of subjects exhibiting an anamnestic response by ELISA at 5 days post-second injection in comparison with baseline (just before second injection); 4) proportion of subjects exhibiting an anamnestic response 5 days post-second injection in comparison with baseline (just before second injection.

STUDY RATIONALE

This trial to be conducted in Singapore focuses on demonstrating the ability of the vaccine to effectively prime for an anamnestic response to a booster dose administered 90, 180, or 365 days after the initial vaccination. The study will be initiated once the optimal vaccine schedule and dose are determined from the phase 2 trial in adults in Bangladesh. The trial will also provide data on antibody persistence (3, 6 and 12 months after the initial vaccination), including ELISA and neutralization titers.

STUDY DESIGN

The trial will be a double-blind, randomized, placebo-controlled, dose-escalation study testing the vaccine dose level selected in the phase 2 study in Bangladesh in three separate cohorts. All enrolled subjects will receive an initial dose (100 ug, if tolerated in the phase 2) on day 1, and will be boosted at either day 91, day 181 or day 365 with the same dose of vaccine. Each cohort will consist of 25 participants (20 vaccine recipients and 5 placebo recipients).

HeV-sG vaccine	n subjects		Vaccine dose	Vaccination schedule	
Phase 1 Singapore	Vaccine	Placebo		dose 1 (day)	dose 2 (day)
Group 1	20	5	100 ug	1	91
Group 2	20	5	100 ug	1	181
Group 3	20	5	100 ug	1	366

Blood samples for immunogenicity analysis will be obtained at baseline for and on Days 6, 29, 91, 96 and 119 (group 1); days 1, 6, 29, 181, 186, and 214 (group 2), and on days 1, 6, 29, 365, 370 and 393 (group 3)

IMMUNOLOGICAL ASSESSMENTS

- Anti-HeV-sG IgG antibodies by ELISA
- Neutralization activity against Nipah and Hendra virus using attenuated VSV-recombinants carrying the corresponding glycoproteins.

STATISTICAL CONSIDERATIONS

Safety: With 20 vaccine recipients per dose group, this study will provide a greater than 90% chance of observing an AE that has an 11% rate of occurrence. Conversely, if no SAEs are observed among the 20 vaccine recipients per group, the study will be able to rule out SAEs occurring at a rate of approximately 14% based on the upper bound of the one-sided 95% confidence interval.

Immunogenicity: Based on the results in non-human primates receiving the HeV-sG vaccine, specific seroresponse rates are expected to be ≥80% for the optimal dose group. For comparisons between the optimal dose group and the combined placebo groups, this study will provide ≥90% power to detect ≥62 percentage point difference or 80% power to detect a ≥56 percentage point difference.

For the Geometric Mean Titer (GMT) endpoint, the $\log_{10}$ standard deviations will be descriptively estimated for each group. No comparison hypotheses will be applied.

4.6. Strategy for regulatory approval of trials and product

Describe the proposed strategy you will take to be under review within WHO emergency use assessment and listing procedure (EUAL), when applicable[1]: Profectus BioSciences has developed a vaccine that protects against disease caused by both Nipah and Hendra viruses. This vaccine has been designed to meet or exceed WHO's proposed Target Product Profile (TPP) for Nipah. Prior to Phase 1, Profectus and PATH will meet with WHO to share our TPP and to confirm that it adequately aligns with WHO Prequalification requirements. We will also share our clinical and regulatory plans for Licensure.

While Nipah is listed on WHO's R&D Blueprint to Action to Prevent Epidemics, it is not currently on WHO's list of diseases which are of "Public Health Emergency of International Concern". Application will be made for listing under the EUAL (Emergency Use Assessment and Listing Procedure) in case it becomes appropriate to use the vaccine before it has been licensed by FDA, or another functional regulatory authority. The availability of early safety data and correlate of protection studies will help determine suitability of use and appropriate dose level prior to completion of Phase 2/3 safety and immunogenicity studies. To meet WHO's requirements for an EUAL in the event of an outbreak, information submitted to the IND can be shared with WHO for review. This would include summary information on available preclinical and clinical data; and manufacturing, testing and stability data. The manufacturing facility would also be available for inspection. Early in clinical development, we would like to meet with WHO to establish acceptable wording for labeling, and put a plan in place for conducting pharmacovigilance and effectiveness studies under outbreak conditions if needed.

Provide the plans for regulatory approval to conduct clinical trials before and during the outbreak: Nipah is not a prevalent disease or endemic to any region, but outbreaks incur high mortality. Since efficacy studies are not feasible and challenge studies are not ethical, registration will be sought with the US FDA by the Animal Rule. Phase 1 First-in-Human safety studies assessing dose level and dosing interval will be conducted in adults in the US. Subsequent studies will be conducted in countries that have experienced outbreaks in the past (e.g. Bangladesh and Singapore) and where the vaccine is most likely to be used. CTA submissions or Ethics Committee contact for ex-US countries as required will be made for these studies, however, all studies will be filed to the US IND. Initial clinical strategy will be reviewed with FDA in a preIND meeting, and prior to the start of Phase 2/3 safety and immunogenicity studies once the previous study's clinical data and subsequent study design is available. We will also seek alignment on how an ongoing clinical study might be adapted in the event of an outbreak to minimize delays in an emergency.

If an outbreak occurs during Phase 1 and available clinical results indicate vaccine safety, we would aim to rapidly initiate a Phase 2/3 study to obtain safety and efficacy data in the field setting, which would potentially support "emergency use" of the investigational vaccine. If the outbreak occurs during Phase 2 or 3, and adequate safety, immunogenicity, and correlate of protection data are available, the investigational vaccine could be considered for emergency use. In that event, a pharmacovigilance and effectiveness study would be conducted. Once dose level and dose interval have been established in adults, we propose to conduct dose escalation studies in the pediatric population to enable vaccination of children during an outbreak scenario. These data would be supported by the results of the nonclinical Correlate of Protection study that will be conducted upon completion of Phase 1 studies. Additionally, after the vaccine has been demonstrated to be safe and an effective dose has been identified in adults and reproductive toxicology data is available, a Phase 1 study may be considered in pregnant women to support vaccination of this population during an outbreak.

Define the proposed strategy for regulatory approval for licensure. Include any major assumptions, including which National Regulatory Authority will be the target of regulatory approval applications: The Nipah/Hendra vaccine consists of a recombinant surface glycoprotein in suspension with an aluminum hydroxide adjuvant. While there have been no discussions with regulatory bodies

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[1] http://www.who.int/medicines/news/public_consult_med_prods/en/

to date on this specific vaccine, Profectus has had discussions with FDA on a similar protein vaccine for HIV (FLSC-IHV01). The technology platform is not novel, however, we will seek confirmation with FDA on all proposed testing in a preIND meeting.

For the Nipah/Hendra vaccine, licensure will be sought under the Animal Rule. After completion of Phase 2/3 clinical studies that demonstrate safety and immunogenicity and identify an effective dose based on the correlate of protection studies; plus lot consistency and nonclinical efficacy, registration will be sought with the US FDA. After FDA approval, an official request will be made for WHO Prequalification. Registration can also be pursued with relevant country regulatory body such as Singapore's Health Sciences Authority (HAS) or other countries that require a CPP (Certificate of Pharmaceutical Product) once FDA approval has been obtained.

Our regulatory strategy targets registration of the Nipah/Hendra vaccine in the US, with global access for outbreaks through WHO Prequalification. Nipah is on WHO's 2017 Blueprint Priority List as needing urgent R&D attention. This priority list seeks to promote epidemic preparedness by focusing accelerated R&D on emerging pathogens likely to cause severe outbreaks in the near future, for which few or no medical countermeasures exist [http://www.who.int/blueprint/priority-diseases/en/]. The vaccine may also be licensed for use directly in countries where the disease is endemic or outbreaks are likely to occur.

The design of initial preclinical and clinical studies for the Nipah/Hendra will based on regulatory advice previously received for other vaccines which consist of glycoproteins adsorbed to aluminum, however, a preIND meeting would be requested with FDA to confirm our strategy. As clinical development progresses, additional meetings will be sought to prepare for later stage studies and to achieve concurrence on a registration pathway. We will also meet with WHO to align on prequalification requirements.

The clinical studies would be conducted under a US IND, with the First-in-Human study in the US. Phase 2 confirmation of dose and dosing interval, and Phase 2/3 safety and immunogenicity studies will be conducted in Bangladesh, where recurrent outbreaks of Nipah disease have occurred. Countries that have experienced earlier disease outbreaks (e.g. Singapore, Malaysia, and India) may also be considered as clinical study sites, or for future registration.

A Phase 1 dose ranging pediatric study should be conducted after initial safety, dose, and dose interval data are available from adult studies (during Adult Phase 2). Reproductive toxicology studies will be conducted prior to the start of Phase 3, or sooner if funds are available and a dose and dosing interval have been identified. Preclinical correlate of protection studies will be conducted to help predict the protective antibody range in humans (using high titer serum harvested in Phase 1) and as a basis for registration by the Animal Rule. This information could also be helpful in the event of an outbreak (emergency use) that occurred prior to Phase 2/3 completion and vaccine registration, and will be supportive of later stage clinical design.

Studies in pregnant or lactating women would not be conducted until after studies in healthy adults are completed. A pregnancy registry will be established to monitor the outcome of pregnancies that may occur during the clinical studies. Studies in other populations (e.g. elderly, immunocompromised, etc.) can be conducted after registration, or earlier if supported.

In the US, a pediatric plan must be in place prior to Biologics License Application (BLA) submission. However, it is imperative that pediatric studies are conducted upon demonstration of safety and identification of an appropriate dose in adults to prepare for emergency use in children in the event of an outbreak.

4.7. Chemistry, Manufacturing & Control (CMC) Development

Although a MCB has been manufactured, tested, characterized, and release for future cGMP production, a stability study was not funded. The following table lists the proposed specifications of the WCB stability program for which funding is requested in the present application.

Specification of WCB

Test	Purpose	Target Specification	Testing Site
Appearance	Visual	Report Result	Catalent
Cell Viability	MCB integrity	> 70%	Catalent
Growth Rate	MCB integrity	Report Results	Catalent

Our current reference standard was manufactured at Profectus in 2014. A new reference standard is required and will prepared using the drug substance produced in an Engineering Run, i.e., finalized upstream and downstream process and characterized using qualified test methods as specified below.

Specification of Reference Standard

Test	Purpose	Target Specification	Testing Site
Appearance	Visual	Report Result	Catalent
Protein Concentration by A_{280}	Potency	0.9 - .1.1 mg/mL	Catalent
pH	MCB integrity	Doubling time	Catalent
Bioassay, ED_{50}	Potency	TBD	Catalent
SDS-PAGE (Reduced, Non-Reduced)	Purity	TBD	Catalent
SEC-HPLC (% Tetramer, Dimer, and Monomer)	Purity	TBD	Catalent

SEC-HPLC, retention times	Identity	TBD	Catalent
Charge distribution by HPLC or cIEF	Characterization	TBD	Catalent
Glycosylation profile	Characterization	TBD	Catalent
Peptide mapping	Characterization	TBD	Catalent

The testing strategy for in-process, release, and stability testing of **DRUG SUBSTANCE** is outlined in the following table. The tests will be conducted at the Drug Substance Manufacturer, Catalent, or at Charles River Labs (Malvern or Wilmington).

Proposed Analytical Testing of HeV-sG In-Process and Drug Substance

Test	Purpose	Target Specification	Testing Site
In process Control during Cell Culture			
Viable Cell Density	Monitor growth	Report results	Catalent
Cell Viability	Monitor growth	Report results	Catalent
Metabolites	Monitor growth	Report results	Catalent
Productivity	Monitor growth	Report results	Catalent
Post-Production Cells or Unprocessed Harvest			
Sterility	Safety	Absence of Growth	CRL
Isoenzyme analysis	Identity	Human Origin	CRL
DNA Finger printing	Identity	Human Origin	CRL
Mycoplasma (EP, USP, PTC,JP) (with Mycoplasmastatsis)	Safety	No evidence of mycoplasma	CRL
In vivo Adventitious Agents	Safety	No evidence of virus	CRL
In vitro Adventitious Agents	Safety	No evidence of virus	CRL
Transmission Electron Microscopy	Safety	No viruses, mycoplasmas, fungi, yeasts, bacteria	CRL
Assays for human viruses	Safety	No evidence of virus	CRL
Assays for Retrovirus	Safety	No evidence of virus	CRL
Bovine Virus assay	Safety	No evidence of virus	CRL
Porcine Virus assay	Safety	No evidence of virus	CRL
Porcine Circovirus assay	Safety	No evidence of virus	CRL
In process Control during Purification			
Protein Concentration by A_{280}	Potency	Per batch record specifications	Catalent
pH	General Quality	Per batch record specifications	Catalent
SDS-PAGE (Reduced, Non-Reduced)	Purity	TBD	Catalent
Host Cell Protein (HEK-293)	Safety	Report results	Catalent
Drug Substance			
Appearance*	Visual	Clear, colorless solution with no foreign particles	Catalent
Protein Concentration by A_{280}*	Potency	0.9 - .1.1 mg/mL	Catalent
pH*	General Quality	6.7 - 7.5	Catalent
Bioassay, ED_{50}*	Potency	TBD	Catalent
SDS-PAGE (Reduced, Non-Reduced)*	Purity	TBD	Catalent
SEC-HPLC (% Tetramer, Dimer, and Monomer)*	Purity	TBD	Catalent
SEC-HPLC, retention times*	Identity	TBD	Catalent
Charge distribution by HPLC or cIEF*	Characterization	TBD	Catalent
Glycosylation profile	Characterization	Report Result	Catalent
Peptide mapping	Characterization	Report Results	Catalent
Residual HEK DNA	Safety	≤ 50 ng/dose	Catalent
Residual HEK Host Cell Protein (HCP)	Safety	Report Results	Catalent
Bioburden*	Safety	≤ 50 CFU/mL	Catalent
Endotoxin*	Safety	≤ 30 EU/mL	Catalent

* Method used in stability testing

The testing strategy for in-process, release, and stability testing of **DRUG PRODUCT** is outlined in the following table. The tests will be conducted at the Drug Product Manufacturer, GRAM, or at Charles River Labs (Malvern or Wilmington).

Proposed Analytical Testing of HeV-sG Vaccine In-Process and Drug Product

In process Control during filling			
Protein Concentration by A_{280}	Potency	Per batch record specifications	GRAM
pH	General Quality	Per batch record specifications	GRAM
SDS-PAGE (Reduced, Non-Reduced)	Purity	TBD	CRL
Bioburden (pre-filtration samples)	Safety	≤ 50 CFU/mL	GRAM
Suspension homogeneity by A_{670}	Purity	Per batch record specifications	GRAM
Drug product			
Appearance*	Visual	White to off-white suspension with no foreign particles	GRAM
Protein Concentration by HPLC*	Potency	0.085 - 0.115 mg/mL (85 - 115 µg/mL)	GRAM
pH*	General Quality	6.7 - 7.5	GRAM
Bioassay, ED_{50}*	Potency	TBD	CRL
SDS-PAGE (Reduced, Non-Reduced)*	Purity	TBD	CRL
SEC-HPLC (% Tetramer, Dimer, and Monomer)*	Purity	TBD	GRAM
SEC-HPLC, retention times*	Identity	TBD	GRAM
Charge distribution by HPLC or cIEF*	Characterization	TBD	CRL
Withdrawable volume	General Quality	≥ 1.0 mL/vial	CRL
Sterility*	Safety	Absence of growth	CRL
Endotoxin*	Safety	≤ 30 EU/mL	CRL

* Method used in stability testing

Drug product will be stored at 2-8°C. The stability program will include accelerated temperatures of 25° and 37°C to definite the type of HEATmarker® vaccine vial monitor to be used to ensure the product quality during shipping and field use at the clinical sites.

Drug product vials will be shipped in bulk to Fisher BioServices for long term storage. Fisher BioServices will label, package, and ship to clinical sites to conduct Phase 1 and 2 clinical trials. During labelling, the appropriate HEATmarker® vaccine vial monitor will be applied to each individual vial to ensure product quality during the entire supply chain.

Profectus has supervised by man-in-plant many production runs of recombinant proteins at various scales and stages (development or clinical) at Catalent for two protein vaccines. For HeV-sG, the removal of host cell protein (HCP) was the more challenging step, but pooling of selected fractions has been identified as an effective method to achieve low levels of residual HEK HCP. The production of HeV-sG in 2x10L bioreactor and the subsequent purification steps were successful, and the resulting DP was used in a GLP toxicological study. The scale up study in 100L bioreactor is currently in progress.

As the HeV-sG vaccine project is in the pre-clinical stage at Profectus, there is limited processing data. After completing the process development at Catalent, the potential risks can be appropriately identified and a detailed risk mitigation plan can be developed prior to the manufacture of CTM. As the manufacturing processes are not defined yet and the demands (patient population and frequency of outbreaks) are unpredictable, it is difficult to calculate the COGS. For HeV-sG vaccine, realistic COGS can be calculated when the vial quantity for stockpiling and product shelf-life are established, most likely after during or after the Phase 2 development.

5. Timeline and decision/stage criteria

5.1. Work breakdown Structure

Task	Task Name	Start	Finish
	CEPI Nipah Virus (HeV-sG) Project Plan	Mon 1/7/13	Thu 4/13/23
		Mon 1/1/18	Mon 1/1/18
1	Production and Release of HeV-sG/Alhydrogel DS and DP	Mon 1/7/13	Tue 12/6/22

	non-cGMP Man/Purif/Release @500L (Catalent)		
	cGMP Man/Purif/Release DS Lot#1 @500L (Catelent)	Mon 1/29/18	Sat 1/8/22
	F/F/Release PhI CTM (3,000 vials @100mcg/vial, GRAM)	Mon 1/7/13	Tue 9/6/22
2	Clinical Assay Dev/Qual (7mon, PBS)	Mon 1/1/18	Fri 7/20/18
3	Prep, submit IND (2 mon, PBS/BCG)	Thu 10/4/18	Sun 12/30/18
4	Ph I Clininical Trial USA, N=78 (9 mon, PATH)	Fri 8/31/18	Wed 10/9/19
5	Ph I Clininical Trial Singapore N=78 (9 mon, Duke/NUS)	Sat 4/13/19	Thu 4/13/23
6	Ph I Clinical Sample Analysis (PBS)	Mon 1/7/13	Tue 3/31/20
	Phase I Clinical sample analysis from USA Trial @PBS, USA	Tue 4/30/19	Sun 10/27/19
	Phase I Clinical sample analysis from Singapore Trial @PBS, USA	Tue 9/3/19	Sun 3/1/20
	Go/NoGo to Phase 2	Sun 3/1/20	Tue 3/31/20
		Mon 1/7/13	Mon 1/7/13
7	Clinical Assay Qualification (PBS)	Tue 3/31/20	Thu 1/7/21
8	Phase 2 Adult and Phase 1 and 2 Pediatric IND/Regularoty Filings (Amendment, PBS/BCG)	Tue 3/31/20	Wed 2/3/21
9	Adult Ph II and Pediatric Ph I and II Clininical testing Singapore (16 mon, PATH)	Thu 4/2/20	Tue 5/24/22
10	Clinical Sample Analysis - Adult Phase 2 and Pediatric Phase I and II Trial	Wed 7/7/21	Fri 8/5/22
11	Emergenccy Use Stockpile (50,000 vials, GRAM)	Tue 3/31/20	Tue 7/7/20
	Engineering Run, 50,000 vials	Tue 3/31/20	Tue 4/28/20
	CTM, 50,000 vials	Tue 4/28/20	Tue 5/5/20
	Release testing - Outsourced	Tue 5/5/20	Tue 7/7/20
12	Exploratory/CoP Assay Development (12 mon, USU)	Mon 1/1/18	Thu 12/27/18
	Activities to Support Licensure	Mon 1/1/18	Mon 1/1/18
13	NHP NiV Correlate of Protection Studies	Thu 9/27/18	Sat 12/26/20
	NHP NiV CoP Study (6 mon, UTMB)	Thu 9/27/18	Tue 3/26/19
	NHP HeV CoP Study (6 mon, UTMB)	Tue 3/31/20	Sun 9/27/20
	NHP Sample analysis (3 mon, USU)	Sun 9/27/20	Sat 12/26/20
14	Phase 1/NHP CoP Sample Analysis	Tue 4/30/19	Thu 6/24/21
	NHP Sample analysis (3 mon, USU)	Sat 12/26/20	Thu 6/24/21
	Ph I Sample analysis (8 mon, USU)	Tue 4/30/19	Mon 7/29/19
15	Production and Release of Consistency Lots of DS and DP	Sat 11/2/19	Fri 12/4/20
	cGMP Man/Purif/Release DS Lot#2 @500L (Catelent)	Sat 11/2/19	Fri 12/4/20
	Pre-process validation readiness	Tue 3/31/20	Sat 5/30/20
	Production - Consistency Lot #2	Sat 11/2/19	Fri 12/4/20
	F/F/Release - Consistency Lot #2 (50,000 vials, GRAM)	Tue 9/1/20	Tue 12/1/20
	CTM, 50,000 vials	Tue 9/1/20	Tue 9/8/20
	Release testing - Outsourced	Tue 9/8/20	Tue 12/1/20
	cGMP Man/Purif/Release DS Lot#3 @500L (Catelent)	Mon 6/1/20	Fri 1/29/21
16	Phase 2 Consistency Lot Trial	Fri 7/3/20	Tue 11/30/21
	IND Amendment to support Ph II lot consistency (PBS/BCG)	Fri 7/3/20	Wed 12/2/20
	Ph II Clininical Trial Lot#2/#3 Singapore (12 mon, PATH)	Tue 8/4/20	Tue 11/30/21
17	Ph II Clinical Sample testing USA (PBS)	Thu 5/6/21	Sat 1/1/22

5.2. Decision/stage gate criteria

Each of the tasks comprising this proposal, as described in Section 4, contain a listing of Deliverables and Go/No Go criteria. Thus, the Go/No Go criteria provide the gate criteria for advancing the program.

5.3. Costs – *use budget narrative and template:*

These are uploaded as separate standalone documents.

6. Product Profile

Target Product Profile: NIPAH VACCINE

For reactive use in outbreak settings with rapid onset of immunity

Vaccine characteristic	**Expected**	**Rational / Argument**	**Variations from WHO preferred TPP**
Indication for use	For active immunization of at-risk persons in the area of an on-going outbreak for the prevention of NiV or HeV disease; to be used in conjunction with other control measures to curtail or end an outbreak.	Also protective against disease caused by Hendra virus.	Exceeds WHO preferred criteria.
Target population	Healthy adults and children, excluding pregnant and lactating women. A pregnancy registry will be set up to monitor outcomes of any pregnancies that occur during the clinical studies.	The addition of pregnant and lactating women will depend upon the outcome of reproductive toxicology studies and clinical studies in this population.	Meets WHO critical criteria.
Safety/Reactogenicity	Safety and reactogenicity sufficient to provide a highly favorable benefit/risk profile in the context of observed vaccine efficacy; ideally with only mild, transient adverse events related to vaccination and no serious AEs related to vaccination.		Meets WHO preferred criteria.
Measures of efficacy	At least 70% efficacy in preventing infection or disease with one dose. Onset of immunity in less than 10 days. Since demonstration of clinical efficacy is likely not feasible, nonclinical immunogenicity and efficacy in a standardized and relevant animal model together with clinical immunogenicity will be considered. If regulatory authorization is provided without clinical efficacy data, effectiveness data are to be generated during use in a future outbreak to the extent possible.		Meets WHO preferred criteria.
Dose regimen	Single dose primary series. A second optional dose may be administered to enhance longevity of immunity 1 to 3 months after the first dose.		Meets WHO preferred criteria.
Duration of protection	Confers protection of at least 3 months, or of at least 1 year with the optional second dose.	One year protection can be obtained with 2 doses. One year protectin may be feasible with a single dose, but that data is not yet available.	Meets WHO critical criteria.
Route of administration	IM delivery ≤1mL		Meets WHO preferred criteria.

Coverage	Monovalent against both Nipah and Hendra viruses with documentation of neutralization of NiV Bangladesh, NiV Malaysia and Hendra virus.	Also protective against Hendra virus.	Exceeds WHO preferred TPP	
Product stability and storage	Alhydrogel suspension with shelf life of at least 12 months at 2-8°C and 24 hours at ambient room temperature. An accelerated stability study will be conducted to assess thermostability at higher temperatures. A vaccine vial monitor will be on each vial of vaccine. Preservatives are not required for single dose vials.	Shelf life may be longer than 12 months at 2-8°C but those data are not yet availalble.	Meets WHO critical criteria.	
Presentation	Vaccine will be presented as a liquid suspension in single dose presentation to provide a dose injection volume of ≤1.0 mL.	In the first Phase 1 study, vaccine volume will likely be 1mL. However, ongoing formulation development is expected to achieve a 0.5mL injection volume	Meets WHO critical criteria.	
Registration and prequalification	Should be WHO pre-qualified according to the process outlined in Procedures for assessing the acceptability, in principle, of vaccines for purchase by United Nations agencies (WHO/BS/10.2155). Nipah is on the list of Blueprint Priority diseases.		Meets WHO preferred criteria.	

7. Risk assessment

Describe the top risks (1 minimum for each functional area) that have moderate to high impact to the development program. Consider risks in different functional areas (i.e. Clinical, CMC, Regulatory, Delivery, Global Access and Partner Management etc.) and how they inter-relate to each other). Add additional rows as needed to describe the risks

- Group responsible for preparation indicated

Functional Domain	Risk Factor	Probability	Likely Impact	Magnitude	Mitigation plan
Regulatory	Cannot establish correlate of protection in two animal species to predict efficacious dose in humans.	Low	Could not register by the animal rule	High	Align early with FDA on correlate of protection study design, choice of animal model(s), and the need for demonstration in two species.
Regulatory	Cannot induce immune response in humans correlated with protection in animal models	Low	Could not register by Animal Rule	High	Explore adequate dose range and dose intervals to maximize immune response
Research	Complete				
Clinical	Vaccine is overly reactogenic and/or safety signals are detected	Low		High	Test lower vaccine dose for which safety signals may be absent
Clinical	Neutralizing antibody does not correlate with protection in the animal model	Low		High	Explore other immune functions that may be better markers of

					protection (B cells, T cells, non-neutralizing antibody functions, et.)
Clinical	Delays due to extended timelines regulatory and ethical approval of clinical trials	High		Medium	Build extra time into study planning and start-up phase to allow for potential delays.
Clinical	Limited vaccine immunogenicity	Low		High	Could be compensated by the use of novel adjuvants (e.g., oil in water emulsions), higher doses and/or multiple dosing
CMC	Manufacturing process does not scale up	Medium	Cost of goods higher than desired	Medium	Several alternative DSP methods are available
Partner Management	Insufficient communication to ensure work achieved in a timely manner	Low	Timeline slippage	Low to High	The Contract Management Team holds regularly scheduled meetings with agendas, minutes and time constrained action items
Product Strategy	PBS				
Delivery	Vaccine proves unstable at refrigerator temperature	Low	Vaccine would require cold chain	High	Comprehensive testing of stabilizers planned and preliminary results good
IP	Third party controls enabling technology		Would require license negotiation and cost	Low to High	Comprehensive review of the IP landscape shows freedom to operate

8. Anticipated use potential

8.1. Suitability of the vaccine candidate for use in disease outbreak settings

Alhydrogel™ formulated HeV-sG remains 100% bound and intact at either 4⁰C or 21⁰C for 32 weeks. Thus, HeV-sG/Alhydrogel™ provides a product with projected multi-year stability at refrigerator temperature, that can withstand extended handling at room temperature, and that will likely withstand multi-day handling at 37⁰C. NHP studies have shown that a single dose of vaccine is able to protect against lethal NiV and HeV challenge. In addition, the human immune response to accidental inoculation (N=2) with the veterinary vaccine has been shown to be potent and to cross-neutralize both NiV and HeV (I. Smith and C. Broder, unpublished). In aggregate, these data indicate that this vaccine can be effectively used in an outbreak setting. Current USP and DSP yields predict a 1,000L fermenter run will provide 120 gm of product. Assuming the intermediate 33.0 µg dose proves to offer adequate immunogenicity, a single 1,000L run is projected to provide ~360,000 doses of vaccine. Therefore, the most practical approach to providing large-scale coverage would be to produce and store bulk drug product (BDP) that could be rapidly filled upon need. Assuming storage stability of 5 years, a run every 3 years would provide an overlapping supply.

8.2. Suitability of the vaccine candidate for use in endemic settings

The HeV-sG/Alhydrogel™ vaccine could be effectively used in an expanded program on immunization that targets those regions that suffer regular outbreaks of NiV disease. Available clinical data indicate that repeated boosting with this vaccine vector is effective, and periodic boosting can be implemented, if needed. In addition, it is anticipated that the AE profile of this vaccine will allow paediatric use.

8.3. Efficacy trials in outbreak situation

It may be possible to use a ring vaccination strategy to demonstrate the efficacy of this vaccine candidate. However, the sporadic nature and small size of the natural outbreaks to date make it more likely that licensure will need to be obtained under “animal rule” studies.

8.4. Application to other pathogens

HeV-sG/Alhydrogel™ is a subunit vaccine specific to NiV and HeV. As such it is not applicable to other pathogens. However, the subunit vaccine base technology has been successful with multiple viral pathogens, including HBV, HPV, and influenza virus.

9. Communication and results

9.1. Dissemination plan of study results

The collaborating partners agree to the timely dissemination of study results via public meetings and publication.

9.2. Data sharing

At the end of each study, final clinical results will be described in a study report and submitted to the IND/CTA and to the BLA for licensure. Anonymized clinical data may be share in publications. The sponsor (or the sponsor's representatives), monitors, auditors, regulatory agencies and ethics committees may have access to the medical records from the study to carry out their obligations or as required by law.

9.3. Comparison of results

Acceptable to the collaborating partners.

10. Other information

10.1. Any other information of relevance for the application

- *Any critical areas where you are likely to require assistance in completing the project, indicating specific roles that CEPI could play to facilitate you getting this type of assistance. Include examples from R&D, manufacturing, regulatory or product delivery domains*
- *Donations or other non-monetized contributions to the project by applicants and their partner(s)*
- *Other information that may be relevant for CEPI's consideration*

10.2. Has the applicant received funding for the proposed project from others sources to date? *(tick one box below)*

√YES	☐ NO

Profectus has received funding from the US National Institutes of Health to advance the HeV-sG/Alhydrogel™ vaccine through scale-up development, IND-enabling toxicology, and NHP protection studies. These activities enable the activities proposed in this contract application, but do not overlap with the current proposal.

10.3. Has the applicant applied for funding of the proposed project to others which is currently pending an award notification? *(tick one box below)*

YES	√NO

10.4. Animal welfare

The **UTMB Animal Resource Center** (ARC) is responsible for housing and care to all animals on campus. The ARC encompasses 89,694 square feet in nine facilities across the UTMB campus. UTMB is a registered Research Facility under the Animal Welfare Act. It has a current Letter of Assurance on file with the Office for Protection from Research Risks. The Geisbert laboratory and the ARC have a documented ability to conduct work in accordance with guidelines for *Biosafety in Microbiological and Biomedical Laboratories* (Fifth Edition, December 2009) and the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (Revised 2015). The Department of Defense, CDC, and USDA have approved our laboratories for work with select agents and AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care International) has certified our animal facilities (Accreditation #000870). UTMB follows the:

- *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (2015 reprint)
- *Guide for the Care and Use of Laboratory Animals*, Eighth Edition (2011)
- US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- USDA-administered Animal Welfare Act (Public Law [PL] 89-544 as amended by PL 91-579 [1970], PL 94-279 [1976], and PL 99-198 [1985]) and published in 9 CFR Parts 1-3 on Final Rules regarding Animal Welfare
- Health Research Extension Act of 1985 (PL 99-158) regarding "Animals in Research"; and
- PL 103-43 regarding the "Plan for Use of Animals in Research" (1993).

UTMB's ARC follows all standards for AAALAC and Institutional Animal Care and Use Committee compliance. Depending on space availability, animals may be housed in ARC facilities with animal care provided by the ARC staff including two full-time veterinarians. These facilities are capable of housing NHPs, rats, mice, hamsters and guinea pigs under BSL2/3 biohazard containment, and encompass 72,847 square feet. All facilities feature full-service animal care procedural and maintenance facilities, including laminar flow isolator caging systems, sterilization equipment, procedure rooms, cage-washing, and autoclaving.

√YES	☐ NO

(If no, please indicate your reasons).

From: John Eldridge on behalf of John Eldridge <eldridge@profectusbiosciences.com>
To: Broder, Christopher; twgeisbe@UTMB.EDU; Antony Dimitrov; Jeffrey Meshulam; Boxley, Kimberly
Subject: RE: CEPI and CETR U19
Date: Tuesday, December 18, 2018 4:26:25 PM
Attachments: CEPI Nipah Full Proposal FINAL.pdf

Hi Chris,

Please find attached the Final Nipah vaccine Technical Proposal submitted by Profectus to CEPI. At the request of our attorney I have deleted any reference to the cost of the program and have marked the document Profectus confidential. As you correctly point out, the Specific Aims of the CEPI and NIAID contracts are completely different, with the exception of developing your Cedar virus-based assay. Let me know if this is adequate to address all overlap concerns.

We will get you BioSketches with updated other support by COB tomorrow.

Best,

John

From: Broder, Christopher <christopher.broder@usuhs.edu>
Sent: Thursday, December 13, 2018 1:02 PM
To: twgeisbe@UTMB.EDU; John Eldridge <eldridge@profectusbiosciences.com>; Antony Dimitrov <dimitrov@profectusbiosciences.com>; Jeffrey Meshulam <meshulam@profectusbiosciences.com>; Boxley, Kimberly <kimberly.boxley.ctr@usuhs.edu>
Subject: CEPI and CETR U19

hi Tom and John (Jeff and Toni cc'd)

NIAID was satisfied with my revised SAC for the CETR U19. We are uploading today. I will send another msg to all partners today.

Kim Boxely (here) will be reaching out to our CETR partners (PIs and their POCs) for JIT other support docs of all
key personnel today/tomorrow.

Micheal sent msg below this AM. This specifically relates to only me (USU), Profectus and UTMB - as we are all parties to the CEPI-funded Nipah vaccine
so you both as key personnel need to put together your other support pages. and address any overlap more specifically.

Scientifically. as far as the Profecus CETR project (RP1) goes (and UTMB/GNL) (Core C) there is no scientific overlap with CEPI project (the goals and proposed studies are totally different.
CEPI project is just gearing up HeV-sG production as GMP material and alum vaccine formulation and assay development, and clinical trials.

There is only slight overlap with USU CETR project (RP3) regards to making just two Cedar chimeric viruses which i will address.

John, as you can see from the msg from NIAID, I need the final funded CEPI application for them., Is this possible? ASAP.

I will be email Micheal back shortly and give him a heads up.

v/r
Chris

Hi Chris,

We are aware that you and additional key personnel of your proposed CETR are engaged in a recently funded CEPI project to develop a Nipah virus vaccine. At this stage of CETR payplan processing, we need to assess potential scientific and budgetary overlap between the CEPI and CETR projects. Accordingly, please provide a copy of the funded CEPI application (uploaded as a JIT document vie eCommons) as soon as possible. Feel free to contact me if you have any questions.
Regards,
Michael

Michael R. Schaefer, Ph.D.
Chief, Translational Centers of Excellence and Research Coordination Section
Office of Biodefense, Research Resources and Translational Research
DMID/NIAID/NIH
--

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Version 1.2

A Research and Development (R&D) Roadmap for Nipah Virus

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The Nipah Virus R&D Roadmap Taskforce[5]

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[Note: Degrees and affiliations will be added once received from TF members.]

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Kim Halpin

Stephen Luby

Gregg Milligan

Version 1.2

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The work for this project was completed with generous support from the Wellcome Trust.

The authors would also like to acknowledge Mr. Carlos R. Cruz; CIDRAP, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

Version 1.2

Word Count: 3,695 (text only, not including References or Tables)

Introduction

Nipah virus (NiV), a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chua 1999, Chew 2000, Parashar 2000). At least 265 human cases of encephalitis were identified in Malaysia and most had close contact with pigs (Chua 2010, Parashar 2000). In addition, 11 abattoir workers in Singapore became ill following contact with pigs imported from Malaysia; eight patients presented with signs of encephalitis and three presented with atypical pneumonia (one of which later also had evidence of encephalitis) (Paton 1999). No new outbreaks have been reported in these countries since May 1999; however, NiV infection was subsequently recognized in Bangladesh in 2001 (Hsu 2004) and nearly annual outbreaks have occurred in that country since then (WHO 2018). Outbreaks also have been recognized in eastern India and, in 2018, an outbreak occurred in the southern Indian state of Kerala (Arunkumar 2018, Sharma 2018, Chattu 2018). To date, nearly 350 cases of NiV infection have been identified in Bangladesh and India (WHO 2018). Possible emergence of NiV in a third geographic area is evidenced by an outbreak of an unidentified henipavirus that occurred among horses and humans in the Philippines in 2014 (Ching 2015). Although detailed genomic information for the virus is limited, experts agree that the causative agent was either NiV or a virus closely related to NiV. The Henipavirus genus includes another pathogen, Hendra virus, which predominantly causes infection in horses in Australia and can also lead to human disease, usually following contact with infected horses (Ksiazek 2011).

The most common clinical syndrome associated with NiV infection is severe, rapidly progressive encephalitis (Goh 2000, Hossain 2008). The main presenting features include fever, headache, dizziness, vomiting, and altered mental status. NiV infection may result in late-onset

encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae (Tan 2002, Goh 2000). Some patients also have respiratory involvement or respiratory illness alone, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The overall case-fatality rate for the Malaysia outbreak was approximately 40%, whereas case-fatality rates during outbreaks in Bangladesh and India have generally ranged from 65% to 100% (Hossain 2008, WHO 2018). Genomic sequencing has demonstrated that there are multiple strains of NiV, which may account for the differences noted in clinical features and outcomes between Malaysia and Bangladesh (Mire 2016).

Fruit bats of the Pteropus genus are considered the primary natural reservoir for NiV. In the Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and possibly limited human-to-human NiV transmission (Chua 2010). Additionally, several other domestic animal species in the outbreak area (including horses, cats, and dogs) were found to be infected with NiV (Chua 2000). In outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap (Islam 2016, Luby 2006, Rahman 2012) and subsequent person-to-person transmission. While person-to-person transmission was apparently minimal in the Malaysia outbreak, it has played a larger role in amplifying outbreaks in Bangladesh and India (Gurley 2007, Luby 2009, Chadha 2006, Arunkumar 2018). This may, in part, be related to the greater propensity toward respiratory disease in NiV strains found in South Asia and transmission via droplet spread (Spiropoulou 2018). The potential for droplet spread is supported by a recent study that demonstrated hamsters could be infected when exposed to NiV via a small-particle aerosol (Escaffre 2018). Given the potential for respiratory transmission of NiV and the recent experience with Ebola in West

Africa, some have raised concerns about the possibility of larger NiV outbreaks if the virus takes hold in a crowded or urban area (Donaldson 2018) or about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Pteropus fruit bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Other fruit bats of the Pteropodidae family also may harbor NiV, and such bats can be found across Africa and parts of the Middle East. Evidence of NiV infection (via virus isolation, serologic testing, or nucleic acid detection using real-time polymerase chain reaction [RT-PCR]) has been found in Pteropus bat species and other bat species in geographically diverse regions, including South Asia (Bangladesh and India); Southeast Asia (Cambodia, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam); the Western Pacific region (Papua New Guinea); and Africa (Madagascar and Ghana) (Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2018, Yob 2001). This wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.

WHO has determined that Nipah virus is a priority disease for epidemic preparedness (WHO 2018). To improve epidemic response for this infection, in the fall of 2017, WHO embarked on development of a research and development (R&D) roadmap for NiV disease; this report provides a summary of the key elements of that roadmap, including important challenges and barriers that need to be addressed to ensure that diagnostics, therapeutics, and vaccines are

developed, manufactured, and readily available when needed *[provide link to full roadmap here when available]*.

The NiV R&D roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one medical countermeasure category), diagnostics, therapeutics, and vaccines. The strategic goals and milestones identified in each section are focused on key achievements for the next 5 years; ~~the roadmap milestones will be tracked over time,~~ the roadmap milestones will be tracked over time, with periodic assessment of progress and updates to the roadmap as needed. The roadmap is specific to R&D for disease in humans; however, further research of NiV in animal species, including development of appropriate medical countermeasures targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV in humans and virus transmission can occur at the human-animal interface.

Cross-Cutting Issues

A major challenge for development of diagnostics, therapeutics, and vaccines for NiV is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Advocacy to policy makers in affected countries and to global stakeholders is needed to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures. As part of this process, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species and proximate

human populations in areas of predicted risk. Human health, animal health, and wildlife officials should be engaged in these efforts as part of a long-term collaborative One Health approach (Chattu 2018, Kulkarni 2013).

Another important issue is the need to optimize relevant animal challenge models for promoting development and evaluation of medical countermeasures for NiV infection, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval (Rockx 2014). For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for therapeutic or vaccine development; (3) determine when after challenge medical countermeasures should be administered in animal models to best mimic realistic timing of such use in humans; (4) bridge data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human doses; and (5) identify the best models for studying chronic (relapsing) infection (Bossart 2012, Debuysscher 2013, Dhondt 2013, Johnston 2015, Mathieu 2015, Satterfield 2016). Currently, the African green monkey is regarded as the most relevant animal model for evaluating candidate therapeutics and vaccines intended for use in humans (Geisbert 2010, Johnston 2015), and studies involving nonhuman primates may be required for licensure of therapeutics and vaccines via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns, however, constrain the use of this animal model. In addition to optimizing animal models, standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains are needed to move R&D of medical countermeasures for NiV infection forward (Satterfield 2016).

To inform development of optimal NiV medical countermeasures, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. This includes issues such as evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection.

To respond to these challenges, the strategic goals and milestones for cross-cutting issues in the NiV R&D roadmap focus primarily on developing a sustainable value proposition and identifying funding sources to promote R&D, availability, and accessibility of NiV medical countermeasures, optimizing animal models for research, and generating standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV medical countermeasures (Table 1).

Diagnostics

The most commonly used methods for diagnosing NiV infection include virus isolation, enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies, and real time-polymerase chain reaction (RT-PCR) nucleic acid testing. Virus isolation requires biosafety level 4 (BSL-4) capabilities and is generally only performed in reference laboratories for specific purposes, such as to confirm a new outbreak or to identify the virus in new host species (Wang 2012). During the Malaysia outbreak, the US Centers for Disease Control and Prevention (CDC) developed a capture ELISA for detection of IgM antibodies and an indirect ELISA for detection of IgG antibodies (Daniels 2001); since then, additional ELISA tests have been developed (Wang 2012) and serologic testing is often used for

case confirmation during outbreaks (Chadha 2006, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009). RT-PCR, which can be performed on throat and nasal swabs, cerebrospinal fluid, urine, and blood, is also often used for diagnostic testing (Chadha 2006, Guillaume 2004, Lo 2012). Quantitative RT-PCR tests have been developed for quantifying viral replication; such tools can be used to measure viral load reduction when screening vaccine candidates and potential therapeutics (Jensen 2018). More recently, pseudotyped-based serum neutralization assays for NiV detection that use vesicular stomatitis virus systems have been developed; these assays potentially can be used under BSL2 conditions in laboratories with limited diagnostic capabilities and appear to offer improved sensitivity and specificity (Kaku 2009, Kaku 2012, Tamin 2009). Antigen-capture ELISAs using monoclonal antibodies also have been developed, which could result in more rapid diagnosis (Chiang 2010). These newer technologies hold promise; however, more information is needed regarding their performance characteristics, particularly under field conditions .

Commented [DTM1]: Development and validation of immunohistochemical staining tests can facilitate studies on pathogenesis of Nipah infections and might also be useful for primary diagnosis in low-resource settings.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation, which can delay outbreak detection and implementation of infection control measures. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. To address these issues, rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes, once therapeutic options become available.

Commented [DTM2]: Where feasible, prospective surveillance of susceptible animal species (e.g., bats, pigs, equids) with potential for human spillover of Nipah infection may be implemented, in order to evaluate risk profiles and patterns of NiV circulation.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of such a repository requires addressing the following: (1) determining what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outlining the purposes of sample collection; (3) determining what organizations will be responsible for activities related to creating and maintaining the repositories; (4) establishing standardized protocols for sample collection and maintenance; (5) establishing an appropriate governance structure; (6) identifying who would have access to the samples; (7) prioritizing use of samples and sample distribution; and (8) ensuring that material transfer agreements are in place. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

To address these issues, the strategic goals and milestones for diagnostic testing outlined in the NiV R&D roadmap focus on creation of a virtual reference repository, development of highly sensitive and specific point-of-care or near-patient NiV diagnostic tests, and enhancement of laboratory diagnostic preparedness to promote early detection of NiV in areas of known or potential spillover risk (Table 2).

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients; findings from treated patients were compared to those of 54 historical controls who became ill before ribavirin was available or who refused treatment (Chong 2001).

Commented [DTM3]: (1) Clinicians, especially in endemic areas, should be asked to maintain high vigilance for cases of acute febrile encephalitic syndrome, in particular, with respiratory symptoms.
(2) Effective and appropriate risk communication strategies should be implemented in endemic areas. Recommendations on behavioral modifications should be communicated to susceptible populations, wherever Nipah infections have occurred after indigenous practices like consumption of raw date palm sap.

Results demonstrated a statistically significant reduction in mortality among treated patients (36%); however, the use of historical controls may have biased this finding. No additional clinical studies using ribavirin have been conducted, and limited studies in animal models have not demonstrated efficacy of ribavirin following NiV or Hendra virus challenge (Rockx 2010, Georges-Courbot 2006). Chloroquine also has been studied in animal models, but has not shown any therapeutic benefit, either alone or in combination with ribavirin (Freiberg 2010, Pallister 2009). Based on these findings, these approaches are not considered viable options for treatment of NiV infection.

A more promising therapeutic option is the development of monoclonal antibodies (mAbs) against viral envelop proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). Therapy with anti-NiV mAbs may be appropriate for early treatment and for post-exposure prophylaxis (PEP) to prevent illness in exposed persons, such as healthcare workers, family caregivers, and persons exposed to infected livestock. Researchers have demonstrated that the human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009), and it has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Recently, a phase 1 clinical trial of m102.4 with 40 human participants was completed in Australia, but results are not yet available (ANZCTR). M102.4 was deployed for use during the 2018 NiV outbreak in Kerala, India, but was not used because the outbreak was brought under control quickly (Spiropoulou 2018). One recent study in nonhuman primates showed that the therapeutic window for m102.4 may vary by virus strain, which is a consideration for further preclinical and clinical study of this agent (Mire 2016).

Commented [DTM4]: Given the limited availability of m102.4, it may also be worthwhile to explore development of alternate monoclonal antibodies against NiV, and to evaluate approaches like cocktails of monoclonal antibodies, purified convalescent human sera, egg yolk-derived immunoglobulins etc. for Immunoprophylaxis against it in humans and/or animals.

Other potential options for NiV treatment include favipiravir (a small-molecule antiviral that inhibits RNA-dependent RNA polymerase), fusion inhibitory peptides (peptides that inhibit virus-mediated membrane fusion), and remdesivir (also known as GS-5734, which is a monophosphoramidate prodrug of an adenosine analogue). Researchers recently demonstrated efficacy of favipiravir in hamsters challenged with a lethal dose of NiV (Dawes 2018), supporting proof-of-concept for favipiravir as a possible therapy against NiV infection. Studies in animal models also have been conducted for fusion inhibitory peptides; for example, investigators recently reported that lipopeptides delivered via the respiratory route can prevent lethal NiV infection in hamsters and nonhuman primates (Mathieu 2018). Remdesivir is a small-molecule antiviral that is currently being evaluated in clinical studies as a therapeutic option for treating Ebola virus disease. Investigators recently demonstrated in vitro inhibition by remdesivir against a number of different viruses, including paramyxoviruses (Lo 2017). While these agents are promising, further preclinical data are needed to assess their utility. In addition, efforts are needed to expand the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents that may be more aligned with optimal and desired characteristics for anti-NiV therapy.

Key additional considerations for moving NiV therapeutics forward include: (1) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising novel treatments for NiV disease; (2) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (3) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in both endemic and at-risk areas. To address these issues,

the goals and milestones for therapeutics in the NiV R&D roadmap focus on continuing to move promising agents forward into clinical trials and on enhancing preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Satterfield 2016). A Hendra virus subunit vaccine involving soluble glycoprotein (sG) has been developed and is commercially available for use in horses (Middleton 2014), which supports this option as a promising approach for developing a human NiV vaccine.

The Coalition for Epidemic Preparedness Innovations (CEPI), which is a partnership of government agencies, industry, academia, philanthropy, and intergovernmental institutions aimed at providing funding for R&D of new vaccines against pathogens with epidemic potential, has recently provided financial support to promote the development of several NiV candidate vaccines. In May 2018, CEPI awarded a US$ 25 million contract to Profectus BioSciences, Inc. and Emergent BioSolutions Inc. to develop an NiV vaccine that is based on the same technology

as that used for the Hendra virus vaccine (CEPI 2018). In September 2018, CEPI announced plans to award approximately US$ 2 million to the Jenner Institute at the University of Oxford and Janssen Vaccines & Prevention BV for preclinical development of a replication-deficient simian adenoviral-vectored vaccine, derived from an adenovirus originally isolated from chimpanzees (CEPI 2018). These efforts will support preclinical and early clinical development of NiV vaccines.

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012,

Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research in this area should focus primarily on the humoral immune response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

The immediate goals and milestones in the vaccine section of the NiV R&D roadmap focus on moving vaccine candidates forward into clinical trials and promoting development of guidance on requirements for clinical trials, regulatory pathways, and other considerations relevant to licensure of a vaccine against NiV.

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures forward. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 31 October 2018 [Web page]

Arunkumar G, Chandni R, DT Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

CEPI (Coalition for Epidemic Preparedness Innovations). CEPI awards $25 million contract to Profectus BioSciences and Emergent BioSolutions to develop Nipah virus vaccine. 2018 May 24 [Full text]

CEPI. CEPI awards contract worth up to USD$19 million to Oxford University and Janssen Vaccines to develop MERS, Lassa, and Nipah vaccines. 2018 Sep 27 [Full text]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in Fruit Bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C_{12}U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis 2014;20:372-9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6): 30916 [Full text]

Pallister J, Middleton D, Crameri G. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. J Virol 2009 Nov;83(22):11979-82 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. List of Blueprint priority diseases; accessed October 28, 2018 [Webpage]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed October 26, 2018 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Tables

Table 1: Goals and Milestones for NiV Infection Cross-Cutting Issues

Goals and Milestones for NiV Infection Cross-Cutting Issues **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.	1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
	2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.
2. Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.	1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
	2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Table 2: Goals and Milestones for NiV Diagnostics

Goals and Milestones for NiV Diagnostics **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.	1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).
	2. By 2021, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.
2. Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are	1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.
	2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
	3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.	4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
3. Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.	1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to ~~diagnos~~diagnose~~is~~ NiV infection during future outbreaks.

Table 3: Goals and Milestones for NiV Therapeutics

Goals and Milestones for NiV Therapeutics **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.	1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	4. By 2020, generate a reliable source of m102.4 to be used in clinical trials.
2. Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.	1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
	2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
	3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Table 4: Goals and Milestones for NiV Vaccines

Goals and Milestones for NiV Vaccines
Nipah Virus Roadmap

STRATEGIC GOALS	MILESTONES
1. Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.	1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
	2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as the FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.
2. Develop and evaluate NiV vaccines for prevention of NiV disease in humans.	1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
	2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
	3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

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Version 1.2

A Research and Development (R&D) Roadmap for Nipah Virus

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The work for this project was completed with generous support from the Wellcome Trust.

The authors would also like to acknowledge Mr. Carlos R. Cruz; CIDRAP, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

Word Count: 3,695 (text only, not including References or Tables)

Introduction

Nipah virus (NiV), a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chua 1999, Chew 2000, Parashar 2000). At least 265 human cases of encephalitis were identified in Malaysia and most had close contact with pigs (Chua 2010, Parashar 2000). In addition, 11 abattoir workers in Singapore became ill following contact with pigs imported from Malaysia; eight patients presented with signs of encephalitis and three presented with atypical pneumonia (one of which later also had evidence of encephalitis) (Paton 1999). No new outbreaks have been reported in these countries since May 1999; however, NiV infection was subsequently recognized in Bangladesh in 2001 (Hsu 2004) and nearly annual outbreaks have occurred in that country since then (WHO 2018). Outbreaks also have been recognized in eastern India and, in 2018, an outbreak occurred in the southern Indian state of Kerala (Arunkumar 2018, Sharma 2018, Chattu 2018). To date, nearly 350 cases of NiV infection have been identified in Bangladesh and India (WHO 2018). Possible emergence of NiV in a third geographic area is evidenced by an outbreak of an unidentified henipavirus that occurred among horses and humans in the Philippines in 2014 (Ching 2015). Although detailed genomic information for the virus is limited, experts agree that the causative agent was either NiV or a virus closely related to NiV. The Henipavirus genus includes another pathogen, Hendra virus, which predominantly causes infection in horses in Australia and can also lead to human disease, usually following contact with infected horses (Ksiazek 2011).

The most common clinical syndrome associated with NiV infection is severe, rapidly progressive encephalitis (Goh 2000, Hossain 2008). The main presenting features include fever, headache, dizziness, vomiting, and altered mental status. NiV infection may result in late-onset

encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae (Tan 2002, Goh 2000). Some patients also have respiratory involvement or respiratory illness alone, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The overall case-fatality rate for the Malaysia outbreak was approximately 40%, whereas case-fatality rates during outbreaks in Bangladesh and India have generally ranged from 65% to 100% (Hossain 2008, WHO 2018). Genomic sequencing has demonstrated that there are multiple strains of NiV, which may account for the differences noted in clinical features and outcomes between Malaysia and Bangladesh (Mire 2016).

Fruit bats of the Pteropus genus are considered the primary natural reservoir for NiV. In the Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and possibly limited human-to-human NiV transmission (Chua 2010). Additionally, several other domestic animal species in the outbreak area (including horses, cats, and dogs) were found to be infected with NiV (Chua 2000). In outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap (Islam 2016, Luby 2006, Rahman 2012) and subsequent person-to-person transmission. While person-to-person transmission was apparently minimal in the Malaysia outbreak, it has played a larger role in amplifying outbreaks in Bangladesh and India (Gurley 2007, Luby 2009, Chadha 2006, Arunkumar 2018). This may, in part, be related to the greater propensity toward respiratory disease in NiV strains found in South Asia and transmission via droplet spread (Spiropoulou 2018). The potential for droplet spread is supported by a recent study that demonstrated hamsters could be infected when exposed to NiV via a small-particle aerosol (Escaffre 2018). Given the potential for respiratory transmission of NiV and the recent experience with Ebola in West

Africa, some have raised concerns about the possibility of larger NiV outbreaks if the virus takes hold in a crowded or urban area (Donaldson 2018) or about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Pteropus fruit bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Other fruit bats of the Pteropodidae family also may harbor NiV, and such bats can be found across Africa and parts of the Middle East. Evidence of NiV infection (via virus isolation, serologic testing, or nucleic acid detection using real-time polymerase chain reaction [RT-PCR]) has been found in Pteropus bat species and other bat species in geographically diverse regions, including South Asia (Bangladesh and India); Southeast Asia (Cambodia, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam); the Western Pacific region (Papua New Guinea); and Africa (Madagascar and Ghana) (Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2018, Yob 2001). This wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.

WHO has determined that Nipah virus is a priority disease for epidemic preparedness (WHO 2018). To improve epidemic response for this infection, in the fall of 2017, WHO embarked on development of a research and development (R&D) roadmap for NiV disease; this report provides a summary of the key elements of that roadmap, including important challenges and barriers that need to be addressed to ensure that diagnostics, therapeutics, and vaccines are

developed, manufactured, and readily available when needed *[provide link to full roadmap here when available]*.

The NiV R&D roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one medical countermeasure category), diagnostics, therapeutics, and vaccines. The strategic goals and milestones identified in each section are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, the roadmap milestones will be tracked over time, with periodic assessment of progress and updates to the roadmap as needed. The roadmap is specific to R&D for disease in humans; however, further research of NiV in animal species, including development of appropriate medical countermeasures targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV in humans and virus transmission can occur at the human-animal interface.

Cross-Cutting Issues

A major challenge for development of diagnostics, therapeutics, and vaccines for NiV is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Advocacy to policy makers in affected countries and to global stakeholders is needed to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures. As part of this process, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species and proximate

human populations in areas of predicted risk. Human health, animal health, and wildlife officials should be engaged in these efforts as part of a long-term collaborative One Health approach (Chattu 2018, Kulkarni 2013).

Another important issue is the need to optimize relevant animal challenge models for promoting development and evaluation of medical countermeasures for NiV infection, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval (Rockx 2014). For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for therapeutic or vaccine development; (3) determine when after challenge medical countermeasures should be administered in animal models to best mimic realistic timing of such use in humans; (4) bridge data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human doses; and (5) identify the best models for studying chronic (relapsing) infection (Bossart 2012, Debuysscher 2013, Dhondt 2013, Johnston 2015, Mathieu 2015, Satterfield 2016). Currently, the African green monkey is regarded as the most relevant animal model for evaluating candidate therapeutics and vaccines intended for use in humans (Geisbert 2010, Johnston 2015), and studies involving nonhuman primates may be required for licensure of therapeutics and vaccines via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns, however, constrain the use of this animal model. In addition to optimizing animal models, standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains are needed to move R&D of medical countermeasures for NiV infection forward (Satterfield 2016).

To inform development of optimal NiV medical countermeasures, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. This includes issues such as evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection.

To respond to these challenges, the strategic goals and milestones for cross-cutting issues in the NiV R&D roadmap focus primarily on developing a sustainable value proposition and identifying funding sources to promote R&D, availability, and accessibility of NiV medical countermeasures, optimizing animal models for research, and generating standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV medical countermeasures (Table 1).

Diagnostics

The most commonly used methods for diagnosing NiV infection include virus isolation, enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies, and real time-polymerase chain reaction (RT-PCR) nucleic acid testing. Virus isolation requires biosafety level 4 (BSL-4) capabilities and is generally only performed in reference laboratories for specific purposes, such as to confirm a new outbreak or to identify the virus in new host species (Wang 2012). During the Malaysia outbreak, the US Centers for Disease Control and Prevention (CDC) developed a capture ELISA for detection of IgM antibodies and an indirect ELISA for detection of IgG antibodies (Daniels 2001); since then, additional ELISA tests have been developed (Wang 2012) and serologic testing is often used for

case confirmation during outbreaks (Chadha 2006, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009). RT-PCR, which can be performed on throat and nasal swabs, cerebrospinal fluid, urine, and blood, is also often used for diagnostic testing (Chadha 2006, Guillaume 2004, Lo 2012). Quantitative RT-PCR tests have been developed for quantifying viral replication; such tools can be used to measure viral load reduction when screening vaccine candidates and potential therapeutics (Jensen 2018). More recently, pseudotyped-based serum neutralization assays for NiV detection that use vesicular stomatitis virus systems have been developed; these assays potentially can be used under BSL2 conditions in laboratories with limited diagnostic capabilities and appear to offer improved sensitivity and specificity (Kaku 2009, Kaku 2012, Tamin 2009). Antigen-capture ELISAs using monoclonal antibodies also have been developed, which could result in more rapid diagnosis (Chiang 2010). These newer technologies hold promise; however, more information is needed regarding their performance characteristics, particularly under field conditions.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation, which can delay outbreak detection and implementation of infection control measures. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. To address these issues, rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes, once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of such a repository requires addressing the following: (1) determining what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outlining the purposes of sample collection; (3) determining what organizations will be responsible for activities related to creating and maintaining the repositories; (4) establishing standardized protocols for sample collection and maintenance; (5) establishing an appropriate governance structure; (6) identifying who would have access to the samples; (7) prioritizing use of samples and sample distribution; and (8) ensuring that material transfer agreements are in place. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

To address these issues, the strategic goals and milestones for diagnostic testing outlined in the NiV R&D roadmap focus on creation of a virtual reference repository, development of highly sensitive and specific point-of-care or near-patient NiV diagnostic tests, and enhancement of laboratory diagnostic preparedness to promote early detection of NiV in areas of known or potential spillover risk (Table 2).

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients; findings from treated patients were compared to those of 54 historical controls who became ill before ribavirin was available or who refused treatment (Chong 2001).

Results demonstrated a statistically significant reduction in mortality among treated patients (36%); however, the use of historical controls may have biased this finding. No additional clinical studies using ribavirin have been conducted, and limited studies in animal models have not demonstrated efficacy of ribavirin following NiV or Hendra virus challenge (Rockx 2010, Georges-Courbot 2006). Chloroquine also has been studied in animal models, but has not shown any therapeutic benefit, either alone or in combination with ribavirin (Freiberg 2010, Pallister 2009). Based on these findings, these approaches are not considered viable options for treatment of NiV infection.

A more promising therapeutic option is the development of monoclonal antibodies (mAbs) against viral envelop proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). Therapy with anti-NiV mAbs may be appropriate for early treatment and for post-exposure prophylaxis (PEP) to prevent illness in exposed persons, such as healthcare workers, family caregivers, and persons exposed to infected livestock. Researchers have demonstrated that the human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009), and it has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Recently, a phase 1 clinical trial of m102.4 with 40 human participants was completed in Australia, but results are not yet available (ANZCTR). M102.4 was deployed for use during the 2018 NiV outbreak in Kerala, India, but was not used because the outbreak was brought under control quickly (Spiropoulou 2018). One recent study in nonhuman primates showed that the therapeutic window for m102.4 may vary by virus strain, which is a consideration for further preclinical and clinical study of this agent (Mire 2016).

Other potential options for NiV treatment include favipiravir (a small-molecule antiviral that inhibits RNA-dependent RNA polymerase), fusion inhibitory peptides (peptides that inhibit virus-mediated membrane fusion), and remdesivir (also known as GS-5734, which is a monophosphoramidate prodrug of an adenosine analogue). Researchers recently demonstrated efficacy of favipiravir in hamsters challenged with a lethal dose of NiV (Dawes 2018), supporting proof-of-concept for favipiravir as a possible therapy against NiV infection. Studies in animal models also have been conducted for fusion inhibitory peptides; for example, investigators recently reported that lipopeptides delivered via the respiratory route can prevent lethal NiV infection in hamsters and nonhuman primates (Mathieu 2018). Remdesivir is a small-molecule antiviral that is currently being evaluated in clinical studies as a therapeutic option for treating Ebola virus disease. Investigators recently demonstrated in vitro inhibition by remdesivir against a number of different viruses, including paramyxoviruses (Lo 2017). While these agents are promising, further preclinical data are needed to assess their utility. In addition, efforts are needed to expand the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents that may be more aligned with optimal and desired characteristics for anti-NiV therapy.

Key additional considerations for moving NiV therapeutics forward include: (1) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising novel treatments for NiV disease; (2) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (3) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in both endemic and at-risk areas. To address these issues,

the goals and milestones for therapeutics in the NiV R&D roadmap focus on continuing to move promising agents forward into clinical trials and on enhancing preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Satterfield 2016). A Hendra virus subunit vaccine involving soluble glycoprotein (sG) has been developed and is commercially available for use in horses (Middleton 2014), which supports this option as a promising approach for developing a human NiV vaccine.

The Coalition for Epidemic Preparedness Innovations (CEPI), which is a partnership of government agencies, industry, academia, philanthropy, and intergovernmental institutions aimed at providing funding for R&D of new vaccines against pathogens with epidemic potential, has recently provided financial support to promote the development of several NiV candidate vaccines. In May 2018, CEPI awarded a US$ 25 million contract to Profectus BioSciences, Inc. and Emergent BioSolutions Inc. to develop an NiV vaccine that is based on the same technology

as that used for the Hendra virus vaccine (CEPI 2018). In September 2018, CEPI announced plans to award approximately US$ 2 million to the Jenner Institute at the University of Oxford and Janssen Vaccines & Prevention BV for preclinical development of a replication-deficient simian adenoviral-vectored vaccine, derived from an adenovirus originally isolated from chimpanzees (CEPI 2018). These efforts will support preclinical and early clinical development of NiV vaccines.

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012,

Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research in this area should focus primarily on the humoral immune response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

The immediate goals and milestones in the vaccine section of the NiV R&D roadmap focus on moving vaccine candidates forward into clinical trials and promoting development of guidance on requirements for clinical trials, regulatory pathways, and other considerations relevant to licensure of a vaccine against NiV.

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures forward. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 31 October 2018 [Web page]

Arunkumar G, Chandni R, DT Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

CEPI (Coalition for Epidemic Preparedness Innovations). CEPI awards $25 million contract to Profectus BioSciences and Emergent BioSolutions to develop Nipah virus vaccine. 2018 May 24 [Full text]

CEPI. CEPI awards contract worth up to USD$19 million to Oxford University and Janssen Vaccines to develop MERS, Lassa, and Nipah vaccines. 2018 Sep 27 [Full text]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in Fruit Bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C_{12}U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis 2014;20:372-9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6): 30916 [Full text]

Pallister J, Middleton D, Crameri G. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. J Virol 2009 Nov;83(22):11979-82 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. List of Blueprint priority diseases; accessed October 28, 2018 [Webpage]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed October 26, 2018 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Tables

Table 1: Goals and Milestones for NiV Infection Cross-Cutting Issues

Goals and Milestones for NiV Infection Cross-Cutting Issues **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.	1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
	2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.
2. Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.	1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
	2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Table 2: Goals and Milestones for NiV Diagnostics

Goals and Milestones for NiV Diagnostics **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.	1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).
	2. By 2021, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.
2. Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are	1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.
	2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
	3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.	4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
3. Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.	1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks.

Table 3: Goals and Milestones for NiV Therapeutics

Goals and Milestones for NiV Therapeutics **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.	1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	4. By 2020, generate a reliable source of m102.4 to be used in clinical trials.
2. Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.	1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
	2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
	3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Table 4: Goals and Milestones for NiV Vaccines

Goals and Milestones for NiV Vaccines

Nipah Virus Roadmap

STRATEGIC GOALS	MILESTONES
1. Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.	1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
	2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as the FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.
2. Develop and evaluate NiV vaccines for prevention of NiV disease in humans.	1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
	2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
	3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

Nipah WHO R&D Roadmap Taskforce Members

First Name	Middle Initial	Last Name	Degrees	Affiliation	City and country of affiliation
Christopher		Broder			
Kaw Bing		Chua			
Emmie		de Wit			
Emily		Gurley			
Kim		Halpin			
Stephen		Luby			
Gregg		Milligan			
Devendra		Mourya			
Shahana		Parveen			
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Lin-Fa		Wang			
Hana		Weingartl			

October 2018

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs)—diagnostics, therapeutics, and vaccines—against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001, and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (*Pteropus* species and several other bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species (including horses, cats, and dogs) were found to be infected with NiV on the farms involved in the outbreak. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen—Hendra virus (HeV)—which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat; disease also occurred in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

NiV infection in humans results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India. Some differences have been noted in the clinical features of infection, with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of MCMs to enable effective and timely emergency response to infectious disease epidemics. NiV infection is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this roadmap ranges from basic research to late-stage development of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include minimizing zoonotic NiV transmission, improving use of personal protective equipment (PPE), ensuring adequate hand hygiene and environmental hygiene, promoting effective community engagement, implementing adequate infection prevention and control practices, developing adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and promoting workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap but need to be addressed as part of a broader public health control strategy. Further research of NiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV (or related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

VISION

Robust MCMs to detect, prevent, and control human outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research represents a substantial challenge, since economic incentives to invest in Nipah research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with small and sporadic outbreaks. The development of a sustainable value proposition for industry and international philanthropic public-private partnerships is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate.
- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (biosafety level 4 [BSL-4]) conditions, which can increase the cost of MCM development.
- To date, NiV spillovers to human communities have been identified most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the *Pteropus* genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to the lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- While ferrets, Syrian hamsters, and interferon-alpha/beta receptor knockout (IFNAR-KO) mice are well-established animal models for NiV research, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection occurs in relatively small, focal outbreaks, the low disease incidence poses a major

challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop and manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries and to global stakeholders to ensure they understand the potential health, societal, and economic benefits of devoting resources to improving NiV surveillance, detection, prevention, and control measures.
- Obtaining additional prospective serosurveillance data of henipavirus exposure from susceptible animal species and proximate human populations in areas of predicted risk should be explored as a strategy to assess the potential of human spillover and to build preparedness for detection of human cases and for limiting exposure.
- Standardized and well-characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. Assays that can be used at lower biosafety levels are an important priority. WHO international standards should be used (when available) as calibrators and reported in units/ml to harmonize assay results.
- Clear criteria for downselection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or other regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.

- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, depend on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of *Pteropus* bats and potentially other bat species.
- Additional research is needed to optimize relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) for promoting development and evaluation of MCMs, particularly if investigators are required to use an alternative pathway (such as the United States Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after-challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunologic clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurologic sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes that may affect the epidemiologic and clinical features of disease over time and thereby influence MCM development.

- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole-genome sequencing of NiV are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume date palm sap, healthcare workers, and workers at the human-animal interface) and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify sources of private- and public-sector funding and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.

2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and that may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.
- **Generate** research tools to promote R&D of MCMs for NiV infection (e.g., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability of henipaviruses to improve understanding of their global distribution.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for downselection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.

- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an “affordable and fair” price is one that can reasonably be paid by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

DIAGNOSTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific, and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and investigation into the cause of infection is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.

- Owing to the biosafety precautions necessary when working with NiV, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods is problematic, given the rapid disease progression of NiV infection.
- *Pteropus* species (and perhaps other bat species) appear to carry other henipaviruses in addition to NiV and HeV, some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate using serologic assays which henipaviruses are in circulation. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.
- A TPP for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples to assess and validate diagnostic tests. As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6) identify who would have access to the samples; (7) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature), and have a high sensitivity and specificity for different NiV strains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

- Improved diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, particularly given the costs of regulatory approval.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotype neutralization assays and antigen-capture enzyme-linked immunosorbent assays [ELISAs]) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries: Bangladesh and India.

2. By 2021, identify funding and initiate creation of the virtual reference repository in Bangladesh and India, with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.

2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnose NiV infection during future outbreaks.

Priority Areas/Activities

Research

- **Create** a virtual repository of clinical reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- **Develop and evaluate** point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed.

Policy and commercialization

- **Develop** guidance on optimal strategies for the deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

THERAPEUTICS

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment of patients and PEP for exposed persons.
- In NiV-endemic areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis but do not have NiV infection. Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore, accurate and rapid diagnosis is critical.
- Studies in animals often evaluate the usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well a therapeutic agent will work in the field.
- NiV can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control– programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas, particularly during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to further assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed that the therapeutic window for m102.4 against a strain from Bangladesh/India was shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection and strengthen the therapeutic pipeline.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamic (PK/PD) relationship of promising therapeutic candidates.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will affect the response to therapeutic candidates and results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials to inform best-practice guidelines and evidence-based policy decisions.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.

2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials.

Strategic Goal 2: Develop and evaluate therapeutic agents for the treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for the treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as m102.4 and favipiravir) for treating and preventing NiV infection, including conducting studies in animal models and clinical trials as appropriate and feasible.
- **Continue to expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Develop, evaluate, and license** safe and effective therapeutic agents for the treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in affected areas to improve clinical management and supportive care of patients with NiV infection.

- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- **Generate** a reliable source of m102.4 to be used in clinical trials.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

VACCINES

Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development, and few companies are willing to invest in the generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could diminish the acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for the timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on the use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac HeV from Zoetis).

- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA’s Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen, and therefore multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed involving target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations and identify additional areas of potential NiV spillover.
- Additional sociological research is needed to explore perceptions and concerns of at-risk populations regarding NiV vaccine implementation.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will affect licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the need for licensure and future use of NiV vaccines. This consultation should include representatives from in-country regulators, other regulatory

agencies (such as the FDA or the European Medicines Agency), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of the humoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serologic assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.

- **Evaluate** the feasibility of generating multivalent vaccines (i.e., that protect against more than one disease) and determine whether or not such vaccines would be useful in future NiV control efforts.

Product development

- **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptance and promotion within the community.
- **Consider** developing a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for faster spread.

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

WHO. Nipah baseline situational analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017 [Full text]

Other Publications:

Arunkumar G, Chandni R, D T Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Med 2016;9(7):630-4 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 29 Nov 2016; site accessed 31 October 2018 [Web page]

Baseler L, Scott DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov 3;10(11):e0005120 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Pathog 2009 Oct 30;5(10):e1000642 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weir DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (US Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 20 Mar 2014 [Web page]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2):235–240 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-283 [Full text]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of infectious diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PKG, de los Reyes V, Sucaldito M, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015 Feb;21(2):328-31 [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia. Malaysian J Pathol 2010; 32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microb Infect 2013 Jun;2(6):e37 [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Curr Opin Virol 2017 Feb;22:97-104 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8:7604 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024 [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Abstract]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global virology I — identifying and investigating viral diseases. New York, NY: Springer US; 2015:125-46

Deka MA, Morshed N. Mapping disease transmission risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2).pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in fruit bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug;14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-7 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37. [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The role of animals in emerging viral diseases. Amsterdam: Elsevier; 2014:293-315 [Abstract]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerg Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis. 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2):e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lancet editors. Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137):2295 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55. [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013 Oct;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug; 15(8): 1229–1235 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015 Mar;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-227 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis. 2014;20:372–9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;6:30916 [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13;10:353 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9. Epub 2000 May 15 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sep 1;157(9):1635-41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Rahman SA. National guideline for management, prevention and control of Nipah virus infection including encephalitis. Dec 2011 [Full text]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-4 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Schmidt R, Beltzig LC, Sawatsky B, et al. Generation of therapeutic antisera for emerging viral infections. NPJ Vaccines 2018 Oct 5;3:42 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013; 8(7): e69544. Published online 2013 Jul 22 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Epub ahead of print]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Steffen DL, Xu K, Nikolov DB, et al. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Sun B, Jia L, Liang B, et al. Phylogeography, transmission, and viral proteins of Nipah virus. Virol Sin 2018; Oct 11; Epub ahead of print [Full text]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

Yu J, Lv X, Yang Z, et al. The main risk factors of Nipah disease and its risk analysis in China. Viruses 2018 Oct 19;10(10). pii:E572 [Full text] van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, influenza, Nipah, and Semliki Forest chimeric viruses with vesicular stomatitis virus: actions in the brain. J Virol 2017 Feb 28;91(6):e02154-16 [Full text]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah Virus, Thailand. Emerg Infect Dis 2005 Dec; 11(12):1949–1951 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

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Subject: Re: Nipah R&D roadmap and draft manuscript
Date: Monday, December 10, 2018 8:34:32 AM
Attachments: Nipah Roadmap manuscript.V1.2 Mourya.docx
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ATT00004.bin
Importance: High

Kristine Moore, MD, MPH

Medical Director

Dear Prof. Moore:

Sorry for responding late. I had been traveling therefore could not send my comments.

I have gone through the manuscript on the roadmap for R & D on Nipah. It is well-written and provides a comprehensive coverage of the topic. I have a few suggestions, indicated in-line in the attached manuscript.

With best regards,

D T Mourya

Version 1.2

A Research and Development (R&D) Roadmap for Nipah Virus

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The work for this project was completed with generous support from the Wellcome Trust.

The authors would also like to acknowledge Mr. Carlos R. Cruz; CIDRAP, University of Minnesota; Minneapolis, Minnesota, USA, for project management related to the WHO R&D Roadmaps project.

Version 1.2

Word Count: 3,695 (text only, not including References or Tables)

Introduction

Nipah virus (NiV), a paramyxovirus of the Henipavirus genus, was first identified after an outbreak of respiratory illness in pigs and severe encephalitic disease in humans occurred in Malaysia and Singapore in 1998-1999 (Chua 1999, Chew 2000, Parashar 2000). At least 265 human cases of encephalitis were identified in Malaysia and most had close contact with pigs (Chua 2010, Parashar 2000). In addition, 11 abattoir workers in Singapore became ill following contact with pigs imported from Malaysia; eight patients presented with signs of encephalitis and three presented with atypical pneumonia (one of which later also had evidence of encephalitis) (Paton 1999). No new outbreaks have been reported in these countries since May 1999; however, NiV infection was subsequently recognized in Bangladesh in 2001 (Hsu 2004) and nearly annual outbreaks have occurred in that country since then (WHO 2018). Outbreaks also have been recognized in eastern India and, in 2018, an outbreak occurred in the southern Indian state of Kerala (Arunkumar 2018, Sharma 2018, Chattu 2018). To date, nearly 350 cases of NiV infection have been identified in Bangladesh and India (WHO 2018). Possible emergence of NiV in a third geographic area is evidenced by an outbreak of an unidentified henipavirus that occurred among horses and humans in the Philippines in 2014 (Ching 2015). Although detailed genomic information for the virus is limited, experts agree that the causative agent was either NiV or a virus closely related to NiV. The Henipavirus genus includes another pathogen, Hendra virus, which predominantly causes infection in horses in Australia and can also lead to human disease, usually following contact with infected horses (Ksiazek 2011).

The most common clinical syndrome associated with NiV infection is severe, rapidly progressive encephalitis (Goh 2000, Hossain 2008). The main presenting features include fever, headache, dizziness, vomiting, and altered mental status. NiV infection may result in late-onset

encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae (Tan 2002, Goh 2000). Some patients also have respiratory involvement or respiratory illness alone, which can progress to acute respiratory distress syndrome (Hossain 2008, Paton 1999). The overall case-fatality rate for the Malaysia outbreak was approximately 40%, whereas case-fatality rates during outbreaks in Bangladesh and India have generally ranged from 65% to 100% (Hossain 2008, WHO 2018). Genomic sequencing has demonstrated that there are multiple strains of NiV, which may account for the differences noted in clinical features and outcomes between Malaysia and Bangladesh (Mire 2016).

Fruit bats of the Pteropus genus are considered the primary natural reservoir for NiV. In the Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and possibly limited human-to-human NiV transmission (Chua 2010). Additionally, several other domestic animal species in the outbreak area (including horses, cats, and dogs) were found to be infected with NiV (Chua 2000). In outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap (Islam 2016, Luby 2006, Rahman 2012) and subsequent person-to-person transmission. While person-to-person transmission was apparently minimal in the Malaysia outbreak, it has played a larger role in amplifying outbreaks in Bangladesh and India (Gurley 2007, Luby 2009, Chadha 2006, Arunkumar 2018). This may, in part, be related to the greater propensity toward respiratory disease in NiV strains found in South Asia and transmission via droplet spread (Spiropoulou 2018). The potential for droplet spread is supported by a recent study that demonstrated hamsters could be infected when exposed to NiV via a small-particle aerosol (Escaffre 2018). Given the potential for respiratory transmission of NiV and the recent experience with Ebola in West

Africa, some have raised concerns about the possibility of larger NiV outbreaks if the virus takes hold in a crowded or urban area (Donaldson 2018) or about the pandemic potential of NiV if a more human-adapted strain emerges (Luby 2013).

Pteropus fruit bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Other fruit bats of the Pteropodidae family also may harbor NiV, and such bats can be found across Africa and parts of the Middle East. Evidence of NiV infection (via virus isolation, serologic testing, or nucleic acid detection using real-time polymerase chain reaction [RT-PCR]) has been found in Pteropus bat species and other bat species in geographically diverse regions, including South Asia (Bangladesh and India); Southeast Asia (Cambodia, Indonesia, Malaysia, the Philippines, Thailand, and Vietnam); the Western Pacific region (Papua New Guinea); and Africa (Madagascar and Ghana) (Breed 2010, Epstein 2008, Hasebe 2012, Hayman 2008, Homaira 2010, Iehlé 2007, Reynes 2005, Sendow 2013, Wacharapluesadee 2005, WHO 2018, Yob 2001). This wide potential range of the NiV natural reservoir raises concerns about the eventual occurrence of outbreaks in new geographic areas (Deka 2018, Donaldson 2018), which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.

WHO has determined that Nipah virus is a priority disease for epidemic preparedness (WHO 2018). To improve epidemic response for this infection, in the fall of 2017, WHO embarked on development of a research and development (R&D) roadmap for NiV disease; this report provides a summary of the key elements of that roadmap, including important challenges and barriers that need to be addressed to ensure that diagnostics, therapeutics, and vaccines are

developed, manufactured, and readily available when needed *[provide link to full roadmap here when available]*.

The NiV R&D roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one medical countermeasure category), diagnostics, therapeutics, and vaccines. The strategic goals and milestones identified in each section are focused on key achievements for the next 5 years; ~~the roadmap milestones will be tracked over time,~~ the roadmap milestones will be tracked over time, with periodic assessment of progress and updates to the roadmap as needed. The roadmap is specific to R&D for disease in humans; however, further research of NiV in animal species, including development of appropriate medical countermeasures targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV in humans and virus transmission can occur at the human-animal interface.

Cross-Cutting Issues

A major challenge for development of diagnostics, therapeutics, and vaccines for NiV is accessing adequate funding to support research. Economic incentives to invest in such research are not readily apparent because the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence has, so far, been low with only small, sporadic outbreaks. Advocacy to policy makers in affected countries and to global stakeholders is needed to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures. As part of this process, the risk profile for NiV and related henipaviruses needs to be further defined through improving disease surveillance in areas of known risk and obtaining prospective serosurveillance data on henipavirus exposure for susceptible animal species and proximate

human populations in areas of predicted risk. Human health, animal health, and wildlife officials should be engaged in these efforts as part of a long-term collaborative One Health approach (Chattu 2018, Kulkarni 2013).

Another important issue is the need to optimize relevant animal challenge models for promoting development and evaluation of medical countermeasures for NiV infection, particularly if investigators are required to use an alternative pathway (such as the US Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval (Rockx 2014). For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for therapeutic or vaccine development; (3) determine when after challenge medical countermeasures should be administered in animal models to best mimic realistic timing of such use in humans; (4) bridge data from animal models to humans, such as identifying thresholds of vaccine protection to determine appropriate human doses; and (5) identify the best models for studying chronic (relapsing) infection (Bossart 2012, Debuysscher 2013, Dhondt 2013, Johnston 2015, Mathieu 2015, Satterfield 2016). Currently, the African green monkey is regarded as the most relevant animal model for evaluating candidate therapeutics and vaccines intended for use in humans (Geisbert 2010, Johnston 2015), and studies involving nonhuman primates may be required for licensure of therapeutics and vaccines via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns, however, constrain the use of this animal model. In addition to optimizing animal models, standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains are needed to move R&D of medical countermeasures for NiV infection forward (Satterfield 2016).

To inform development of optimal NiV medical countermeasures, additional research also is needed on the virology, immunology, and pathogenesis of NiV in humans and animals. This includes issues such as evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection.

To respond to these challenges, the strategic goals and milestones for cross-cutting issues in the NiV R&D roadmap focus primarily on developing a sustainable value proposition and identifying funding sources to promote R&D, availability, and accessibility of NiV medical countermeasures, optimizing animal models for research, and generating standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV medical countermeasures (Table 1).

Diagnostics

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The most commonly used methods for diagnosing NiV infection include virus isolation, enzyme-linked immunosorbent assays (ELISAs) for serologic detection of IgM or IgG antibodies, and real time-polymerase chain reaction (RT-PCR) nucleic acid testing. Virus isolation requires biosafety level 4 (BSL-4) capabilities and is generally only performed in reference laboratories for specific purposes, such as to confirm a new outbreak or to identify the virus in new host species (Wang 2012). During the Malaysia outbreak, the US Centers for Disease Control and Prevention (CDC) developed a capture ELISA for detection of IgM antibodies and an indirect ELISA for detection of IgG antibodies (Daniels 2001); since then, additional ELISA tests have been developed (Wang 2012) and serologic testing is often used for

case confirmation during outbreaks (Chadha 2006, Gurley 2007, Islam 2016, Lo 2012, Luby 2006, Luby 2009). RT-PCR, which can be performed on throat and nasal swabs, cerebrospinal fluid, urine, and blood, is also often used for diagnostic testing (Chadha 2006, Guillaume 2004, Lo 2012). Quantitative RT-PCR tests have been developed for quantifying viral replication; such tools can be used to measure viral load reduction when screening vaccine candidates and potential therapeutics (Jensen 2018). More recently, pseudotyped-based serum neutralization assays for NiV detection that use vesicular stomatitis virus systems have been developed; these assays potentially can be used under BSL2 conditions in laboratories with limited diagnostic capabilities and appear to offer improved sensitivity and specificity (Kaku 2009, Kaku 2012, Tamin 2009). Antigen-capture ELISAs using monoclonal antibodies also have been developed, which could result in more rapid diagnosis (Chiang 2010). These newer technologies hold promise; however, more information is needed regarding their performance characteristics, particularly under field conditions.

Initial signs and symptoms of NiV infection are nonspecific (CDC 2014) and the diagnosis often is not considered at the time of presentation, which can delay outbreak detection and implementation of infection control measures. Furthermore, laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited (Chua 2013, Wang 2012); thus, etiologic diagnosis is not always pursued. To address these issues, rapid point-of-care or near-patient diagnostic tests that involve minimal requirements for laboratory infrastructure are needed to identify cases quickly, promote outbreak detection, ensure timely implementation of infection control measures, and ultimately improve patient outcomes, once therapeutic options become available.

Development of new diagnostics is impeded by lack of clinical samples for assay assessment and validation; therefore, a virtual repository (with specimens being held and maintained in the countries of origin) of clinical samples is needed to move NiV diagnostic testing forward. Development of such a repository requires addressing the following: (1) determining what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid); (2) outlining the purposes of sample collection; (3) determining what organizations will be responsible for activities related to creating and maintaining the repositories; (4) establishing standardized protocols for sample collection and maintenance; (5) establishing an appropriate governance structure; (6) identifying who would have access to the samples; (7) prioritizing use of samples and sample distribution; and (8) ensuring that material transfer agreements are in place. A target product profile (TPP) for NiV diagnostics that identifies the key use cases and optimal and desirable characteristics also is needed to guide the development of promising new diagnostic assays.

To address these issues, the strategic goals and milestones for diagnostic testing outlined in the NiV R&D roadmap focus on creation of a virtual reference repository, development of highly sensitive and specific point-of-care or near-patient NiV diagnostic tests, and enhancement of laboratory diagnostic preparedness to promote early detection of NiV in areas of known or potential spillover risk (Table 2).

Therapeutics

Safe, well-tolerated, and effective therapeutic agents are not readily available for treatment of NiV infection. During the 1998-1999 NiV outbreak in Malaysia, ribavirin was used to treat 140 patients; findings from treated patients were compared to those of 54 historical controls who became ill before ribavirin was available or who refused treatment (Chong 2001).

Results demonstrated a statistically significant reduction in mortality among treated patients (36%); however, the use of historical controls may have biased this finding. No additional clinical studies using ribavirin have been conducted, and limited studies in animal models have not demonstrated efficacy of ribavirin following NiV or Hendra virus challenge (Rockx 2010, Georges-Courbot 2006). Chloroquine also has been studied in animal models, but has not shown any therapeutic benefit, either alone or in combination with ribavirin (Freiberg 2010, Pallister 2009). Based on these findings, these approaches are not considered viable options for treatment of NiV infection.

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A more promising therapeutic option is the development of monoclonal antibodies (mAbs) against viral envelope proteins that can neutralize NiV via passive administration (Broder 2012, Guillaume 2004). Therapy with anti-NiV mAbs may be appropriate for early treatment and for post-exposure prophylaxis (PEP) to prevent illness in exposed persons, such as healthcare workers, family caregivers, and persons exposed to infected livestock. Researchers have demonstrated that the human mAb, m102.4, offers protection against lethal NiV challenge in animal models (Geisbert 2014, Bossart 2009), and it has been provided under compassionate use for a small number of individuals exposed to henipaviruses (Broder 2013). Recently, a phase 1 clinical trial of m102.4 with 40 human participants was completed in Australia, but results are not yet available (ANZCTR). M102.4 was deployed for use during the 2018 NiV outbreak in Kerala, India, but was not used because the outbreak was brought under control quickly (Spiropoulou 2018). One recent study in nonhuman primates showed that the therapeutic window for m102.4 may vary by virus strain, which is a consideration for further preclinical and clinical study of this agent (Mire 2016).

Other potential options for NiV treatment include favipiravir (a small-molecule antiviral that inhibits RNA-dependent RNA polymerase), fusion inhibitory peptides (peptides that inhibit virus-mediated membrane fusion), and remdesivir (also known as GS-5734, which is a monophosphoramidate prodrug of an adenosine analogue). Researchers recently demonstrated efficacy of favipiravir in hamsters challenged with a lethal dose of NiV (Dawes 2018), supporting proof-of-concept for favipiravir as a possible therapy against NiV infection. Studies in animal models also have been conducted for fusion inhibitory peptides; for example, investigators recently reported that lipopeptides delivered via the respiratory route can prevent lethal NiV infection in hamsters and nonhuman primates (Mathieu 2018). Remdesivir is a small-molecule antiviral that is currently being evaluated in clinical studies as a therapeutic option for treating Ebola virus disease. Investigators recently demonstrated in vitro inhibition by remdesivir against a number of different viruses, including paramyxoviruses (Lo 2017). While these agents are promising, further preclinical data are needed to assess their utility. In addition, efforts are needed to expand the therapeutic drug pipeline for treatment of NiV by supporting basic science research toward discovery of additional novel agents that may be more aligned with optimal and desired characteristics for anti-NiV therapy.

Key additional considerations for moving NiV therapeutics forward include: (1) obtaining additional preclinical and clinical data on the safety, tolerability, pharmacokinetics, pharmacodynamics, and efficacy of the most promising novel treatments for NiV disease; (2) ensuring that vetted and approved protocols for conducting clinical efficacy trials of promising therapeutics are in place in at-risk countries to be implemented during future NiV outbreaks; and (3) determining which agents may be appropriate for PEP, what types of exposures warrant PEP, and the feasibility of PEP distribution in both endemic and at-risk areas. To address these issues,

the goals and milestones for therapeutics in the NiV R&D roadmap focus on continuing to move promising agents forward into clinical trials and on enhancing preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Vaccines

In June 2017, WHO released a TPP for NiV vaccine (WHO 2017) and several candidate vaccines are in the pipeline (Satterfield 2016); however, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generating new NiV vaccines, which creates an important barrier to ensuring that safe and effective NiV vaccines are made available. To date, clinical trials involving NiV vaccines have not been conducted and vaccines are still in preclinical stages of research. The two primary approaches for NiV vaccine development include creating a subunit vaccine against one or both of the outer-membrane proteins (the glycoprotein [G] or the fusion protein [F]) and using live-virus vectored vaccine platforms, including vesicular stomatitis virus, adenovirus, rabies virus, measles virus, Venezuelan equine encephalitis virus, canarypox virus, and Newcastle disease virus (Broder 2013, Satterfield 2016). A Hendra virus subunit vaccine involving soluble glycoprotein (sG) has been developed and is commercially available for use in horses (Middleton 2014), which supports this option as a promising approach for developing a human NiV vaccine.

The Coalition for Epidemic Preparedness Innovations (CEPI), which is a partnership of government agencies, industry, academia, philanthropy, and intergovernmental institutions aimed at providing funding for R&D of new vaccines against pathogens with epidemic potential, has recently provided financial support to promote the development of several NiV candidate vaccines. In May 2018, CEPI awarded a US$ 25 million contract to Profectus BioSciences, Inc. and Emergent BioSolutions Inc. to develop an NiV vaccine that is based on the same technology

as that used for the Hendra virus vaccine (CEPI 2018). In September 2018, CEPI announced plans to award approximately US$ 2 million to the Jenner Institute at the University of Oxford and Janssen Vaccines & Prevention BV for preclinical development of a replication-deficient simian adenoviral-vectored vaccine, derived from an adenovirus originally isolated from chimpanzees (CEPI 2018). These efforts will support preclinical and early clinical development of NiV vaccines.

Although phase 1 and 2 clinical vaccine trials can be conducted in either endemic or non-endemic regions, phase 3 clinical efficacy trials will need to be conducted in endemic areas. Because NiV infection has, to date, occurred as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting such trials, in terms of achieving a sufficient sample size to estimate vaccine efficacy with adequate statistical power. It may be possible to address this issue by enhancing case detection through improved surveillance and by combining clinical trial data over time, including across outbreaks. While it is critical to focus on conducting ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not feasible.

Additional research is needed in several areas to support development of NiV vaccines. First, identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed, particularly to expedite possible licensing through nontraditional regulatory pathways (Satterfield 2016). Correlates of protection may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized. Second, additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV (Mathieu 2015, Prescott 2012,

Satterfield 2016). Since neutralizing antibodies are likely the primary mediator of protection against NiV infection (Satterfield 2016), research in this area should focus primarily on the humoral immune response for driving vaccine development. Third, further work in animal models is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date (Satterfield 2016). Finally, mathematical modelling and forecasting may be useful in creating a current estimate of the reproductive number for NiV, gauging the potential impact of NiV vaccines, and assessing needs for a vaccine stockpile (WHO 2017).

The immediate goals and milestones in the vaccine section of the NiV R&D roadmap focus on moving vaccine candidates forward into clinical trials and promoting development of guidance on requirements for clinical trials, regulatory pathways, and other considerations relevant to licensure of a vaccine against NiV.

Conclusion

The NiV R&D roadmap contains a series of concrete steps to be accomplished over the next 5 years to accelerate the development of NiV medical countermeasures forward. NiV infection is likely more prevalent than currently recognized; therefore, rapid, easy to use point-of-care or near-patient diagnostic tests and enhanced case detection and surveillance in areas of known or suspected NiV spillover will improve understanding of the true burden of NiV disease. These efforts, in turn, may provide information to further support the public health value proposition for NiV medical countermeasure development and, by increasing case detection, may enhance feasibility of conducting clinical trials of promising vaccines and therapeutics in endemic areas.

References

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 31 October 2018 [Web page]

Arunkumar G, Chandni R, DT Mourya DT, et al. Outbreak investigation of Nipah virus disease in Kerala, India, 2018. J Infect Dis 2018; published ahead of print 26 October 2018 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10):e1000642 [Full text]

Breed AC, Meng Yu, Barr, et al. Prevalence of Henipavirus and Rubulavirus antibodies in Pteropid bats, Papua New Guinea. Emerg Infect Dis 2010 Dec;16(12):1997-9 [Full text]

Broder CC. Henipavirus outbreaks to antivirals: the current status of potential therapeutics. Curr Opin Virol 2012 Apr 2(2):176-87 [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13 [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

CEPI (Coalition for Epidemic Preparedness Innovations). CEPI awards $25 million contract to Profectus BioSciences and Emergent BioSolutions to develop Nipah virus vaccine. 2018 May 24 [Full text]

CEPI. CEPI awards contract worth up to USD$19 million to Oxford University and Janssen Vaccines to develop MERS, Lassa, and Nipah vaccines. 2018 Sep 27 [Full text]

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis 2006 Feb;12(2): 235-40 [Full text]

Chattu VK, Kumar R, Kumary S, et al. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. J Family Med Prim Care 2018 Mar-Apr;7(2):275-83 [Full text]

Chew MH, Arguin PM, Shay DK, et al. Risk factors for Nipah virus infection among abattoir workers in Singapore. J Infect Dis 2000 May;181(5):1760-3 [Abstract]

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7:115 [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331 [Full text]

Chong HT, Kmarulzaman A, Tan CT. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol 2001 Jun;49(6):810-3 [Abstract]

Chua KB. Epidemiology, surveillance and control of Nipah virus infections in Malaysia Malaysian J Pathol 2010;32(2):69-73 [Full text]

Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. Science 2000 May 26;288:1432-35 [Full text]

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet 1999 Oct 9;354(9186):1257-9 [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):e37 [Full text]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001 Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;8(1):7604 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

Deka MA, Morshed N. Mapping diseaset risk of Nipah virus in South and Southeast Asia. Trop Med Infect Dis 2018 May 30;3(2):pii:E57 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

Donaldson H, Lucey D. Enhancing preparation for large Nipah outbreaks beyond Bangladesh: Preventing a tragedy like Ebola in West Africa. Int J Infect Dis 2018 Jul;72:69-72 [Full text]

Epstein JH, Prakash V, Smith CS, et al. Henipavirus Infection in Fruit Bats (Pteropus giganteus), India. Emerg Infect Dis 2008 Aug; 14(8):1309–1311 [Full text]

Escaffre O, Hill T, Ikegami T, et al. Experimental infection of Syrian hamsters with aerosolized Nipah virus. J Infect Dis 2018 Oct 5;218(10):1602-10 [Abstract]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690 [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25;6(242):242ra82 [Full text]

Georges-Courbot MC, Contamin H, Faure C, et al. Poly(I)-Poly(C_{12}U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2006 May; 50(5):1768–1772 [Full text]

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35 [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Guillaume V, Lefeuvre A, Faure C, et al. Specific detection of Nipah virus using real-time RT-PCR (TaqMan). J Virol Methods 2004 Sep 15;120(2):229-37 [Abstract]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-7 [Full text]

Hasebe F, Thuy NT, Inoue S, et al. Serologic evidence of Nipah virus infection in bats, Vietnam. Emerg Infect Dis 2012:18:536–7 [Full text]

Hayman DT, Suu-Ire R, Breed AC, et al. Evidence of henipavirus infection in West African fruit bats. PloS One 2008 Jul 23;3(7):e2739 Full text]

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis 2008 Apr 1;46(7):977-84 [Full text]

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect 2010 Nov;138(11):1630-6 [Abstract]

Hsu VP, Hossain MJ, Parshar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. Emerge Infect Dis 2004 Dec;10(12):2082-7 [Full text]

Iehlé C, Razafitrimo G, Razainirina, et al. Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. Emerg Infect Dis 2007 Jan;13(1):159-61 [Full text]

Islam MS, Sazzad HM, Satter SM, et al. Nipah virus transmission from bats to humans associated with drinking traditional liquor made from date palm sap, Bangladesh, 2011-2014. Emerg Infect Dis 2016 Apr;22(4):664-70 [Full text]

Jensen KS, Adams R, Bennett RS, et al. Development of a novel real-time polymerase chain reaction assay for the quantitative detection of Nipah virus replicative viral RNA. PLoS One 2018 Jun 19;13(6):e0199534 [Full text]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817 [Full text]

Kaku Y, Noguchi A, Marsh GA, et al. Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. J Virol Methods 2012 Jan;179(1):226-32 [Abstract]

Kaku Y, Noguchi A, Marsh GA, et al. A neutralization test for specific detection of Nipah virus antibodies using pseudotyped vesicular stomatitis virus expressing green fluorescent protein. J Virol Methods 2009 Sep;160(1-2):7-13 [Abstract]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Res 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Lo MK, Jordan R, Arvey A, et al. GS-5734 and its parent nucleoside analog inhibit Filo-, Pneumo-, and Paramyxoviruses. Sci Rep 2017; 7: 43395 [Full text]

Lo MK, Lowe L, Hummel KB, et al. Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerg Infect Dis 2012;18(2):248–55 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Abstract]

Luby SP, Hossain MJ, Gurley ES, et al. Recurrent Zoonotic Transmission of Nipah Virus into Humans, Bangladesh, 2001–2007. Emerg Infect Dis 2009 Aug;15(8):1229-35 [Full text]

Luby SP, Rahman M, Hossain MJ, et al. Foodborne transmission of Nipah virus, Bangladesh. Emerg Infect Dis 2006 Dec;12(12):1888-94 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

Mathieu C, Porotto M Fogueira TN, et al. Fusion inhibitory lipopeptides engineered for prophylaxis of Nipah virus in primates. J Infect Dis 2018 Jun 20;218(2):218-27 [Abstract]

Middleton D, Pallister J, Klein R, Feng YR, Haining J, Arkinstall R, et al. Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. Emerg Infect Dis 2014;20:372-9 [Full text]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6): 30916 [Full text]

Pallister J, Middleton D, Crameri G. Chloroquine administration does not prevent Nipah virus infection and disease in ferrets. J Virol 2009 Nov;83(22):11979-82 [Full text]

Parashar UD, Sunn LM, Ong F, et al. Case-control study of risk factors for human infection with a new zoonotic paramyxovirus, Nipah virus, during a 1998-1999 outbreak of severe encephalitis in Malaysia. J Infect Dis 2000 May;181(5):1755-9 [Abstract]

Paton NI, Leo YS, Zaki SR, et al. Outbreak of Nipah-virus infection among abattoir workers in Singapore. Lancet 1999 Oct 9;354(9186):1253-6 [Abstract]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 Sept;157(9):1635–41 [Full text]

Rahman MA, Hossain MJ, Sultana S, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector Borne Zoonotic Dis. 2012 Jan;12(1):65-72 [Abstract]

Reynes JM, Counor D, Ong S, et al. Nipah virus in Lyle's flying foxes, Cambodia. Emerg Infect Dis 2005 Jul;11(7):1042-7 [Full text]

Rockx B. Recent developments in experimental animal models of Henipavirus infection. Pathog Dis 2014 Jul;71(2):199-206 [Abstract]

Rockx B, Bossart KN, Feldmann F, et al. A novel model of lethal Hendra virus infection in African green monkeys and the effectiveness of ribavirin treatment. J Virol 2010 Oct;84(19):9831-9 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-5 [Full text]

Sendow I, Ratnawati A, Taylor T, et al. Nipah Virus in the Fruit Bat *Pteropus vampyrus* in Sumatera, Indonesia PLoS One 2013;8(7):e69544 [Full text]

Spiropoulou C. Nipah virus outbreaks: still small but extremely lethal. J Infect Dis 2018; published ahead of print, 26 October 2018 [Full text]

Sharma V, Kaushik S, Kumar R, et al. Emerging trends of Nipah virus: a review. Rev Med Virol 2018 Sep 24:e2010 [Abstract]

Tamin A, Harcourt BH, Lo MK, et al. Development of a neutralization assay for Nipah virus using pseudotype particles. J Virol Methods 2009 Sep;160(1-2):1-6 [Full text]

Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. Ann Neurol 2002 Jun;51(6):703-8 [Abstract]

Wacharapluesadee S, Lumlertdacha B, Boongird K, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005 Dec;11(12):1949-51 [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

WHO. List of Blueprint priority diseases; accessed October 28, 2018 [Webpage]

WHO. Target product profile for Nipah virus vaccine. June 2017 [Full text]

WHO. Nipah virus; accessed October 26, 2018 [Webpage]

WHO. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis in WHO South-East Asia region, 2001-2018. May 2018 [Full text]

Yob JM, Field H, Rashdi AM, et al. Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. Emerg Infect Dis 2001;7:439–41 [Full text]

Tables

Table 1: Goals and Milestones for NiV Infection Cross-Cutting Issues

Goals and Milestones for NiV Infection Cross-Cutting Issues **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.	1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
	2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.
2. Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.	1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
	2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Table 2: Goals and Milestones for NiV Diagnostics

Goals and Milestones for NiV Diagnostics **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.	1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).
	2. By 2021, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.
2. Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are	1. By 2019, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.
	2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
	3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.	4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
3. Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.	1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks.

Table 3: Goals and Milestones for NiV Therapeutics

Goals and Milestones for NiV Therapeutics **Nipah Virus Roadmap**	
STRATEGIC GOALS	**MILESTONES**
1. Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.	1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas and develop plans for operationalizing the protocol.
	4. By 2020, generate a reliable source of m102.4 to be used in clinical trials.
2. Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.	1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
	2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
	3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and (possibly) efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Table 4: Goals and Milestones for NiV Vaccines

Goals and Milestones for NiV Vaccines
Nipah Virus Roadmap

STRATEGIC GOALS	MILESTONES
1. Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.	1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
	2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as the FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.
2. Develop and evaluate NiV vaccines for prevention of NiV disease in humans.	1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
	2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
	3. By 2023, complete the following if phase 3 trials are considered feasible: (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a collaborative and transparent strategy for determining which candidate vaccines will go forward into phase 3 trials.

Nipah WHO R&D Roadmap Taskforce Members

First Name	Middle Initial	Last Name	Degrees	Affiliation	City and country of affiliation
Christopher		Broder			
Kaw Bing		Chua			
Emmie		de Wit			
Emily		Gurley	MPH, PhD	Johns Hopkins Bloomberg School of Public Health	Baltimore, Maryland, USA
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To:	Steve Luby; Kris Moore
Cc:	christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; kim.halpin@csiro.au; gnmillig@utmb.edu; mahmudur57@gmail.com; dtmourya@gmail.com; pyr3@cdc.gov; shahana@icddrb.org; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca; K.Cook@wellcome.ac.uk; J.Golding@wellcome.ac.uk; benassiv@who.int; jto@umn.edu; mto@umn.edu; preziosim@who.int
Subject:	RE: Nipah R&D roadmap and draft manuscript
Date:	Tuesday, December 4, 2018 2:24:41 PM
Attachments:	Nipah Roadmap manuscript.V1.2_eg.docx Nipah TF members.degrees and affiliations sl_eg.docx

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Dear Kris,

I agree that the manuscript reads well. I've attached a couple of minor comments and edits and my affiliation information.

I also agree with Steve's suggestion about how to address the limitations for development of therapeutics.

All the best,
Emily

From: Steve Luby [mailto:sluby@stanford.edu]
Sent: Friday, November 30, 2018 10:08 PM
To: Kris Moore <kamoore@umn.edu>
Cc: christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; Emily Gurley <egurley1@jhu.edu>; kim.halpin@csiro.au; gnmillig@utmb.edu; mahmudur57@gmail.com; dtmourya@gmail.com; pyr3@cdc.gov; shahana@icddrb.org; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca; K.Cook@wellcome.ac.uk; J.Golding@wellcome.ac.uk; benassiv@who.int; jto@umn.edu; mto@umn.edu; preziosim@who.int
Subject: Re: Nipah R&D roadmap and draft manuscript

Kris,

The manuscript reads well. Thanks for all of your effort in pulling these ideas together and communicating this so clearly.

I agree with the Wellcome reviewer that there is some risk of favoring one therapeutic by including a specific milestone to advance it.

If the R&D roadmap is successful in in catalyzing the development of multiple therapeutic agents a limiting constraint to assessing their effectiveness will be the number of patients with Nipah who can be enrolled. A broader discussion on how best to allocate such a scarce resource seems more appropriate than assuming that we should focus all resources on the first available agent.

Personally, I would favor an R&D roadmap for therapeutics that noted this issue, and proposed an approach to engage in a broad open conversation on how we allocate multiple scarce resources, donor interest, donor funding, and patients identified early enough to be candidates for therapeutic trials.

I have attached my identifying information.

Steve

Kris Moore wrote on 11/20/2018 2:07 AM:

> Dear Nipah R&D Roadmap Taskforce members:
>
> On behalf of CIDRAP, Wellcome, and WHO, attached please find the final version of the Nipah R&D Roadmap, a draft manuscript for publication, and a table for you to complete with personal information.
>
> - The roadmap is undergoing final review and clearance at WHO.
> - The draft manuscript is also being reviewed by WHO; their edits are not yet reflected in this version, so it may change. Before submission, once we have incorporated all comments, we will send a final version to you again for your approval and sign off.
>
> Here are some high-level thoughts regarding the manuscript:
>
> - We thought it was essential to provide some context for where things stand with the current status of diagnostics, therapeutics, and vaccines, so we added a few short paragraphs in order to provide this perspective. This was done for the MERS-CoV roadmap and we all agreed that this was the right approach.
> - We also decided to highlight some of the key challenges and barriers, as we did in each of the roadmaps—so we used our best judgement to focus on what we thought were the most critical issues. We also did quite a bit of reworking to put the material into a more readable format.
> - With regard to the roadmap itself, we decided to include tables of the goals and milestones, since it wasn't possible to cover everything and these seem to be the most critical. We should be able to provide a link to the full roadmap once it's posted, assuming each is posted before publication.
> - We decided that the manuscripts should be no more than about 3,500 words, so we aimed for that length.
>
> We would very much appreciate your help by doing the following:
>
> 1. Review the manuscript and submit your comments via track changes on the Word document.
> 2. Complete the attached table, so you are cited correctly in the manuscript.
> 3. If, as you review the manuscript, you find anything glaring that needs to

> change in the roadmap, please let us know. We still have time to make minor changes to the roadmap. Again, you can use track changes on the Word document of the roadmap.

Please also address the following question from Wellcome regarding the roadmap: For Nipah therapeutics we mention "By 2020 generate a reliable source of m102.4 to be used in clinical trials. We have not mentioned a specific vaccine or therapeutic for Lassa—is there a risk of promoting one candidate over others? This may be a redundant question since the Nipah field is far less advanced than other diseases."

WE WOULD VERY MUCH APPRECIATE YOUR COMMENTS AND FEEDBACK BY MONDAY, DECEMBER 3.

Thank you again for your support of this important effort, and thank you in advance for reviewing the manuscript. Please send any comments to me at kamoore@umn.edu.

Warm regards,

Kris

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BOHRN

THE BAT / ONE HEALTH RESEARCH NETWORK

OVERVIEW

There is a long tradition of international cooperation in scientific research. Scientific networks can be instrumental to bridge cultural boundaries and build trust, addressing the global threat of emerging infectious diseases. Current trends in scientific research funding, specifically competition for ever-decreasing research budgets, necessitate international collaborations focused around specific and prioritized research questions.

Scientists posit that the Ebola outbreak of 2014 began with a Guinean toddler playing in a bat roost amongst fruit bats that had migrated 2,500 miles from Central Africa. Understanding bat migration patterns, the effect of humans on those patterns, and the challenges of conducting disease surveillance in free-range bat populations, will enable relevant policy makers to better identify, plan, and prepare for the next pandemic. Additionally, research coordinated networks have the ability to significantly impact threat reduction by identifying and prioritizing coordinated approaches to close these and other pressing knowledge gaps.

The Defense Threat Reduction Agency (DTRA) Biological Threat Reduction Program (BTRP) is sponsoring a multi-regional disease surveillance research coordinated network to mitigate the threat of bat-associated pathogens of security concern. This threat reduction network will identify and connect interdisciplinary expertise, convening an agile group to adapt to a wide spectrum of arising challenges and threats. The Bat / One Health Research Network (BOHRN) will enable shared learning and research opportunities, establish new research projects, and facilitate joint applications for funding; thus increasing the opportunity for peer review, especially if a cross-regional and multi-disciplinary team of authors are involved

The BOHRN kick-off meeting coincided with the 2nd International Symposium on Infectious Diseases of Bats in Fort Collins, CO on 29 June 2017. During this meeting, the group began preliminary actions to build a self-sustainable disease surveillance network and identified initial network objectives needed to develop a comprehensive research strategy to address bat-associated disease threats and mitigation solutions.

NETWORK OBJECTIVES

- Facilitate interdisciplinary collaboration to identify research goals and needs for bat-borne disease research and broader threat reduction
- Create a common action plan that yields collaborative and sustainable projects which: (1) better inform policy makers; (2) better inform scientific community regarding funding targets and gaps in areas of research and development; (3) better define threat to global health security from bat-associated pathogens; and (4) improve national, regional, and global capacity to detect and respond to pathogens of security concern
- Enable better communication, coordination, and outreach at the research and conservation interface

APPROACH AND IMPACT

BOHRN has four thematic focus areas, which were characterized and developed into research Working Groups at the kick-off meeting. These Working Groups (described below) will operationalize the network objectives by serving as subdivisions to the overall network to foster multi-national and multidisciplinary participation and mentorship. Each member of the BOHRN will identify with at least one Working Group based on field of research/practice. Working Group members will identify and prioritize research gaps and needs, and research project ideas will be solicited from BOHRN membership to address the identified gaps and needs.

Working Group 1: Host / pathogen biology interactions; specifically: (1) Bat physiology and immunology; (2) Bat pathogen community biology (e.g., co-infections and co-morbidities); and (3) Distribution of pathogens among species

Working Group 2: Pathogen surveillance, diagnostic capacity, and epidemiology; specifically: (1) Molecular epidemiology; (2) Distribution of pathogens geographically and phylogenetically; and (3) Detection, diagnosis, and reporting of bat-associated pathogens

Working Group 3: Ecology (bat, domesticated animal, and wildlife interface); specifically: (1) Bat behavior; (2) Domesticated animal and wildlife behavior, distribution, and movement impact; and (3) The effect of anthropogenic disturbance and modification on pathogen dynamics and spillover risks

Working Group 4: Human-bat interactions; specifically: (1) Hunting and commodity chain (e.g., bushmeat, guano, and pet trade); (2) Ecotourism; and (3) Interactions in human dwellings

Why Bats?

Bats act as natural reservoirs for over 60 pathogens, including some of the world’s most deadly viruses, such as Nipah, Hendra, Marburg, and SARS viruses. Understanding the role of bats as a reservoir and the risk of pathogen transmission from bats to humans and other animals could be a key to discovering novel pathogens, mitigating the impact of emerging and re-emerging pathogens, and preventing future pandemics.

There are a number of factors which make bats unique disease reservoirs, including their social behavior, distinct physiology and metabolism, ability to travel long distances, nocturnal activity, species diversity, and long life span (10-20 years). These specific bat characteristics, coupled with the impact of human-mediated interactions and environmental changes, create research challenges to understanding the role of bats in global zoonotic disease ecology. BOHRN will create opportunities for policy makers, scientists, conservationists, funders, and students to identify community challenges, develop priority research lists and implement associated action plans that target needs and gaps. The opportunities created will work at all levels to build awareness of bat-associated disease burden and transmission risks and improve the prevention, detection, diagnosis, and reporting of pathogens of security concern.

U.S. DoD and Health Security

BOHRN outcomes will also support the Global Health Security Agenda (GHSA) Zoonotic Disease Action Package, which has a five-year target for countries to adopt measured behaviors, policies, and/or practices that minimize the spillover of zoonotic diseases from lower animals into human populations.

Although not directly involved in implementation, DTRA BTRP supports the GHSA goals and milestones, and synchronizes with GHSA country projects through the DoD GHSA Coordination Cell. DTRA BTRP is the DoD’ premier biological nonproliferation division protecting the United States and its allies from especially dangerous pathogens by collaborating with partner countries and the international community to minimize the threat of deliberate, accidental, and natural infectious disease outbreaks through enhanced biosafety, security, and surveillance measures. DTRA BTRP investments build capacity to detect, diagnose, and report disease events and help reduce the magnitude and response costs of biological incidents.

Additionally, DTRA BTRP promotes scientific and technical collaborations among partner nations and the international community in the disciplines of biological safety, security, and surveillance to build constructive and sustainable international partnerships that address threats posed to health security. These science diplomacy-based activities engage scientists in peaceful application of biotechnology; building partner country disease surveillance capabilities; promoting adherence to international codes of conduct, security, and safety; and enhancing transparency and confidence building.

Although DTRA BTRP is committed to supporting BOHRN, there is no guarantee or obligation for DTRA BTRP to fund projects resulting from the network or its members.

Point of Contact

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BHORN Website

https://www.bohrn.net

Fact Sheet References

Hayman, D. 2016. *As the Bat Flies*. Science 02 Dec 2016: Vol. 354, Issue 6316, pp. 1099-1100`
DOI: 10.1126/science.aaj1818

GHSA. 2017. *Global Health Security Agenda: Zoonotic Disease Action Package (GHSA Action Package Prevent 2)*.
https://www.ghsagenda.org/packages/p2-zoonotic-disease

Kingston T., et. al. 2016. *Networking networks for global bat conservation. Bats in the Anthropocene: Conservation of Bats in a Changing World*. Springer Open.

Saez AM, et.al. 2014. *Investigating the zoonotic origin of the West African Ebola epidemic*. EMBO Mol Med. Vol 7, Iss 1:17-23. DOI: 10.15252/emmm.201404792

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Quad Chart Instructions: Please fill out all four portions of the quad chart. The chart is read in a clockwise direction starting with the technical description. This activity is intended to provide a big picture overview and not an in-depth report of your research. Please limit the text to provide only the most important aspects of each quad. You will be given 5 minutes to present the information within this chart. Refer to the questions in each box for more guided assistance.

TECHNICAL DESCRIPTION AND OBJECTIVES

Briefly describe the research you are currently conducting and why. What questions are you trying to answer and what is the importance of this research to your field and the region? What are the specific aims of your research? What is currently known about your research? Consider the following:

1. **Objectives of research**
2. **Current state of understanding**
3. **Location of research (city/country and or coordinates)**

KEY PARTNERS AND REGIONS OF STUDY

Briefly describe the key partners and funders in your research. List the region(s) your research is being conducted in and any networks you are working with formally or informally. Consider the following:

1. **Who are the key partners involved in your research?**
2. **Who are the key funders involved in your research?**
3. **What region(s) is your research being conducted in?**
4. **Are you working with any networks (formally or informally)?**

MILESTONES, STATUS, AND CHALLENGES

Briefly explain the timeline of your research. When do you anticipate your research to be completed? Are there deliverables or steps along the way that will show substantial progress? Identify the challenges you will face. Consider the following:

1. **Provide timeline for delivery**
2. **Overview on project status**
3. **List challenges or needs in your research**

REGIONAL IMPACT

Describe the potential regional impact of your research. What will the impact be for the regional area? Will this lead to the need for future studies? Consider the following:

1. **Define the quantitative impact of project.**
2. **Define the regional impact.**

TECHNICAL DESCRIPTION AND OBJECTIVES

KEY PARTNERS AND REGIONS OF STUDY

MILESTONES, STATUS, AND CHALLENGES

REGIONAL IMPACT

From: Megan Hudson on behalf of Megan Hudson <megan.hudson@globalsyseng.com>
To: christopher.broder@usuhs.edu
Cc: Stokes, Martha M CIV (US); Becker, Stephen M CTR DTRA J3-7 (US); Katie Leahy; Caitlin Devaney
Subject: Invitation to the Bat One Health Research Network Workshop in Vienna
Date: Monday, October 29, 2018 11:57:55 AM
Attachments: PAOBOHRNFactSheet copy.pdf
BOHRN_QUADCHART[2].docx
image001.png

Dear Dr. Broder,

I wanted to follow up on your earlier discussion with Dr. Marty Stokes, the Southeast Asia Science Manager for the Defense Threat Reduction Agency, Biological Threat Reduction Program (formerly Cooperative Biological Engagement Program or CBEP), regarding the Bat One Health Research (BOHRN) Workshop in Vienna.

On behalf of Dr. Stokes we would like to extend an invitation for you to the BOHRN 1st Annual Workshop in Vienna, Austria on 8-9 November 2018. The two day meeting will be held at the Imlauer Wien hotel.

Established 2016, BOHRN convenes multi-disciplinary and One Health-focused scientists, policy makers, research scientists, and medical/veterinary practitioners with interests in bat-related research involving pathogens of security concern. The network builds on community standards and best practices for research. BOHRN identifies and shares information on research funding opportunities offered by multiple institutions. Most importantly, this network fosters international relationships among collaborators, agencies, and organizations, which can produce long-term, sustainable partnerships that withstand changes in government and organization budgets, priorities, postures, and policies. Please see the attached fact sheet for more information on BOHRN.

DTRA BTRP sponsors BOHRN a global networks that aims to characterize global threats of bat-borne pathogens and formalize community standards and conservation- conscious practices for One Health disease research. We hope to achieve the following objectives during the 1st
Annual BOHRN Workshop:

(1) Facilitate a multi-disciplinary forum for discussion on research methods and practices to characterize bat-borne pathogen spillover threats

(2) Engage available funding program representatives with interests in conservation-conscious bat-borne pathogen research

(3) Characterize global research interests and priorities, and align them with network research focus areas and available funding programs

(4) Discuss upcoming planning opportunities

Attached you will find the network fact sheet; you can also visit www.bohrn.net for more information.

We have secured a hotel room for you the nights of 7-8 November at the Imlauer hotel in Vienna prior to the IMED meeting. In addition, **please find a quad chart attached, we ask that you please fill in the attached quad chart and return as soon as possible.**

Each participant will be asked to present the attached quad chart during the workshop day 1 event (about five minutes per chart). The information presented will be used to aid breakout group and large group discussions. Along with the quad chart, we are requesting that you send a picture of yourself. The quad charts and pictures will be printed to display around the room during the two day workshop.

Please let us know if you have any questions regarding this meeting or the quad chart.

Kind Regards,

Megan

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NipahResearch and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs) – diagnostics, therapeutics, and vaccines – against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness,cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India; some differences have been noted in the clinical features of infection with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in thise roadmap ranges from basic research to late-stage development~~, licensure, and early use ~~of MCMs to prevent and control NiV outbreaks and endemic diseasein humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updatingas needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing zoonotic NiV transmission, improved personal protective equipment (PPE), ensure hand hygiene and environmental hygiene, effective community engagement, adequate infection prevention and control practices,adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy. Further research ofNiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV(or related henipavirus species)in humans and virus transmission can occur at the human-animal interface.

Commented [EG1]: I would argue that enhanced surveillance systems are also crucial to the goals of development of MCM. Without active and effective surveillance, we do not have a platform with which to develop and test these new technologies. In addition, expanded surveillance could have an impact on the business case for these technologies. I think it's important to have this argument clearly articulated in the roadmap.

Commented [KM2]: Issues related to MCM development for animals have been deleted, since the decision was made at the July meeting to focus on human disease. However, further research in animals is critical, so this sentence was added.

VISION

Robust MCMs to detect, prevent, and control human outbreaks of NiV infection(and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research re~~s~~presents a substantial challenge, since e~~E~~conomic incentives to invest in Nipah research are not readily apparent because~~, as~~ the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence ~~is~~ has, so far, beenlow with small and sporadic outbreaks. ~~; therefore, securing funding for Nipah research represents a substantial challenge.~~ The development of a sustainable value propositionfor industry and international philanthropic public-private partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition ~~would ideally~~should be informed by a robust assessment of the risk of future outbreaksand the economic, societal,and health impacts that such outbreaks could generate. ~~and will likely require new systematic surveillance studies in humans and susceptibleanimal hosts in affected areas to strengthen the evidence base.~~

Commented [KM3]: Taken out because this is too long term for creating the value proposition.

- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions, which increases~~. This raises~~ the cost of MCM development.
- To date, NiV spillovers to human communities have been identified ~~almost exclusively~~ most commonly in rural communities in Bangladesh andIndia; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- ~~Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs older than 4 weeks old), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before~~

~~livestock are tested for NiV, which could cause large financial losses forlivestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.~~

- ~~NiV disease occurs in both humans and animals (particularly livestock). This creates two streams of research, which complicates the overall approach for moving NiV MCMs forward. For example, animal models for human disease will have different parameters than animal models for disease in animal species.~~
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research, ~~applicable to humans (and pigs and horses for research applicable to disease in animals),~~ the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions;however, phase 3 clinical efficacy trials will need to be conducted in endemic areas~~.~~ ~~However, B~~because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence ~~in endemic areas~~ poses a major challenge for conducting ~~phase 3 clinical efficacy~~suchtrials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by combining clinical trial data across outbreaks over time. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not ~~applicable~~feasible.

Commented [EG4]: And, possibly by expanding surveillance areas.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop an~~m~~d manufacture NiVMCMs.
- Enhanced clinical, laboratory, and public healthinfrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures.
- Additional prospective serosurveillance data from susceptible animal species and proximate human populations in areas of predicted risk to determine the level of human spillover and to build preparedness for detection of human cases and for limiting exposure. This is particularly important in areas where public health surveillance programs are not feasible.

Commented [KM5]: Even though this bullet deals primarily with animals, I left it in, since it also deals with the human-animal interface.

- Standardized and ~~validated~~ well characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)WHO

international standards should be used (when available) as calibrators and reported in Units/ml to harmonize assay results.

- ~~Efforts to establish the natural history of NiV infection in animals and define the animal modelsthat are most reflective of human disease.~~
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- ~~Efforts to design clinical efficacy trials in affected areas that are ethical, interpretable, and feasible. Researchers should explore the potential for conducting clinical trials before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).~~
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to cliniciansand community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and ~~facilitate effective communication with communities to~~ strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturingto endemic and at-risk areas.

Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus)is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of Pteropus bats and potentially other batspecies.

Commented [KM6]: Moved to vaccines.

- Additional research is needed to~~refine, standardize, and validate~~ optimize relevant animal challenge models(e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) ~~to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for~~ to promote development and evaluation of MCMs, particularly if investigators intend to use an alternative pathway(such as the United States Food and Drug Administration's [FDA's] Animal Rule)to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3)determine when after challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data ~~between~~ from animal models ~~and~~ to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hostsand pathogenicity.
- Additional studies applying whole genome sequencing of NiV viruses are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume data palm sap and workers at the human-animal interface) ~~pig farmers, hunters, abattoir workers)~~ and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiVMCMs, especially if therapeutics and vaccines do not consistently prevent disease.
- ~~Efforts are needed to: (1) assess potential barriers for conducting clinical trials, (2) assess MCM acceptability in at-risk populations, (3) determine culturally appropriate messaging to enhance MCM acceptance, and (4) identify public health strategies to promote vaccine use.~~

Commented [SP7]: May also need to include healthcare workers at hospital setting

Commented [KM8]: This is relatively redundant to the bullet above, so was deleted.

Strategic Goals and Aligned Milestones

Strategic Goal 1:Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Commented [KM9]: In the Lassa taskforce meeting, we discussed the need for a value proposition document, which can help justify the funding plan, so that was added here. We also talk about the value proposition at the beginning of this document. There actually is a template for a value proposition for vaccines (created by a WHO group), so perhaps that document could be used as a starting point.

Strategic Goal 2:Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiVsurveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the diseaseburden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform**phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.

- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability henipaviruses in order to improve understanding of their global distribution.
- ~~**Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.~~
- ~~**Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.~~
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Commented [KM10]: These two bullets were moved to vaccines.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote**early communication between developers and appropriate NRAs forclarity and guidance on the regulatory aspects ofMCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacityto: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiVsurveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiVspillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiVtherapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.

- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorizationfrom appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an “affordable and fair” price is one that can reasonably be funded by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify**the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Critical Path Analysis

[See accompanying table.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and etiologic diagnosis is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species)appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate which henipaviruses are in circulation using serologic assays. Capacity to identify additional

pathogenic henipaviruses is an important challenge forensuring diagnostic preparednessto respond tofuture outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.

Commented [KM11]: Moved up from further down

- A TPP for NiV diagnostics~~,identifying~~ that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A ~~biobank~~virtual repository (with specimens being held and maintained in the countries of origin) of ~~human and animal~~clinical samples to assess and validate diagnostic tests.~~ and a process for how best to judiciously use the samples.~~ As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governace structure; (6~~3~~) identify who would have access to the samples; ~~and~~ (7~~4~~) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place.(Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- ~~Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.~~

Commented [KM12]: Moved up and shortened.

- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course,are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), ~~can be applied in both human and animal populations,~~ and have a high degree of sensitivity and specificity for different NiVstrains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

- Improved ~~D~~diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses ~~in humans and animals~~ as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiVdiagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- ~~If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of public health concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.~~
- ~~If NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals (such as the Differentiating Infected from Vaccinated Animals (DIVA) test) will be needed.~~

Commented [KM13]: The group decided to take out any reference to multiplex assays, given the 5 year timeframe of the roadmap, so this was deleted.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. ~~Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.~~
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudo typed neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- ~~A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).

2. By 2020, identify funding and initiate creation ofthe virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2:Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2018, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.

2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.

3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3:Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks.For example, this may include strategies for development of mobile laboratories that can be deployed when outbreaks occur.

Priority Areas/Activities

Research

- **Create** a ~~biobank~~ virtual repositoryof clinical ~~human and animal~~reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- ~~**Continue to research** cross-reactivity of diagnostic tests in animal populations.~~

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- ~~**Define** use cases for diagnostic assays.~~
- **Develop** and ~~,~~ **evaluate**~~, and validate~~ point-of-care or near-patient rapid diagnostic tests for NiVinfection that are affordable, highly sensitive and specific (as needed, depending on their intended use), ~~available for use in humans and animals, and~~ can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.
- ~~**Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.~~
- ~~**Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.~~
- ~~**Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).~~

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed. ~~in humans and animals.~~

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiVdiagnostic tests across different geographic areas, as such tests become available.

Critical Path Analysis

[See accompanying table.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment and PEP to exposed persons.

- In ~~the~~ NiV-endemic ~~region of Bangladesh~~areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. ~~In the absence of confirmatory testing, t~~Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well ~~an~~ a therapeutic agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control ~~systems~~ programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- ~~A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.~~
- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- ~~Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.~~
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- ~~Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection may be warranted.~~
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to assess the safety, tolerability, and efficacyof m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the mostpromising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against a strain from Bangladesh/India to be shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- ~~Additional studies, as needed, of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.~~
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer.This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will impact response to therapeutic candidates and influence results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies,diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines and evidence-based policy decisions.

Commented [KM14]: Taken out because this was redundant to other statements above.

Strategic Goals and Aligned Milestones

Strategic Goal 1:Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2018, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.

3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented during future NiV outbreaks and develop plans for operationalizing the protocol.
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.

Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as ~~ribavirin and~~ m102.4 and favipiravir) for treating and preventing NiV infection, including conducting ~~animal~~ studies in animal models andclinical trialsas appropriate and feasible.
- **Continue to** ~~**identify**~~**expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- ~~**Generate** a TPP for NiV infection therapeutics.~~
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized in advance of future outbreaks, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.

- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- ~~**Identify** a company or joint partnership to advance therapeutic use of m102.4 (including conduct of additional collaborative research) and **secure** financing for its manufacture, and distribution, and post-marketing surveillance.~~
- **Generate** a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.
- **Develop**guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Critical Path Analysis

[See accompanying table.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could impact acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.

- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac® HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.These specific correlates may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed in target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populationsand identify additional areas of potential NiV spillover.

Commented [SP15]: It may also worthy to explore perceptions and/or concerns of the population/communities at risk for future vaccine implementation

- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiVand HeV or NiV and measles virus [MV]) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available),(4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- ~~Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses and available evidence suggests this vaccine is cross-protective against NiV. Ongoing research into developing NiV/HeV vaccines for livestock(or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.~~
- ~~Additional research is needed to determine if development of multivalent vaccines for animals(that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.

2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2:Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.

2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.

3. By 2023, complete the followingif phase 3 trials are considered feasible (per the first Strategic Goal): (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of thehumoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serological assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.

- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.
- ~~**Explore**whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.~~
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.

Product development

- ~~**License**~~ **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans. ~~and animals.~~
- ~~**License** safe and effective multivalent vaccines for use in humans that protect against more than one disease for use in human populations (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.~~
- ~~**Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a sustainable approach.~~

Key capacities

- **Improve**surveillance capabilities to assess the impact of vaccine use and vaccination strategies(once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of ~~licensed~~ NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide**guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptation and promotion within the community.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for more rapid spread.

Critical Path Analysis

[See accompanying table.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine.Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifyingearly target cells of Nipah virus infection in Syrian hamsters. PLoSNegl Trop Dis2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10): e1000642. doi: 10.1371/journal.ppat.1000642. [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. SciTransl Med 2012 Aug 8;4(146):146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95[Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;(8). doi: 10.1038/s41598-018-25780-3 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoSNegl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al.Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295.[Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. SciTransl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. EmergInfec Dis 2007 Jul;13(7):1031-1037 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE.A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B.Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137). doi: 10.1016/S0140-6736(18)31264-9 [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-2975 [Full text]

Satterfield BA, Geisbert TW, Mire CE.Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top MicrobiolImmunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. CurrOpinVirol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

Critical Path Analysis for Development of Nipah Virus Diagnostics, Therapeutics, and Vaccines

The table below provides a high-level description of the primary risks and barriers to achieving the milestones identified in the research and development (R&D) roadmap for Nipah virus and also identifies other milestones that are critical dependencies, meaning that in order for the milestone in question to either be initiated or completed, another milestone should be completed first. The focus of this critical path analysis is on R&D of medical countermeasures (MCMs) and does not encompass all aspects of Nipah virus prevention and control.

Critical Path Analysis of Milestones for Strategic GoalsIdentified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
CROSS-CUTTING ISSUES		
Strategic Goal 1: Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.		
1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.	• Lack of resources to create a public value proposition (e.g., funding, availability and commitment of partners with the appropriate expertise) • Lack of accurate data sources and information to support the value proposition	--
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.	• The need to identify and engage appropriate industry partners as part of the process in creating a funding plan • Challenges with identifying funders willing to support elements of the funding plan	• A completed value proposition to support and justify the funding plan (Cross cutting: SG1, MS1)
Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.		
1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.	• Lack of ability to secure funding to enhance NiV surveillance • Challenges with developing consensus on the implementation protocol because of differing clinical and laboratory capabilities across catchment areas • Limited information regarding which areas may be at high risk for NiV spillover	--

Critical Path Analysis of Milestones for Strategic GoalsIdentified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.	• The need to educate clinicians and community health workers about NiV infection to increase their index of suspicion and improve case detection	• A completed and agreed upon surveillance plan and related protocols(Cross cutting: SG2, MS1) • Improved diagnostic assays to rapidly and accurately identify cases (Diagnostics: SG2, MS4) • Adequate laboratory capacity for diagnostic testing (Diagnostics: SG3, MS1)
Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.		
1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.	• Limited BSL-4 capacity • Lack of dedicated funding to create research tools • Lack of consensus on criteria for standardization and characterization	• Availability of well-characterized clinical samples (including representation of the different NiV strains)(Diagnostics: SG1, MS2)
2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.	• Limited BSL-4 capacity • Regulations and restrictions applicable to animal research, including ethical considerations regarding use of NHPs • The need for agreed-upon benchmark parameters (i.e., challenge strain, route, timing, and dose)	• Availability of standardized assays and challenge strains for animal research (Cross cutting: SG3, MS1) • Availability of well-characterized clinical samples (including representation of the different NiV strains) (Diagnostics: SG1, MS2)
DIAGNOSTICIS		
Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.		
1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).	• Complexities involved with creating a virtual reference repository • The need for coordination across multiple organizations and agencies within each of the primary NiV-affected countries • The need for international and in-country leadership to drive the process	--
2. By 2020, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.	• Lack of funding and resources to create the in-country repositories • Lack of adequate case numbers for sample collection (in part owing to lack of surveillance capabilities) • Challenges with international regulations for transport of clinical samples and other biologic	• Completion of the plans and protocols for creating the virtual reference repository (Diagnostics: SG1, MS1)

Critical Path Analysis of Milestones for Strategic GoalsIdentified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
	materials • Challenges in completing the steps necessary to operationalize the plans (e.g., ensuring laboratory capacity, conducting training, having MTAs in place, having the governance structure in place)	
Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient-care NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.		
1. By 2018, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.	--	--
2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.	• The need for a coordinated process and leadership to engage and convene the appropriate regulatory agencies and other key partners	--
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.	• Insufficient resources and funding for R&D to produce promising diagnostics • Low engagement in diagnostic development from commercial manufacturers • Adequate supplies of clinical samples or experimentally generated samples from research animals	• Availability of well-characterized samples from patients (Diagnostics: SG1, MS2)
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.	• Lack of clinical and laboratory capacity for conducting field studies in different geographic areas of NiV spillover • The need to ensure proper test performance under field conditions	• Successful completion of preclinical evaluation of promising diagnostic assays (Diagnostics: SG2, MS3) • Adequate laboratory capacity to conduct the field studies (Diagnostics: SG3, MS1)
Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.		
1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks. For example,	• The need for international leadership and engagement to assist with development of the national laboratory strategies • The need to engage and obtain support from key in-country partners	--

Critical Path Analysis of Milestones for Strategic GoalsIdentified in the WHO Nipah Virus R&D Roadmap		
MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
this may include strategies for development of mobile laboratories that can be deployed when outbreaks occur.	• Lack of necessary resources to enhance laboratory capacity	
THERAPEUTICS		
Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.		
1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to the operationalize protocol (e.g., obtaining necessary reviews and approvals, performing training) • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate suppliesnecessary to conduct clinical trial research (Therapeutics: SG2, MS1)
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to operationalize the protocol (e.g., obtaining necessary reviews and approvals, performing training) • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
3. By 2020, complete a broader, regional protocol for conducting clinical trials of promisingtherapeutic candidates to be implemented during future NiV outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to operationalize the protocol (e.g., obtaining necessary reviews and approvals, performing training) • The need to develop consensus across different countries to generate a regional approach and promote adoption by member states • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.	--	--
Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.		
1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.	• Obtaining consensus regarding prioritization of promising candidates • Lack of an adequate pipeline of promising	--

Critical Path Analysis of Milestones for Strategic GoalsIdentified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
	therapeutic agents and lack of R&D resources to strengthen the pipeline	
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.	• Lack of dedicated R&D funding to conduct preclinical evaluation of novel NiV therapeutic agents • Limited BSL-4 capacity • Limited understanding regarding whether or not genetic diversity of NiV strains will impact therapeutic response to new treatment options	• Availability of standardized and well-characterized clinical research tools (e.g., assays, reagents, stocks of NiV challenge strains) (Cross cutting: SG3, MS1)
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.	• Lack of in-country capacity to conduct clinical trials • Hesitancy or opposition to clinical trials being conducted in at-risk populations • Difficulty in receiving approval from in-country NRAs to conduct clinical trials • Identifying sufficient cases to support an informative evaluation, especially if there are multiple potential therapeutic candidates • Develop guidance to engage with families of patients to explain the need for testing therapeutic candidates to avoid any potential mistrust on heath system and healthcare workers	• Successful completion of preclinical evaluation of promising therapeutic candidates (Therapeutics: SG2, MS2) • Improved diagnostic assays to rapidly and accurately identify cases (Diagnostics: SG2, MS4) • Adequate laboratory capacity for diagnostic testing (Diagnostics: SG3, MS1)
VACCINES		
Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.		
1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.	• Need for regulatory engagement and guidance on whether or not clinical efficacy trials are feasible • Need for better characterization of clinical trial capacity in areas of NiV spillover • Lack of accurate information on the disease burden caused by NiV infection, which is important for assessing the likelihood of completing clinical efficacy trials	--

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Critical Path Analysis of Milestones for Strategic GoalsIdentified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as FDA or the EMA), national and international public health agencies and organizations, and local and international researchers.	• Challenges with identifying the appropriate regional and international partners • Challenges with developing consensus among partners	• Determination the feasibility of conducting phase 3 clinical efficacy trials in NIV-affected areas (Vaccines: SG1, MS1)
Strategic Goal 2:Develop and evaluate NiV vaccines for prevention of NiV disease in humans.		
1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.	• Insufficient R&D funding to conduct preclinical evaluation of promising NiV vaccines • Limited BSL-4 capacity for conducting preclinical research on potential NiV vaccines	• Availability of standardized and well-characterized clinical research tools (e.g., assays, reagents, stocks of NiV challenge strains) (Cross cutting: SG3, MS1) • Availability of animal models that bridge to humans and can be used to demonstrate vaccine protection (Cross cutting: SG3, MS2)
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines, including in target populations.	• Hesitancy or opposition to clinical trials being conducted in at-risk populations ~~• Lack of in-country capacity to conduct clinical trials~~ • ~~Difficulty~~ Delaysin receiving approval from in-country NRAs to conduct clinical trials •	• Successful completion of preclinical evaluation of 1 or more promising vaccine candidates (Vaccines: SG2, MS1)
3. By 2023, complete the following if phase 3 trials are considered feasible (per the first Strategic Goal): (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a strategy for determining which candidate vaccines will go forward into phase 3 trials.	• The challenges of engaging partners and completing steps necessary to operationalize clinical trial protocols • The need to develop consensus across different countries • Lack of surveillance capacity and systemic estimates for NiV infection to assess vaccine efficacy and impact over time	• Successful completion of early clinical trials of 1 or more promising vaccine candidates (Vaccines: SG2, MS2)

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Commented [SPL2]: This is relevant neither for Bangladesh nor India which each have a long history of such trials.

Critical Path Analysis of Milestones for Strategic GoalsIdentified in the WHO Nipah Virus R&D Roadmap		
MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
Abbreviations: BSL-4, biosafety level 4 (highest biosafety level); EMA, European Medicines Agency; FDA, US Food and Drug Administration; MCMs, medical countermeasures; MS, Milestone; MTA, material transfer agreement; NHP, nonhuman primate; NiV, Nipah virus; NRA, national regulatory authority; PEP, post-exposure prophylaxis; R&D, research and development; SG, Strategic Goal; TPP, target product profile; WHO, World Health Organization. *These dependencies focus only on the relationships between the milestones to show how the roadmap should be implemented over time; however, there are other critical dependencies for each of the milestones, such as adequate resources and funding.		

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs) – diagnostics, therapeutics, and vaccines – against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India; some differences have been noted in the clinical features of infection with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this~~e~~ roadmap ranges from basic research to late-stage development~~, licensure, and early use~~ of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing zoonotic NiV transmission, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy. Further research of NiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV (or related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

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VISION

Robust MCMs to detect, prevent, and control human outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research respresents a substantial challenge, since e~~E~~conomic incentives to invest in Nipah research are not readily apparent because~~, as~~ the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low with small and sporadic outbreaks. ~~; therefore, securing funding for Nipah research represents a substantial challenge.~~ The development of a sustainable value proposition for industry and international philanthropic public-private partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition ~~would ideally~~should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate. ~~and will likely require new systematic surveillance studies in humans and susceptible animal hosts in affected areas to strengthen the evidence base.~~
- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions, which increases~~. This raises~~ the cost of MCM development.
- To date, NiV spillovers to human communities have been identified ~~almost exclusively~~ most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- ~~Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs older than 4 weeks old), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before~~

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~~livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.~~

- ~~NiV disease occurs in both humans and animals (particularly livestock). This creates two streams of research, which complicates the overall approach for moving NiV MCMs forward. For example, animal models for human disease will have different parameters than animal models for disease in animal species.~~
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research, ~~applicable to humans (and pigs and horses for research applicable to disease in animals),~~ the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. ~~However,~~ B~~b~~ecause NiV infection occurs as relatively small, focal outbreaks, the low disease incidence ~~in endemic areas~~ poses a major challenge for conducting ~~phase 3 clinical efficacy~~such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by combining clinical trial data across outbreaks over time. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not ~~applicable~~feasible.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop amd manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries and to global stakeholder to ensure they understand the potential health, societal, and economic benefits of devoting ~~limited~~ resources to improving NiV surveillance, detection, prevention, and control measures.
- Additional prospective serosurveillance data on henipavirus exposure from susceptible animal species and proximate human populations in areas of predicted risk should be explored as a strategy to ~~determine~~ assess the ~~level~~ risk of human spillover and to build preparedness for detection of human cases and for limiting exposure. ~~This is particularly important in areas where public health surveillance programs are not feasible.~~
- Standardized and ~~validated~~ well characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.) WHO

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international standards should be used (when available) as calibrators and reported in Units/ml to harmonize assay results.

- ~~Efforts to establish the natural history of NiV infection in animals and define the animal models that are most reflective of human disease.~~
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- ~~Efforts to design clinical efficacy trials in affected areas that are ethical, interpretable, and feasible. Researchers should explore the potential for conducting clinical trials before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).~~
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and ~~facilitate effective communication with communities to~~ strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

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Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of Pteropus bats and potentially other bat species.

- Additional research is needed to ~~refine, standardize, and validate~~optimize relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) ~~to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for~~ to promote development and evaluation of MCMs, particularly if investigators intend to use an alternative pathway (such as the United States Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data ~~between~~ from animal models ~~and~~ to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole genome sequencing of NiV viruses are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume data palm sap and workers at the human-animal interface) ~~pig farmers, hunters, abattoir workers)~~ and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.
- ~~Efforts are needed to: (1) assess potential barriers for conducting clinical trials, (2) assess MCM acceptability in at-risk populations, (3) determine culturally appropriate messaging to enhance MCM acceptance, and (4) identify public health strategies to promote vaccine use.~~

Commented [KM7]: This is relatively redundant to the bullet above, so was deleted.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Commented [KM8]: In the Lassa taskforce meeting, we discussed the need for a value proposition document, which can help justify the funding plan, so that was added here. We also talk about the value proposition at the beginning of this document. There actually is a template for a value proposition for vaccines (created by a WHO group), so perhaps that document could be used as a starting point.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.

- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability henipaviruses in order to improve understanding of their global distribution.
- ~~**Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.~~
- ~~**Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.~~
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Commented [KM9]: These two bullets were moved to vaccines.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.

- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an "affordable and fair" price is one that can reasonably be funded by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Critical Path Analysis

[See accompanying table.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and etiologic diagnosis is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate which henipaviruses are in circulation using serologic assays. Capacity to identify additional

pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.
- A TPP for NiV diagnostics~~,~~ ~~identifying~~ that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A ~~biobank~~ virtual repository (with specimens being held and maintained in the countries of origin) of ~~human and animal~~ clinical samples to assess and validate diagnostic tests~~. and a process for how best to judiciously use the samples.~~ As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6~~3~~) identify who would have access to the samples; ~~and~~ (7~~4~~) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- ~~Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.~~
- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), ~~can be applied in both human and animal populations,~~ and have a high degree of sensitivity and specificity for different NiV strains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

Commented [KM10]: Moved up from further down

Commented [KM11]: Moved up and shortened.

- Improved ~~D~~diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses ~~in humans and animals~~ as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- ~~If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of public health concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.~~
- ~~If NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals (such as the Differentiating Infected from Vaccinated Animals (DIVA) test) will be needed.~~

Commented [KM12]: The group decided to take out any reference to multiplex assays, given the 5 year timeframe of the roadmap, so this was deleted.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. ~~Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.~~
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- ~~A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).

Commented [SPL13]: I envision that governance of this virtual repository will be the most difficult aspect. Notably, it will require high-level approval by the Government of India. If we say that we will develop a plan for the governance structure in 2019, the earliest I can imagine approval is 2020, and even this would be quite optimistic. It will require focused attention and advocacy.

2. By 2020, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2018, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.

Commented [SPL14]: Are we on track to do this? Do we want to give ourselves a little more time, perhaps by the first quarter of 2019?

2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.

3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks. For example, this may include strategies for development of mobile laboratories that can be deployed when outbreaks occur.

Commented [SPL15]: This seems to me to be less important, than figuring out a strategy for identifying samples with a high enough pretest probability for Nipah infection so that they are tested. The vast majority of patients with symptoms of encephalitis in the high risk areas do not have diagnostic tests of any type. If we tested all of them, Nipah would be a very low proportion, likely less than 1%. Thus, the key issue in terms of recognizing more Nipah, is figuring out whom to collect samples on and send the reference laboratory.

Priority Areas/Activities

Research

- **Create** a ~~biobank~~ virtual repository of clinical ~~human and animal~~reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- ~~**Continue to research** cross-reactivity of diagnostic tests in animal populations.~~

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- ~~**Define** use cases for diagnostic assays.~~
- **Develop and** ~~,~~ **evaluate**~~, and validate~~ point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), ~~available for use in humans and animals, and~~ can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.
- ~~**Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.~~
- ~~**Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.~~
- ~~**Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).~~

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed. ~~in humans and animals.~~

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Critical Path Analysis

[See accompanying table.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment and PEP to exposed persons.

- In ~~the~~ NiV-endemic ~~region of Bangladesh~~areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. ~~In the absence of confirmatory testing, t~~Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well ~~an~~ a therapeutic agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control ~~systems~~ –programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- ~~A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.~~
- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- ~~Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.~~
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- ~~Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection may be warranted.~~
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against a strain from Bangladesh/India to be shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- ~~Additional studies, as needed, of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.~~

Commented [KM16]: Taken out because this was redundant to other statements above.

- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will impact response to therapeutic candidates and influence results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines and evidence-based policy decisions.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2018, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.

Commented [SPL17]: Outbreaks are chaotic and most are small. I believe that we would be better placed to evaluate therapeutics if we had rapid tests and embedded efficacy trials into our routine surveillance.

Commented [SPL18]: As above. It takes so long to recognize and respond to outbreaks that with Nipah most of them are over by the time we can respond. Instead, if this is part of surveillance for every case we diagnose, case accumulation still might be slow, but we could expect several cases per year, especially if we spread this over multiple improved surveillance sites.

3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented during future NiV outbreaks and develop plans for operationalizing the protocol.
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.

Commented [SPL19]: As above.

Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Commented [SPL20]: These will need to be remarkably efficacious, in order for us to accumulate sufficient sample size to achieve this by 2023. Given the late presentation of patients, this strikes me as quite optimistic.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as ~~ribavirin and~~ m102.4 and favipiravir) for treating and preventing NiV infection, including conducting ~~animal~~ studies in animal models and clinical trials as appropriate and feasible.
- **Continue to ~~identify~~ expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- ~~**Generate** a TPP for NiV infection therapeutics.~~
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized in advance of future outbreaks, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.

Commented [SPL21]: As noted above, the should be part of routine surveillance, not something that has to be implemented during a chaotic large outbreak.

- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- ~~**Identify** a company or joint partnership to advance therapeutic use of m102.4 (including conduct of additional collaborative research) and **secure** financing for its manufacture, and distribution, and post-marketing surveillance.~~
- **Generate** a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Critical Path Analysis

[See accompanying table.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could impact acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.

- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac® HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed in target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations and identify additional areas of potential NiV spillover.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- ~~Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses and available evidence suggests this vaccine is cross-protective against NiV. Ongoing research into developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.~~
- ~~Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.
3. By 2023, complete the following if phase 3 trials are considered feasible (per the first Strategic Goal): (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of the humoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serological assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.

- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.
- ~~**Explore** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.~~
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.

Product development

- ~~**License**~~ **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans. ~~and animals.~~
- ~~**License** safe and effective multivalent vaccines for use in humans that protect against more than one disease for use in human populations (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.~~
- ~~**Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a sustainable approach.~~

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of ~~licensed~~ NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptation and promotion within the community.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for more rapid spread.

Critical Path Analysis

[See accompanying table.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10): e1000642. doi: 10.1371/journal.ppat.1000642. [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;(8). doi: 10.1038/s41598-018-25780-3 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-1037 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137). doi: 10.1016/S0140-6736(18)31264-9 [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-2975 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

Critical Path Analysis for Development of Nipah Virus Diagnostics, Therapeutics, and Vaccines

The table below provides a high-level description of the primary risks and barriers to achieving the milestones identified in the research and development (R&D) roadmap for Nipah virus and also identifies other milestones that are critical dependencies, meaning that in order for the milestone in question to either be initiated or completed, another milestone should be completed first. The focus of this critical path analysis is on R&D of medical countermeasures (MCMs) and does not encompass all aspects of Nipah virus prevention and control.

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
CROSS-CUTTING ISSUES		
Strategic Goal 1: Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.		
1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.	• Lack of resources to create a public value proposition (e.g., funding, availability and commitment of partners with the appropriate expertise) • Lack of accurate data sources and information to support the value proposition	--
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.	• The need to identify and engage appropriate industry partners as part of the process in creating a funding plan • Challenges with identifying funders willing to support elements of the funding plan	• A completed value proposition to support and justify the funding plan (Cross cutting: SG1, MS1)
Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.		
1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.	• Lack of ability to secure funding to enhance NiV surveillance • Challenges with developing consensus on the implementation protocol because of differing clinical and laboratory capabilities across catchment areas • Limited information regarding which areas may be at high risk for NiV spillover	--

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.	• The need to educate clinicians and community health workers about NiV infection to increase their index of suspicion and improve case detection	• A completed and agreed upon surveillance plan and related protocols (Cross cutting: SG2, MS1) • Improved diagnostic assays to rapidly and accurately identify cases (Diagnostics: SG2, MS4) • Adequate laboratory capacity for diagnostic testing (Diagnostics: SG3, MS1)
Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.		
1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.	• Limited BSL-4 capacity • Lack of dedicated funding to create research tools • Lack of consensus on criteria for standardization and characterization	• Availability of well-characterized clinical samples (including representation of the different NiV strains) (Diagnostics: SG1, MS2)
2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.	• Limited BSL-4 capacity • Regulations and restrictions applicable to animal research, including ethical considerations regarding use of NHPs • The need for agreed-upon benchmark parameters (i.e., challenge strain, route, timing, and dose)	• Availability of standardized assays and challenge strains for animal research (Cross cutting: SG3, MS1) • Availability of well-characterized clinical samples (including representation of the different NiV strains) (Diagnostics: SG1, MS2)
DIAGNOSTICIS		
Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.		
1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).	• Complexities involved with creating a virtual reference repository • The need for coordination across multiple organizations and agencies within each of the primary NiV-affected countries • The need for international and in-country leadership to drive the process	--
2. By 2020, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.	• Lack of funding and resources to create the in-country repositories • Lack of adequate case numbers for sample collection (in part owing to lack of surveillance capabilities) • Challenges with international regulations for transport of clinical samples and other biologic	• Completion of the plans and protocols for creating the virtual reference repository (Diagnostics: SG1, MS1)

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap		
MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
	materials • Challenges in completing the steps necessary to operationalize the plans (e.g., ensuring laboratory capacity, conducting training, having MTAs in place, having the governance structure in place)	
Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient-care NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.		
1. By 2018, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.	--	--
2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.	• The need for a coordinated process and leadership to engage and convene the appropriate regulatory agencies and other key partners	--
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.	• Insufficient resources and funding for R&D to produce promising diagnostics • Low engagement in diagnostic development from commercial manufacturers • Adequate supplies of clinical samples or experimentally generated samples from research animals	• Availability of well-characterized samples from patients (Diagnostics: SG1, MS2)
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.	• Lack of clinical and laboratory capacity for conducting field studies in different geographic areas of NiV spillover • The need to ensure proper test performance under field conditions	• Successful completion of preclinical evaluation of promising diagnostic assays (Diagnostics: SG2, MS3) • Adequate laboratory capacity to conduct the field studies (Diagnostics: SG3, MS1)
Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.		
1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks. For example,	• The need for international leadership and engagement to assist with development of the national laboratory strategies • The need to engage and obtain support from key in-country partners	--

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
this may include strategies for development of mobile laboratories that can be deployed when outbreaks occur.	• Lack of necessary resources to enhance laboratory capacity	
THERAPEUTICS		
Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.		
1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to the operationalize protocol (e.g., obtaining necessary reviews and approvals, performing training) • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to operationalize the protocol (e.g., obtaining necessary reviews and approvals, performing training) • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented during future NiV outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to operationalize the protocol (e.g., obtaining necessary reviews and approvals, performing training) • The need to develop consensus across different countries to generate a regional approach and promote adoption by member states • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.	--	--
Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.		
1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.	• Obtaining consensus regarding prioritization of promising candidates • Lack of an adequate pipeline of promising	--

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap		
MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
	therapeutic agents and lack of R&D resources to strengthen the pipeline	
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.	• Lack of dedicated R&D funding to conduct preclinical evaluation of novel NiV therapeutic agents • Limited BSL-4 capacity • Limited understanding regarding whether or not genetic diversity of NiV strains will impact therapeutic response to new treatment options	• Availability of standardized and well-characterized clinical research tools (e.g., assays, reagents, stocks of NiV challenge strains) (Cross cutting: SG3, MS1)
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.	• Lack of in-country capacity to conduct clinical trials • Hesitancy or opposition to clinical trials being conducted in at-risk populations • Difficulty in receiving approval from in-country NRAs to conduct clinical trials • Identifying sufficient cases to support an informative evaluation, especially if there are multiple potential therapeutic candidates	• Successful completion of preclinical evaluation of promising therapeutic candidates (Therapeutics: SG2, MS2) • Improved diagnostic assays to rapidly and accurately identify cases (Diagnostics: SG2, MS4) • Adequate laboratory capacity for diagnostic testing (Diagnostics: SG3, MS1)
VACCINES		
Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.		
1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.	• Need for regulatory engagement and guidance on whether or not clinical efficacy trials are feasible • Need for better characterization of clinical trial capacity in areas of NiV spillover • Lack of accurate information on the disease burden caused by NiV infection, which is important for assessing the likelihood of completing clinical efficacy trials	--
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from	• Challenges with identifying the appropriate regional and international partners • Challenges with developing consensus among partners	• Determination the feasibility of conducting phase 3 clinical efficacy trials in NIV-affected areas (Vaccines: SG1, MS1)

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
the following: in-country regulators, other regulatory agencies (such as FDA or the EMA), national and international public health agencies and organizations, and local and international researchers.		
Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.		
1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.	• Insufficient R&D funding to conduct preclinical evaluation of promising NiV vaccines • Limited BSL-4 capacity for conducting preclinical research on potential NiV vaccines	• Availability of standardized and well-characterized clinical research tools (e.g., assays, reagents, stocks of NiV challenge strains) (Cross cutting: SG3, MS1) • Availability of animal models that bridge to humans and can be used to demonstrate vaccine protection (Cross cutting: SG3, MS2)
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines, including in target populations.	• Hesitancy or opposition to clinical trials being conducted in at-risk populations ~~• Lack of in-country capacity to conduct clinical trials~~ • ~~Difficulty~~ Delays in receiving approval from in-country NRAs to conduct clinical trials •	• Successful completion of preclinical evaluation of 1 or more promising vaccine candidates (Vaccines: SG2, MS1)
3. By 2023, complete the following if phase 3 trials are considered feasible (per the first Strategic Goal): (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a strategy for determining which candidate vaccines will go forward into phase 3 trials.	• The challenges of engaging partners and completing steps necessary to operationalize clinical trial protocols • The need to develop consensus across different countries • Lack of surveillance capacity and systemic estimates for NiV infection to assess vaccine efficacy and impact over time	• Successful completion of early clinical trials of 1 or more promising vaccine candidates (Vaccines: SG2, MS2)

Abbreviations: BSL-4, biosafety level 4 (highest biosafety level); EMA, European Medicines Agency; FDA, US Food and Drug Administration; MCMs, medical countermeasures; MS, Milestone; MTA, material transfer agreement; NHP, nonhuman primate; NiV, Nipah virus; NRA, national regulatory authority; PEP, post-exposure prophylaxis; R&D, research and development; SG, Strategic Goal; TPP, target product profile; WHO, World Health Organization.

*These dependencies focus only on the relationships between the milestones to show how the roadmap should be implemented over time; however, there are

Commented [SPL1]: Not really an issue with CEPI support.

Commented [SPL2]: This is relevant neither for Bangladesh nor India which each have a long history of such trials.

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap		
MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
other critical dependencies for each of the milestones, such as adequate resources and funding.		

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs) – diagnostics, therapeutics, and vaccines – against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India; some differences have been noted in the clinical features of infection with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this~~e~~ roadmap ranges from basic research to late-stage development~~, licensure, and early use~~ of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing zoonotic NiV transmission, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy. Further research of NiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV (or related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

Commented [KM1]: Issues related to MCM development for animals have been deleted, since the decision was made at the July meeting to focus on human disease. However, further research in animals is critical, so this sentence was added.

VISION

Robust MCMs to detect, prevent, and control human outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research respresents a substantial challenge, since e~~E~~conomic incentives to invest in Nipah research are not readily apparent because~~, as~~ the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low with small and sporadic outbreaks. ~~; therefore, securing funding for Nipah research represents a substantial challenge.~~ The development of a sustainable value proposition for industry and international philanthropic public-private partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition ~~would ideally~~should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate. ~~and will likely require new systematic surveillance studies in humans and susceptible animal hosts in affected areas to strengthen the evidence base.~~

Commented [KM2]: Taken out because this is too long term for creating the value proposition.

- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions, which increases~~. This raises~~ the cost of MCM development.
- To date, NiV spillovers to human communities have been identified ~~almost exclusively~~ most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- ~~Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs older than 4 weeks old), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before~~

~~livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.~~

- ~~NiV disease occurs in both humans and animals (particularly livestock). This creates two streams of research, which complicates the overall approach for moving NiV MCMs forward. For example, animal models for human disease will have different parameters than animal models for disease in animal species.~~
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research, ~~applicable to humans (and pigs and horses for research applicable to disease in animals),~~ the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. ~~However,~~ B~~b~~ecause NiV infection occurs as relatively small, focal outbreaks, the low disease incidence ~~in endemic areas~~ poses a major challenge for conducting ~~phase 3 clinical efficacy~~such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by combining clinical trial data across outbreaks over time. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not ~~applicable~~feasible.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop amd manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures.
- Additional prospective serosurveillance data from susceptible animal species and proximate human populations in areas of predicted risk to determine the level of human spillover and to build preparedness for detection of human cases and for limiting exposure. This is particularly important in areas where public health surveillance programs are not feasible.

Commented [KM3]: Even though this bullet deals primarily with animals, I left it in, since it also deals with the human-animal interface.

- Standardized and ~~validated~~ well characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.) WHO

international standards should be used (when available) as calibrators and reported in Units/ml to harmonize assay results.

- ~~Efforts to establish the natural history of NiV infection in animals and define the animal models that are most reflective of human disease.~~
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- ~~Efforts to design clinical efficacy trials in affected areas that are ethical, interpretable, and feasible. Researchers should explore the potential for conducting clinical trials before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).~~
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and ~~facilitate effective communication with communities to~~ strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Commented [KM4]: Moved to vaccines.

Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of Pteropus bats and potentially other bat species.

- Additional research is needed to ~~refine, standardize, and validate~~optimize relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) ~~to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for~~ to promote development and evaluation of MCMs, particularly if investigators intend to use an alternative pathway (such as the United States Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data ~~between~~ from animal models ~~and~~ to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole genome sequencing of NiV viruses are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume data palm sap and workers at the human-animal interface) ~~pig farmers, hunters, abattoir workers)~~ and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.
- ~~Efforts are needed to: (1) assess potential barriers for conducting clinical trials, (2) assess MCM acceptability in at-risk populations, (3) determine culturally appropriate messaging to enhance MCM acceptance, and (4) identify public health strategies to promote vaccine use.~~

Commented [KM5]: This is relatively redundant to the bullet above, so was deleted.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Commented [KM6]: In the Lassa taskforce meeting, we discussed the need for a value proposition document, which can help justify the funding plan, so that was added here. We also talk about the value proposition at the beginning of this document. There actually is a template for a value proposition for vaccines (created by a WHO group), so perhaps that document could be used as a starting point.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.

- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability henipaviruses in order to improve understanding of their global distribution.
- ~~**Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.~~
- ~~**Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.~~

Commented [KM7]: These two bullets were moved to vaccines.

- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.

- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an "affordable and fair" price is one that can reasonably be funded by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Critical Path Analysis

[See accompanying table.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and etiologic diagnosis is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate which henipaviruses are in circulation using serologic assays. Capacity to identify additional

pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.
- A TPP for NiV diagnostics~~,~~ ~~identifying~~ that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A ~~biobank~~ virtual repository (with specimens being held and maintained in the countries of origin) of ~~human and animal~~ clinical samples to assess and validate diagnostic tests.~~, and a process for how best to judiciously use the samples.~~ As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6~~3~~) identify who would have access to the samples; ~~and~~ (7~~4~~) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- ~~Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.~~
- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), ~~can be applied in both human and animal populations,~~ and have a high degree of sensitivity and specificity for different NiV strains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

Commented [KM8]: Moved up from further down

Commented [KM9]: Moved up and shortened.

- Improved ~~D~~diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses ~~in humans and animals~~ as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- ~~If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of public health concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.~~
- ~~If NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals (such as the Differentiating Infected from Vaccinated Animals (DIVA) test) will be needed.~~

Commented [KM10]: The group decided to take out any reference to multiplex assays, given the 5 year timeframe of the roadmap, so this was deleted.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. ~~Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.~~
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- ~~A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).

2. By 2020, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2018, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.

2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.

3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks. For example, this may include strategies for development of mobile laboratories that can be deployed when outbreaks occur.

Priority Areas/Activities

Research

- **Create** a ~~biobank~~ virtual repository of clinical ~~human and animal~~reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- ~~**Continue to research** cross-reactivity of diagnostic tests in animal populations.~~

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- ~~**Define** use cases for diagnostic assays.~~
- **Develop and ~~,~~ evaluate**~~, and validate~~ point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), ~~available for use in humans and animals, and~~ can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.
- ~~**Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.~~
- ~~**Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.~~
- ~~**Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).~~

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed. ~~in humans and animals.~~

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Critical Path Analysis

[See accompanying table.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment and PEP to exposed persons.

- In ~~the~~ NiV-endemic ~~region of Bangladesh~~areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. ~~In the absence of confirmatory testing, t~~Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well ~~an~~ a therapeutic agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control ~~systems~~ programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- ~~A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.~~
- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- ~~Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.~~
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- ~~Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection may be warranted.~~
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against a strain from Bangladesh/India to be shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- ~~Additional studies, as needed, of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.~~
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will impact response to therapeutic candidates and influence results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines and evidence-based policy decisions.

Commented [KM11]: Taken out because this was redundant to other statements above.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2018, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.

3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented during future NiV outbreaks and develop plans for operationalizing the protocol.
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.

Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as ~~ribavirin and~~ m102.4 and favipiravir) for treating and preventing NiV infection, including conducting ~~animal~~ studies in animal models and clinical trials as appropriate and feasible.
- **Continue to ~~identify~~ expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- ~~**Generate** a TPP for NiV infection therapeutics.~~
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized in advance of future outbreaks, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.

- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- ~~**Identify** a company or joint partnership to advance therapeutic use of m102.4 (including conduct of additional collaborative research) and **secure** financing for its manufacture, and distribution, and post-marketing surveillance.~~
- **Generate** a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Critical Path Analysis

[See accompanying table.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could impact acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.

- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac® HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed in target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations and identify additional areas of potential NiV spillover.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- ~~Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses and available evidence suggests this vaccine is cross-protective against NiV. Ongoing research into developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.~~
- ~~Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.

2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.

2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.

3. By 2023, complete the following if phase 3 trials are considered feasible (per the first Strategic Goal): (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of the humoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serological assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.

- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.
- ~~**Explore** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.~~
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.

Product development

- ~~**License**~~ **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans. ~~and animals.~~
- ~~**License** safe and effective multivalent vaccines for use in humans that protect against more than one disease for use in human populations (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.~~
- ~~**Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a sustainable approach.~~

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of ~~licensed~~ NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptation and promotion within the community.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for more rapid spread.

Critical Path Analysis

[See accompanying table.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10): e1000642. doi: 10.1371/journal.ppat.1000642. [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;(8). doi: 10.1038/s41598-018-25780-3 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-1037 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137). doi: 10.1016/S0140-6736(18)31264-9 [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-2975 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

Critical Path Analysis for Development of Nipah Virus Diagnostics, Therapeutics, and Vaccines

The table below provides a high-level description of the primary risks and barriers to achieving the milestones identified in the research and development (R&D) roadmap for Nipah virus and also identifies other milestones that are critical dependencies, meaning that in order for the milestone in question to either be initiated or completed, another milestone should be completed first. The focus of this critical path analysis is on R&D of medical countermeasures (MCMs) and does not encompass all aspects of Nipah virus prevention and control.

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
CROSS-CUTTING ISSUES		
Strategic Goal 1: Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.		
1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.	• Lack of resources to create a public value proposition (e.g., funding, availability and commitment of partners with the appropriate expertise) • Lack of accurate data sources and information to support the value proposition	--
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.	• The need to identify and engage appropriate industry partners as part of the process in creating a funding plan • Challenges with identifying funders willing to support elements of the funding plan	• A completed value proposition to support and justify the funding plan (Cross cutting: SG1, MS1)
Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.		
1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.	• Lack of ability to secure funding to enhance NiV surveillance • Challenges with developing consensus on the implementation protocol because of differing clinical and laboratory capabilities across catchment areas • Limited information regarding which areas may be at high risk for NiV spillover	--

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.	• The need to educate clinicians and community health workers about NiV infection to increase their index of suspicion and improve case detection	• A completed and agreed upon surveillance plan and related protocols (Cross cutting: SG2, MS1) • Improved diagnostic assays to rapidly and accurately identify cases (Diagnostics: SG2, MS4) • Adequate laboratory capacity for diagnostic testing (Diagnostics: SG3, MS1)
Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.		
1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.	• Limited BSL-4 capacity • Lack of dedicated funding to create research tools • Lack of consensus on criteria for standardization and characterization	• Availability of well-characterized clinical samples (including representation of the different NiV strains) (Diagnostics: SG1, MS2)
2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.	• Limited BSL-4 capacity • Regulations and restrictions applicable to animal research, including ethical considerations regarding use of NHPs • The need for agreed-upon benchmark parameters (i.e., challenge strain, route, timing, and dose)	• Availability of standardized assays and challenge strains for animal research (Cross cutting: SG3, MS1) • Availability of well-characterized clinical samples (including representation of the different NiV strains) (Diagnostics: SG1, MS2)
DIAGNOSTICIS		
Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.		
1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).	• Complexities involved with creating a virtual reference repository • The need for coordination across multiple organizations and agencies within each of the primary NiV-affected countries • The need for international and in-country leadership to drive the process	--
2. By 2020, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.	• Lack of funding and resources to create the in-country repositories • Lack of adequate case numbers for sample collection (in part owing to lack of surveillance capabilities) • Challenges with international regulations for transport of clinical samples and other biologic	• Completion of the plans and protocols for creating the virtual reference repository (Diagnostics: SG1, MS1)

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
	materials • Challenges in completing the steps necessary to operationalize the plans (e.g., ensuring laboratory capacity, conducting training, having MTAs in place, having the governance structure in place)	
Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient-care NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.		
1. By 2018, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.	--	--
2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.	• The need for a coordinated process and leadership to engage and convene the appropriate regulatory agencies and other key partners	--
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.	• Insufficient resources and funding for R&D to produce promising diagnostics • Low engagement in diagnostic development from commercial manufacturers • Adequate supplies of clinical samples or experimentally generated samples from research animals	• Availability of well-characterized samples from patients (Diagnostics: SG1, MS2)
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.	• Lack of clinical and laboratory capacity for conducting field studies in different geographic areas of NiV spillover • The need to ensure proper test performance under field conditions	• Successful completion of preclinical evaluation of promising diagnostic assays (Diagnostics: SG2, MS3) • Adequate laboratory capacity to conduct the field studies (Diagnostics: SG3, MS1)
Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.		
1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks. For example,	• The need for international leadership and engagement to assist with development of the national laboratory strategies • The need to engage and obtain support from key in-country partners	--

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap		
MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
this may include strategies for development of mobile laboratories that can be deployed when outbreaks occur.	• Lack of necessary resources to enhance laboratory capacity	
THERAPEUTICS		
Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.		
1. By 2019, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to the operationalize protocol (e.g., obtaining necessary reviews and approvals, performing training) • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to operationalize the protocol (e.g., obtaining necessary reviews and approvals, performing training) • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented during future NiV outbreaks and develop plans for operationalizing the protocol.	• The challenges of engaging partners and completing steps necessary to operationalize the protocol (e.g., obtaining necessary reviews and approvals, performing training) • The need to develop consensus across different countries to generate a regional approach and promote adoption by member states • Potential issues regarding storage of candidate therapeutics and plans for deployment	• Prioritization of promising therapeutic candidates and ensuring adequate supplies necessary to conduct clinical trial research (Therapeutics: SG2, MS1)
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.	--	--
Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.		
1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.	• Obtaining consensus regarding prioritization of promising candidates • Lack of an adequate pipeline of promising	--

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES BY STRATEGIC GOAL	PRIMARY RISKS/BARRIERS	OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES*
	therapeutic agents and lack of R&D resources to strengthen the pipeline	
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.	• Lack of dedicated R&D funding to conduct preclinical evaluation of novel NiV therapeutic agents • Limited BSL-4 capacity • Limited understanding regarding whether or not genetic diversity of NiV strains will impact therapeutic response to new treatment options	• Availability of standardized and well-characterized clinical research tools (e.g., assays, reagents, stocks of NiV challenge strains) (Cross cutting: SG3, MS1)
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.	• Lack of in-country capacity to conduct clinical trials • Hesitancy or opposition to clinical trials being conducted in at-risk populations • Difficulty in receiving approval from in-country NRAs to conduct clinical trials	• Successful completion of preclinical evaluation of promising therapeutic candidates (Therapeutics: SG2, MS2) • Improved diagnostic assays to rapidly and accurately identify cases (Diagnostics: SG2, MS4) • Adequate laboratory capacity for diagnostic testing (Diagnostics: SG3, MS1)
VACCINES		
Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.		
1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.	• Need for regulatory engagement and guidance on whether or not clinical efficacy trials are feasible • Need for better characterization of clinical trial capacity in areas of NiV spillover • Lack of accurate information on the disease burden caused by NiV infection, which is important for assessing the likelihood of completing clinical efficacy trials	--
2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as FDA or the EMA),	• Challenges with identifying the appropriate regional and international partners • Challenges with developing consensus among partners	• Determination the feasibility of conducting phase 3 clinical efficacy trials in NIV-affected areas (Vaccines: SG1, MS1)

Critical Path Analysis of Milestones for Strategic Goals Identified in the WHO Nipah Virus R&D Roadmap		
MILESTONES BY STRATEGIC GOAL	**PRIMARY RISKS/BARRIERS**	**OTHER MILESTONES THAT ARE CRITICAL DEPENDENCIES***
national and international public health agencies and organizations, and local and international researchers.		
Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.		
1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.	• Insufficient R&D funding to conduct preclinical evaluation of promising NiV vaccines • Limited BSL-4 capacity for conducting preclinical research on potential NiV vaccines	• Availability of standardized and well-characterized clinical research tools (e.g., assays, reagents, stocks of NiV challenge strains) (Cross cutting: SG3, MS1) • Availability of animal models that bridge to humans and can be used to demonstrate vaccine protection (Cross cutting: SG3, MS2)
2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines, including in target populations.	• Hesitancy or opposition to clinical trials being conducted in at-risk populations • Lack of in-country capacity to conduct clinical trials • Difficulty in receiving approval from in-country NRAs to conduct clinical trials	• Successful completion of preclinical evaluation of 1 or more promising vaccine candidates (Vaccines: SG2, MS1)
3. By 2023, complete the following if phase 3 trials are considered feasible (per the first Strategic Goal): (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a strategy for determining which candidate vaccines will go forward into phase 3 trials.	• The challenges of engaging partners and completing steps necessary to operationalize clinical trial protocols • The need to develop consensus across different countries • Lack of surveillance capacity and systemic estimates for NiV infection to assess vaccine efficacy and impact over time	• Successful completion of early clinical trials of 1 or more promising vaccine candidates (Vaccines: SG2, MS2)
Abbreviations: BSL-4, biosafety level 4 (highest biosafety level); EMA, European Medicines Agency; FDA, US Food and Drug Administration; MCMs, medical countermeasures; MS, Milestone; MTA, material transfer agreement; NHP, nonhuman primate; NiV, Nipah virus; NRA, national regulatory authority; PEP, post-exposure prophylaxis; R&D, research and development; SG, Strategic Goal; TPP, target product profile; WHO, World Health Organization. *These dependencies focus only on the relationships between the milestones to show how the roadmap should be implemented over time; however, there are other critical dependencies for each of the milestones, such as adequate resources and funding.		

From:	Shahana Parveen on behalf of Shahana Parveen <shahana@icddrb.org>
To:	Kris Moore
Cc:	Steve Luby; christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; egurley1@jhu.edu; kim.halpin@csiro.au; gnmillig@utmb.edu; mahmudur57@gmail.com; dtmourya@gmail.com; pyr3@cdc.gov; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca; mto@umn.edu; Julie Ostrowsky; BENASSI, Virginia; preziosim@who.int; Kori Cook; "Josie Golding"
Subject:	RE: Revised Nipah R&D Roadmap and CPA table for taskforce member review
Date:	Saturday, August 18, 2018 9:59:59 AM
Attachments:	Nipah Roadmap August 2018 with track changes For Review eg SP.doc Nipah revised CPA table August 2018 for Review sl SP.docx

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Dear Kris,

Please see the comments in the attached file. My comments in Nipah roadmap document are combined with Emily's one. For Nipah CPA table I included my comment with Steve's one.

Regards,
Shahana

From: Steve Luby [mailto:sluby@stanford.edu]
Sent: Friday, August 17, 2018 6:18 PM
To: Kris Moore
Cc: christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; egurley1@jhu.edu; kim.halpin@csiro.au; gnmillig@utmb.edu; mahmudur57@gmail.com; dtmourya@gmail.com; pyr3@cdc.gov; Shahana Parveen; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca; mto@umn.edu; Julie Ostrowsky; BENASSI, Virginia; preziosim@who.int; Kori Cook; 'Josie Golding'
Subject: Re: Revised Nipah R&D Roadmap and CPA table for taskforce member review

Dear Kris:

Attached are my comments. I apologize that these are not combined with Emily's. I started working on this a couple of days ago, but only completed it after Emily's comments.

Steve

Kris Moore

Wednesday, August 08, 2018 5:54 AM

Dear Nipah WHO R&D roadmap taskforce members:

Attached please find the following:

- A revised version of the Nipah WHO R&D roadmap, with track changes so you can see the edits that were made on the documents in response to discussions that took place at the July meeting in London. We DID NOT use track changes on the strategic goals and milestones, since these were substantially changed; the goals and milestones are highlighted in gray and we would like you to pay particular attention to those.
- A revised Critical Path Analysis (CPA) table that incorporates the new goals and

> milestones and also looks at critical dependencies among milestones, rather than looking at possible mitigation steps. Please be aware that this version is still in draft and the final version may not use this format.

To those of you who were able to attend the meeting in person, we're very grateful for you time and input—the meeting was extremely valuable. To those of you who could not be there, we're still very interested in your edits and written comments on this next version.

We would very much appreciate your review of the roadmap and the current version of the CPA table. Please send us any edits or comments that you have by **AUGUST 17, 2018.** If we don't hear from you by then, we'll assume that the current drafts are acceptable to you.

Once again, thank you so much for your ongoing involvement and participation in this important process. It's a pleasure to work with all of you.

Warm regards,

Kris

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Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a 5-year framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research toward advanced development, licensure, manufacture, acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs) – diagnostics, therapeutics, and vaccines – against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since. NiV infection has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India. Case-fatality rates during outbreaks in Bangladesh and India have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India; some differences have been noted in the clinical features of infection with different strains in humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in this~~e~~ roadmap ranges from basic research to late-stage development~~, licensure, and early use~~ of MCMs to prevent and control NiV outbreaks and endemic disease in humans. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.) The strategic goals and milestones identified in the roadmap are focused on key achievements for the next 5 years; the roadmap milestones will be tracked over time, with periodic assessment of progress and updating as needed.

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing zoonotic NiV transmission, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy. Further research of NiV in animal species, including development of appropriate MCMs targeted to animal populations, also is needed, since disease in animals may amplify occurrence of NiV (or related henipavirus species) in humans and virus transmission can occur at the human-animal interface.

Commented [EG1]: I would argue that enhanced surveillance systems are also crucial to the goals of development of MCM. Without active and effective surveillance, we do not have a platform with which to develop and test these new technologies. In addition, expanded surveillance could have an impact on the business case for these technologies. I think it's important to have this argument clearly articulated in the roadmap.

Commented [KM2]: Issues related to MCM development for animals have been deleted, since the decision was made at the July meeting to focus on human disease. However, further research in animals is critical, so this sentence was added.

VISION

Robust MCMs to detect, prevent, and control human outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Securing funding for Nipah research re~~s~~presents a substantial challenge, since e~~E~~conomic incentives to invest in Nipah research are not readily apparent because~~, as~~ the disease primarily occurs in under-resourced areas of South Asia and reported disease incidence ~~is~~ has, so far, been low with small and sporadic outbreaks. ~~; therefore, securing funding for Nipah research represents a substantial challenge.~~ The development of a sustainable value proposition for industry and international philanthropic public-private partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition ~~would ideally~~should be informed by a robust assessment of the risk of future outbreaks and the economic, societal, and health impacts that such outbreaks could generate. ~~and will likely require new systematic surveillance studies in humans and susceptible animal hosts in affected areas to strengthen the evidence base.~~

Commented [KM3]: Taken out because this is too long term for creating the value proposition.

- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway, can be prohibitively expensive for product developers in the absence of a predictable demand. In addition, licensure of vaccines and therapeutics using alternative regulatory pathways can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions, which increases~~. This raises~~ the cost of MCM development.
- To date, NiV spillovers to human communities have been identified ~~almost exclusively~~ most commonly in rural communities in Bangladesh and India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- ~~Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs older than 4 weeks old), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before~~

~~livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.~~

- ~~NiV disease occurs in both humans and animals (particularly livestock). This creates two streams of research, which complicates the overall approach for moving NiV MCMs forward. For example, animal models for human disease will have different parameters than animal models for disease in animal species.~~
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research, ~~applicable to humans (and pigs and horses for research applicable to disease in animals),~~ the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions; however, phase 3 clinical efficacy trials will need to be conducted in endemic areas. ~~However,~~ B~~b~~ecause NiV infection occurs as relatively small, focal outbreaks, the low disease incidence ~~in endemic areas~~ poses a major challenge for conducting ~~phase 3 clinical efficacy~~such trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by combining clinical trial data across outbreaks over time. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not ~~applicable~~feasible.

Commented [EG4]: And, possibly by expanding surveillance areas.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop amd manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Advocacy to policy makers in affected countries to ensure they understand the potential health, societal, and economic benefits of devoting limited resources to improving NiV surveillance, detection, prevention, and control measures.
- Additional prospective serosurveillance data from susceptible animal species and proximate human populations in areas of predicted risk to determine the level of human spillover and to build preparedness for detection of human cases and for limiting exposure. This is particularly important in areas where public health surveillance programs are not feasible.

Commented [KM5]: Even though this bullet deals primarily with animals, I left it in, since it also deals with the human-animal interface.

- Standardized and ~~validated~~ well characterized assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.) WHO

international standards should be used (when available) as calibrators and reported in Units/ml to harmonize assay results.

- ~~Efforts to establish the natural history of NiV infection in animals and define the animal models that are most reflective of human disease.~~
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- ~~Efforts to design clinical efficacy trials in affected areas that are ethical, interpretable, and feasible. Researchers should explore the potential for conducting clinical trials before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).~~
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and ~~facilitate effective communication with communities to~~ strengthen disease prevention and preparedness activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Commented [KM6]: Moved to vaccines.

Knowledge gaps

- Continued R&D, improved manufacturing processes, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of Pteropus bats and potentially other bat species.

- Additional research is needed to ~~refine, standardize, and validate~~optimize relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) ~~to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for~~ to promote development and evaluation of MCMs, particularly if investigators intend to use an alternative pathway (such as the United States Food and Drug Administration's [FDA's] Animal Rule) to obtain regulatory approval. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data ~~between~~ from animal models ~~and~~ to humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole genome sequencing of NiV viruses are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage populations at high risk of exposure (such as persons who consume data palm sap and workers at the human-animal interface) ~~pig farmers, hunters, abattoir workers)~~ and vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.
- ~~Efforts are needed to: (1) assess potential barriers for conducting clinical trials, (2) assess MCM acceptability in at-risk populations, (3) determine culturally appropriate messaging to enhance MCM acceptance, and (4) identify public health strategies to promote vaccine use.~~

Commented [KM7]: This is relatively redundant to the bullet above, so was deleted.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

Milestones:

1. By 2019, develop a public value proposition to effectively advocate for the development and sustainability of NiV MCMs that: (1) articulates the potential global threat of NiV infection, (2) outlines the social and economic benefits of generating accessible and affordable NiV MCMs, and (3) details the positive impact on the health systems in affected areas.
2. By 2019, create a funding plan for moving NiV diagnostics, therapeutics, and vaccines toward clinical evaluation, licensure/approval, acceptance, and sustainable access.

Commented [KM8]: In the Lassa taskforce meeting, we discussed the need for a value proposition document, which can help justify the funding plan, so that was added here. We also talk about the value proposition at the beginning of this document. There actually is a template for a value proposition for vaccines (created by a WHO group), so perhaps that document could be used as a starting point.

Strategic Goal 2: Improve understanding of NiV epidemiology and ecology to estimate the relative risk and potential for global spread of NiV outbreaks.

Milestones:

1. By 2021, develop a plan for enhancing NiV surveillance, including securing funding, identifying surveillance catchment areas, engaging key partners in those areas, generating a standardized protocol, and conducting training for implementation.
2. By 2022, initiate enhanced NiV surveillance to better characterize NiV epidemiology (including the potential for spillover events), enhance case detection, and better define the disease burden in different geographic areas.

Strategic Goal 3: Support basic science research to improve understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animal models.

Milestones:

1. By 2020, generate standardized and well-characterized assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.
2. By 2021, optimize animal models that recapitulate disease in humans for use in preclinical studies of NiV MCMs and which may be necessary for licensure of MCM products (particularly vaccines) via non-traditional regulatory pathways.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine and optimize** relevant animal models to support the development and evaluation of NiV MCMs, particularly as needed for licensure, if alternative regulatory pathways are considered.

- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability henipaviruses in order to improve understanding of their global distribution.
- ~~**Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.~~
- ~~**Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.~~
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Commented [KM9]: These two bullets were moved to vaccines.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.

- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an "affordable and fair" price is one that can reasonably be funded by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Critical Path Analysis

[See accompanying table.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and implementation of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection, which can complicate epidemiologic investigation.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and etiologic diagnosis is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate which henipaviruses are in circulation using serologic assays. Capacity to identify additional

pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- Clarification regarding the use cases for different diagnostic assays, since the corresponding performance, validation, and regulatory approval requirements may differ depending on how the tests will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific.
- A TPP for NiV diagnostics~~,~~ ~~identifying~~ that identifies the key use cases and optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A ~~biobank~~ virtual repository (with specimens being held and maintained in the countries of origin) of ~~human and animal~~ clinical samples to assess and validate diagnostic tests. ~~and a process for how best to judiciously use the samples.~~ As part of this process, a clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) determine what organizations will be responsible for the activities related to creating and maintaining the repositories; (4) establish standardized protocols for sample collection and maintenance; (5) establish an appropriate governance structure; (6~~3~~) identify who would have access to the samples; ~~and~~ (7~~4~~) prioritize use of samples and sample distribution; and (8) ensure that material transfer agreements (MTAs) are in place. (Samples obtained from laboratory animals also can be used to assess diagnostic assays during the timeframe when the virtual repository is being created.)
- ~~Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.~~
- Rapid point-of-care or near-patient diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), ~~can be applied in both human and animal populations,~~ and have a high degree of sensitivity and specificity for different NiV strains, as needed, depending on the use cases for each test. Rapid diagnostic capability is needed for early case detection to promote outbreak detection, ensure early implementation of infection control measures, and ultimately to improve patient outcomes, once therapeutic options are available.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic criteria and standardized testing for including patients in clinical trials of therapeutics.

Commented [KM10]: Moved up from further down

Commented [KM11]: Moved up and shortened.

- Improved ~~D~~diagnostic preparedness in at-risk areas to detect NiV, HeV, and other emergent henipaviruses ~~in humans and animals~~ as they arise.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- ~~If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of public health concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.~~
- ~~If NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals (such as the Differentiating Infected from Vaccinated Animals (DIVA) test) will be needed.~~

Commented [KM12]: The group decided to take out any reference to multiplex assays, given the 5 year timeframe of the roadmap, so this was deleted.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. ~~Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.~~
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- ~~A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Support development of diagnostic assays through creation of a virtual reference repository of clinical samples from NiV-infected patients.

Milestones:

1. By 2019, develop and standardize plans and protocols (including the governance structure) for creating a virtual reference repository of well-characterized clinical samples to be maintained in the two primary NiV-affected countries (Bangladesh and India).

2. By 2020, identify funding and initiate creation of the virtual reference repository in the two primary NiV-affected countries (Bangladesh and India), with samples to be collected during future outbreaks and possibly as part of future clinical trials.

Strategic Goal 2: Develop and assess affordable, highly sensitive and specific (as needed depending on intended use), point-of-care or near-patient NiV diagnostic tests that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training.

Milestones:

1. By 2018, generate a TPP for NiV diagnostics that identifies the primary use cases and optimal and desirable characteristics to guide the development of promising NiV diagnostic assays.
2. By 2019, engage appropriate regulatory agencies and NRAs to inform commercialization pathways for NiV diagnostic assays.
3. By 2021, complete preclinical evaluation for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.
4. By 2022, complete field studies for at least two of the most promising NiV point-of-care or near-patient diagnostic assays that align with the TPP.

Strategic Goal 3: Enhance laboratory diagnostic preparedness in areas of known or potential spillover risk to promote early detection of NiV.

Milestones:

1. By 2021, develop national laboratory strategies for NiV detection in the primary affected countries that include plans for enhancing laboratory preparedness to diagnosis NiV infection during future outbreaks. For example, this may include strategies for development of mobile laboratories that can be deployed when outbreaks occur.

Priority Areas/Activities

Research

- **Create** a ~~biobank~~ virtual repository of clinical ~~human and animal~~reference samples for use in researching new diagnostic agents.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- ~~**Continue to research** cross-reactivity of diagnostic tests in animal populations.~~

Product development

- **Generate** a TPP for NiV diagnostics that defines the use cases and addresses the optimal and preferred performance characteristics for different use cases.
- ~~**Define** use cases for diagnostic assays.~~
- **Develop** **and** ~~,~~ **evaluate**~~, and validate~~ point-of-care or near-patient rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific (as needed, depending on their intended use), ~~available for use in humans and animals, and~~ can capture antigenically diverse strains of the virus, and can be performed accurately and safely in remote areas under a variety of circumstances.
- ~~**Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.~~
- ~~**Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.~~
- ~~**Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).~~

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Develop** national laboratory strategies for NiV diagnosis and detection in the primary NiV-affected countries.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses as needed. ~~in humans and animals.~~

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Critical Path Analysis

[See accompanying table.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment and PEP to exposed persons.

- In ~~the~~ NiV-endemic ~~region of Bangladesh~~areas, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. ~~In the absence of confirmatory testing, t~~Treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well ~~an~~ a therapeutic agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control ~~systems~~ –programs in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- ~~A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.~~
- Protocols for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- ~~Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.~~
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- ~~Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection may be warranted.~~
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against a strain from Bangladesh/India to be shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- ~~Additional studies, as needed, of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.~~
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially exposed persons annually that could be candidates for PEP.
- Additional information is needed regarding whether or not strain differences will impact response to therapeutic candidates and influence results from clinical trials.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines and evidence-based policy decisions.

Commented [KM13]: Taken out because this was redundant to other statements above.

Strategic Goals and Aligned Milestones

Strategic Goal 1: Enhance preparedness to conduct clinical trials of therapeutic agents during future NiV outbreaks.

Milestones:

1. By 2018, complete a protocol for conducting safety and efficacy clinical trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.
2. By 2019, complete a protocol for conducting PEP trials of promising therapeutic candidates to be implemented in NiV-affected areas during future outbreaks and develop plans for operationalizing the protocol.

3. By 2020, complete a broader, regional protocol for conducting clinical trials of promising therapeutic candidates to be implemented during future NiV outbreaks and develop plans for operationalizing the protocol.
4. By 2020, generate a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.

Strategic Goal 2: Develop and evaluate therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection.

Milestones:

1. By 2019, create and implement a prioritization process for evaluating promising NiV therapeutic candidates.
2. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.
3. By 2023, complete clinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising therapeutic candidates or combination therapies for treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of available investigational therapies (such as ~~ribavirin and~~ m102.4 and favipiravir) for treating and preventing NiV infection, including conducting ~~animal~~ studies in animal models and clinical trials as appropriate and feasible.
- **Continue to** ~~**identify**~~ **expand** the pipeline of new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- ~~**Generate** a TPP for NiV infection therapeutics.~~
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Ensure** that clinical trial protocols are in place and are ready to be operationalized in advance of future outbreaks, including obtaining appropriate approvals and conducting necessary training.
- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.

- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for further clinical testing and outbreak control.

Policy and commercialization

- ~~**Identify** a company or joint partnership to advance therapeutic use of m102.4 (including conduct of additional collaborative research) and **secure** financing for its manufacture, and distribution, and post-marketing surveillance.~~
- **Generate** a reliable source of m102.4 to be used in clinical trials during future NiV outbreaks.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Critical Path Analysis

[See accompanying table.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Currently, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generation of new NiV vaccines.
- Sociocultural issues may hinder trust in the formal human and veterinary clinical and public health systems, which could impact acceptance of NiV vaccine use.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains, and (3) provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines to include vaccination strategies for special populations (such as children, immunocompromised individuals, and pregnant women); different epidemiologic scenarios; and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since neutralizing antibodies are likely the primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development.

- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac® HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized.
- Pre-licensure evaluation of vaccine safety is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed in target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations and identify additional areas of potential NiV spillover.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- ~~Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses and available evidence suggests this vaccine is cross-protective against NiV. Ongoing research into developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.~~
- ~~Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.~~

Strategic Goals and Aligned Milestones

Strategic Goal 1: Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact licensure of a vaccine against NiV.

Milestones:

1. By 2019, convene an expert working group to assess the feasibility of conducting clinical efficacy trials of NiV vaccines and to determine the most appropriate regulatory pathways for licensure.

2. By 2020, convene a regional consultation to clarify in-country issues around conducting clinical trials (if deemed feasible) and the needs for licensure and future use of NiV vaccines. This consultation should include representatives from the following: in-country regulators, other regulatory agencies (such as FDA or the European Medicines Agency [EMA]), national and international public health agencies and organizations, and local and international researchers.

Strategic Goal 2: Develop and evaluate NiV vaccines for prevention of NiV disease in humans.

Milestones:

1. By 2021, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least two promising NiV vaccine candidates.

2. By 2023, complete phase 1 and phase 2 clinical trials for at least one of the most promising NiV candidate vaccines.

3. By 2023, complete the following if phase 3 trials are considered feasible (per the first Strategic Goal): (1) develop a framework for conducting phase 3 clinical trials of NiV vaccine candidates, (2) develop a regional protocol for conducting phase 3 trials and plans for operationalizing the protocol, and (3) create a strategy for determining which candidate vaccines will go forward into phase 3 trials.

Priority Areas/Activities

Research

- **Improve understanding** of the humoral immune response to infection to inform development and evaluation of NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serological assays for vaccine potency analyses.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.

- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.
- ~~**Explore** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.~~
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.
- **Explore** possible strategies for conducting clinical vaccine efficacy trials in affected areas that are ethical, interpretable, and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.

Product development

- ~~**License**~~ **Develop and clinically evaluate** safe and effective monovalent NiV vaccines for humans. ~~and animals.~~
- ~~**License** safe and effective multivalent vaccines for use in humans that protect against more than one disease for use in human populations (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.~~
- ~~**Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a sustainable approach.~~

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines, if clinical trials are considered feasible.
- **Identify and address** issues with licensure and use of NiV vaccines in affected areas through a coordinated process involving key stakeholders, including in-country NRAs and public health authorities.
- **Support** plans for adequate manufacturing and stockpiling of ~~licensed~~ NiV vaccines for further clinical evaluation and use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptation and promotion within the community.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent or if an NiV strain emerges with increased capacity for person-to-person transmission and potential for more rapid spread.

Critical Path Analysis

[See accompanying table.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10): e1000642. doi: 10.1371/journal.ppat.1000642. [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;(8). doi: 10.1038/s41598-018-25780-3 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-1037 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137). doi: 10.1016/S0140-6736(18)31264-9 [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-2975 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

From: Emily Gurley on behalf of Emily Gurley <egurley1@jhu.edu>
To: Kris Moore; christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; kim.halpin@csiro.au; sluby@stanford.edu; gnmillig@utmb.edu; mahmudur57@gmail.com; dtmourya@gmail.com; pyr3@cdc.gov; shahana@icddrb.org; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca
Cc: mto@umn.edu; Julie Ostrowsky; BENASSI, Virginia; preziosim@who.int; Kori Cook; "Josie Golding"
Subject: RE: Revised Nipah R&D Roadmap and CPA table for taskforce member review
Date: Thursday, August 16, 2018 5:18:19 PM
Attachments: Nipah Roadmap.August 2018.with track changes.For Review_eg.docx

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Kris,

Please find my comments on this draft attached.

Best,
Emily

From: Kris Moore [mailto:kamoore@umn.edu]
Sent: Tuesday, August 7, 2018 8:24 PM
To: christopher.broder@usuhs.edu; chuakb@tll.org.sg; emmie.dewit@nih.gov; Emily Gurley <egurley1@jhu.edu>; kim.halpin@csiro.au; sluby@stanford.edu; gnmillig@utmb.edu; mahmudur57@gmail.com; dtmourya@gmail.com; pyr3@cdc.gov; shahana@icddrb.org; linfa.wang@duke-nus.edu.sg; hana.weingartl@inspection.gc.ca
Cc: mto@umn.edu; Julie Ostrowsky <jto@umn.edu>; BENASSI, Virginia <benassiv@who.int>; preziosim@who.int; Kori Cook <K.Cook@wellcome.ac.uk>; 'Josie Golding' <J.Golding@wellcome.ac.uk>; kamoore@umn.edu
Subject: Revised Nipah R&D Roadmap and CPA table for taskforce member review

Dear Nipah WHO R&D roadmap taskforce members:

Attached please find the following:

- A revised version of the Nipah WHO R&D roadmap, with track changes so you can see the edits that were made on the documents in response to discussions that took place at the July meeting in London. We DID NOT use track changes on the strategic goals and milestones, since these were substantially changed; the goals and milestones are highlighted in gray and we would like you to pay particular attention to those.
- A revised Critical Path Analysis (CPA) table that incorporates the new goals and milestones and also looks at critical dependencies among milestones, rather than looking at possible mitigation steps. Please be aware that this version is still in draft and the final version may not use this format.

To those of you who were able to attend the meeting in person, we're very grateful for you time and input—the meeting was extremely valuable. To those of you who could not be there, we're still very interested in your edits and written comments on this next version.

We would very much appreciate your review of the roadmap and the current version of the

CPA table. Please send us any edits or comments that you have by **AUGUST 17, 2018.** If we don't hear from you by then, we'll assume that the current drafts are acceptable to you.

Once again, thank you so much for your ongoing involvement and participation in this important process. It's a pleasure to work with all of you.

Warm regards,

Kris

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Nipah WHO R&D Roadmap Taskforce Meeting

July 9 and 10, 2018

DAY 1	
8:00 am	**REGISTRATION**
	Session 1: Welcome, Introductions, and Overview
8:30 am	Welcome *(Marie-Pierre Preziosi [WHO] and Charlie Weller [Wellcome])*
8:35 am	Introductions *(Michael Osterholm [CIDRAP])*
8:40 am	Overview and meeting objectives *(Kris Moore [CIDRAP])*
	Session 2: Nipah Cross-Cutting Issues
8:50 am	Comments on current roadmap draft: cross-cutting issues
9:05 am	Milestones for cross-cutting issues
9:35 am	Critical path analysis for cross-cutting issues
10:15 am	**BREAK**
10:30 am	Critical path analysis for cross-cutting issues (cont.)
	Session 3: Nipah Diagnostics
11:20 am	Comments on current roadmap draft: diagnostics
11:30 am	**LUNCH**
12:15 pm	Milestones for diagnostics
12:45 pm	Critical path analysis for diagnostics
2:15 pm	**BREAK**
	Session 3: Nipah Therapeutics
2:30 pm	Comments on current roadmap draft: therapeutics
2:45 pm	Milestones for therapeutics
3:15 pm	Critical path analysis for therapeutics
	Session 4: Nipah Vaccines
4:45 pm	Comments on current roadmap draft: vaccines
5:00 pm	Milestones for vaccines
5:30 pm	**ADJOURN DAY 1**
6:00 pm	**DINNER**
DAY 2	
8:00 am	Welcome: Day 2 *(Michael Osterholm [CIDRAP])*
8:05 am	Critical path analysis for vaccines
9:35 am	**BREAK**
	Session 5: Roadmap Implementation and Next Steps
9:50 am	Discussion: Roadmap implementation *(Louisa Tribe [Wellcome]*)
11:50 am	Discussion: Publication process *(Marie-Pierre Preziosi [WHO])*
12:20 pm	Wrap up and next steps *(Michael Osterholm [CIDRAP])*
12:30 pm	**ADJOURN DAY 2**
12:30 pm	**LUNCH**

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, ~~and~~ acceptance and deployment, and assessment) for accelerating the collaborative development of medical countermeasures (MCMs) – diagnostics, therapeutics, and vaccines – against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since~~, with~~. NiV infection ~~disease~~ has also been identified periodically in eastern India and, in 2018, was identified for the first time in southern India.~~; c~~Case fatality rates during outbreaks in ~~these~~ Bangladesh and India ~~countries~~ have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India; some differences have been noted in the clinical features of infection with different strains ~~these strains provoke distinct but overlapping clinical features~~ in ~~both~~ humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority diseases" (defined as diseases that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority diseases to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease. The roadmap is organized into four main sections: cross-cutting issues (for areas that apply to more than one MCM category), diagnostics, therapeutics, and vaccines. (*Note*: These topics are not presented in order of public health priority.)

Other aspects of public health preparedness and response, in addition to R&D for diagnostics, therapeutics, and vaccines, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing zoonotic NiV transmission, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, adequate infrastructure (such as cold-chain maintenance) to deploy MCMs, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid and ~~,~~ accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING ~~TOPICS AND~~ ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low with small and sporadic outbreaks; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition for industry and international philanthropic public-private partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition would ideally be informed by a robust assessment of the risk of future outbreaks (with risk mapping and identification of hotspots), and will likely require new systematic surveillance studies in humans and susceptible animal hosts in affected areas to strengthen the evidence base.
- Demonstrating whether or not a product provides meaningful benefit without undue risk, which is a key aspect of any regulatory approval pathway~~s for MCMs~~, can be prohibitively expensive for product developers in the absence of a predictable demand. ~~For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore~~In addition, licensure of vaccines and therapeutics using alternative regulatory pathways ~~also~~ can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. This raises the cost of MCM development.
- To date, NiV spillovers to human communities have ~~occurred~~ been identified almost exclusively in rural communities in Bangladesh and ~~East~~ India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs older than 4 weeks old), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- NiV disease occurs in both humans and animals (particularly livestock). This creates two streams of research, which complicates the overall approach for moving NiV MCMs forward. For example, animal models for human disease will have different parameters than animal models for disease in animal species.

- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans (and pigs and horses for research applicable to disease in animals), the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines intended for use in humans. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Phase 1 and 2 clinical trials can be conducted in non-endemic regions or in endemic regions. ~~Conducting phase 1 and phase 2 clinical trials is potentially feasible in endemic regions.~~ However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence in endemic areas poses a major challenge for conducting phase 3 clinical efficacy trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. It may be possible to address this issue by combining clinical trial data across outbreaks over time. While it is critical to focus on approaches that make ethical and scientifically valid clinical trials feasible whenever possible, alternative regulatory pathways ~~and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time)~~ may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Key needs

- Funding sources (such as public-private partnerships, government agencies, and philanthropic organizations) and industry incentives and competitions for non-dilutive funding to encourage innovation and secure private-sector commitments to develop amd manufacture NiV MCMs.
- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, surveillance, and implementation of vaccination programs for NiV prevention and control.
- Additional prospective serosurveillance data from susceptible animal species and proximate human populations in areas of predicted risk to determine the level of human spillover and to build preparedness for detection of human cases and for limiting exposure. This is particularly important in areas where public health surveillance programs are not feasible ~~or justifiable~~.
- Standardized and validated assays (to be further defined based on end use), reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.) WHO international standards should be used (when available) as calibrators and reported in Units/ml to harmonize assay results.
- Efforts to establish the natural history of NiV infection in animals and define the animal models that are most reflective of human disease.
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.

- ~~A determination regarding the feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection which is needed~~ Efforts to design clinical efficacy trials in affected areas that are ethical, interpretable, and feasible. Researchers should explore the potential for conducting clinical trials before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) or regulatory agencies to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities ~~may~~ will vary between countries; therefore, early engagement, potentially with support from WHO, is essential to identify country-specific considerations.
- Outreach and education to clinicians and community health workers to improve NiV awareness, training, and outbreak preparedness (e.g., disease diagnosis, clinical management, and infection prevention and control) and to ensure availability of diagnostic tools ~~to improve NiV awareness and training, and to ensure availability of diagnostic tools~~ in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data ~~under the authority of local leadership~~.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and facilitate effective communication with communities to strengthen disease prevention and preparedness activities. ~~facilitate effective communication with communities regarding disease prevention~~ Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, improved manufacturing processes~~manufacture~~, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of Pteropus bats and potentially other bat species.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for development and evaluation of MCMs. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize

the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when after challenge MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection to determine appropriate human MCM doses; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.

- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole genome sequencing of NiV viruses are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage ~~at-risk~~ populations at high risk of exposure (such as pig farmers, hunters, abattoir workers) and ~~(including~~ vulnerable populations (such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease.
- Efforts are needed to: (1) assess potential barriers for conducting clinical trials, (2) assess MCM acceptability in at-risk populations, (3) determine culturally appropriate messaging to enhance MCM acceptance, and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. ~~Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.”~~ Identify sources of private- and public-sector funding, and develop appropriate incentives and competitions to promote R&D of NiV MCMs.

2. Undertake surveillance activities (including research studies) to estimate the relative risk and global spread of NiV outbreaks and public value of MCM development.

3. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.

4. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.

5. Engage NRAs (particularly in endemic and at-risk areas) and WHO to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

Milestones

1. By 20XX, create a living biobank of human and animal reference samples for development of NiV MCMs.

2. By 20XX, generate standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate R&D of NiV MCMs.

3. By 20XX, standardize animal models for use in preclinical studies of NiV MCMs and for bridging data between animals and humans.

4. By 20XX, complete a multi-center epidemiologic or enhanced surveillance study in areas of known or potential NiV spillover to better characterize NiV epidemiology in humans and animals, including improved understanding of reservoir species.

5. By 20XX, convene an expert working group to assess feasibility of conducting clinical trials of NiV therapeutics and vaccines and to determine the most appropriate regulatory pathways for licensure.

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate (i.e., determine that an animal model resembles disease and therapeutic response in humans), and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability henipaviruses in order to improve understanding of their global distribution.
- ~~**Determine** the feasibility of conducting phase 3 clinical trials or identify~~ **Explore** possible strategies for conducting clinical efficacy trials in affected areas that are ethical, interpretable,

and feasible or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.

- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Support** the development of affordable pricing mechanisms to promote accessibility of NiV MCMs in low- and middle-income at-risk countries. (*Note*: According to WHO, an “affordable

and fair" price is one that can reasonably be funded by patients and health budgets and simultaneously sustains research and development, production, and distribution within a country.)

- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Critical Path Analysis

[See accompanying table.]

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and institution of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and etiologic diagnosis is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate which henipaviruses are in circulation using serologic assays. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- A TPP for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobank of human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of public health concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- If NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals (such as the Differentiating Infected from Vaccinated Animals (DIVA) test) will be needed.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.

2. Develop and assess affordable, highly sensitive and specific, point-of-care NiV diagnostic tests for use in humans and animals that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.

3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.

4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Milestones

1. By 20XX, delineate use cases for NiV diagnostic assays.

2. By 20XX, generate a TPP for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.

3. By 20XX, advance at least 2 promising NiV point-of-care or near-patient-care diagnostic tests through field studies.

4. By 20XX, obtain regulatory clearance/market authorization by a relevant regulatory agency or NRA for at least 1 validated NiV point-of-care or near-patient-care diagnostic assay that is aligned with the TPP.

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.
- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Critical Path Analysis

[See accompanying table.]

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- Sociocultural issues may hinder trust in the formal healthcare and public health systems, which could reduce acceptance of NiV therapeutics.
- The absence of improved diagnostic assays for timely diagnosis and surveillance of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection may be warranted.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided ~~as a compassionate use~~under compassionate use programs for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, favipiravir [an RNA-dependent RNA polymerase inhibitor], and GS-~~4734~~ 5734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against a strain from Bangladesh/India to be shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- Additional studies, as needed, of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines and evidence-based policy decisions.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.

2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Milestones

1. By 20XX, generate a TPP for NiV therapeutics identifying optimal and desirable characteristics.

2. By 20XX, conduct clinical trials on m102.4 for safety, tolerability, and efficacy.

3. By 20XX, identify a company or joint partnerships to advance therapeutic use of m102.4 (including conduct of additional collaborative research) and secure financing for its manufacture, distribution, and post-marketing surveillance.

4. By 20XX, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least 2 new therapies for treatment of NiV infection.

5. By 20XX, complete phase 1 and 2 clinical trials for at least 1 additional promising therapeutic agent for treatment of NiV infection.

6. By 20XX, complete phase 3 clinical trials for at least 1 additional promising therapeutic agent that is aligned with the TPP.

7. By 20XX, obtain regulatory approval for a new NiV therapeutic agent that is aligned with the TPP for treatment of NiV infection.

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ~~ribavirin and~~ m102.4 and possibly ribavirin) for treating and preventing NiV infection, including conduct of animal studies and clinical trials as appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company or joint partnerships to advance therapeutic use of m102.4 (including conduct of additional collaborative research) and **secure** financing for its manufacture, ~~and~~ distribution, and post-marketing surveillance.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Critical Path Analysis

[See accompanying table.]

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human and ~~,~~veterinary ~~,~~clinical and public health systems, which could impact acceptance of NiV vaccines for use in humans or animals.
- Currently, there is no candidate vaccine that is in late-stage development and few companies are willing to invest in generation of new NiV vaccines.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that: (1) are readily accessible with adequate supply chains, (2) can protect against different NiV strains in humans and animals, and (3) ~~that~~ provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines (or broader henipavirus vaccines) to include vaccination strategies, potentially in both humans (including special populations such as children, immunocompromised individuals, and pregnant women) and animals, for different epidemiologic scenarios and different vaccine attributes.
- Public communication outreach strategies that address possible vaccine uptake hesitancy in target populations and guidance for community sensitization to vaccine acceptation and promotion within the community.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- ~~While neutralizing antibodies are likely a primary mediator of protection against NiV infection cellular immunity appears to also play a role.~~ Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV. Since ~~While~~ neutralizing antibodies are likely the~~a~~ primary mediator of protection against NiV infection, research in this area should focus primarily on the humoral immune response for driving vaccine development. ~~cellular immunity appears to also play a role.~~
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.
- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac® HeV from Zoetis).
- Additional research is needed in animal models to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection in humans and animals and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms. These specific correlates may vary by vaccine platform and antigen and, therefore, multiple assays may need to be standardized.
- Pre-licensure ~~E~~evaluation of vaccine safety ~~in target populations~~ is needed to better understand the risk of adverse incidents associated with vaccine use. While phase 1 and phase 2 trials can be performed in non-affected countries, safety trials will also be needed in target populations in endemic regions.
- Further epidemiologic research is needed to better define at-risk populations.
- ~~If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas. (Satterfield 201 6 – status of vaccine R&D [combining NV w/ another vaccine to increase likelihood of financing])~~
- Mathematical modelling and forecasting may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), (4) estimating disease risk based on risk behaviors and practices in communities or specific population groups, and (5~~4~~) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses and available evidence suggests this vaccine is cross-protective against NiV. Ongoing research into

developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.

- Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, ~~and~~ prequalify, deploy, and ensure access to NiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).

2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).

3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Milestones

1. By 20XX, identify and standardize correlates and/or surrogates of protection to facilitate NiV vaccine research.

2. By 20XX, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least 1 new NiV vaccine candidate.

3. By 20XX, complete phase 1 and phase 2 clinical trials for at least 1 of the most promising NiV vaccines.

4. By 20XX, complete phase 3 clinical trials for at least 1 of the most promising candidate NiV vaccines.

5. By 20XX, obtain regulatory approval for least 1 vaccine for NiV via phase 3 clinical trials or an alternative regulatory pathway.

Priority Areas/Activities

Research

- **Improve understanding** of the ~~**Determine** the innate, cell-mediated, and~~ humoral immune response~~s~~ to infection to inform development and evaluation of~~that contribute to protective immunity against NiV infection for use in developing and evaluating~~ NiV vaccines.~~.~~
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Generate** international reference standards to calibrate serological assays for vaccine potency analyses.

- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models (such as through serum transfer studies), correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.
- **Explore** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **License** safe and effective monovalent NiV vaccines for humans and animals.
- **License** safe and effective multivalent vaccines for use in humans that protect against more than one disease for use in human populations (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a sustainable approach.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Develop** guidance for community sensitization to vaccine acceptation and promotion within the community.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Critical Path Analysis

[See accompanying table.]

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Zhu Z, Middleton D, et al. A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. PLoS Path. 2009 Oct 30; 5(10): e1000642. doi: 10.1371/journal.ppat.1000642. [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

Dawes BE, Kalveram B, Ikegami T, et al. Favipiravir (T-705) protects against Nipah virus infection in the hamster model. Sci Rep 2018 May 15;(8). doi: 10.1038/s41598-018-25780-3 [Full text]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infec Dis 2007 Jul;13(7):1031-1037 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Nipah virus control needs more than R&D. Lancet 2018 Jun 9;391(10137). doi: 10.1016/S0140-6736(18)31264-9 [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine 2016 Jun 3;34(26):2971-2975 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

The table below provides a high-level description of the *primary* (or most critical) risks/barriers to achieving the milestones identified in the R&D roadmap for Nipah virus, along with their associated *potential* mitigations that may allow achievement of the milestones by the target deadlines. The focus of this critical path analysis is on R&D of MCMs and it does not encompass all aspects of Nipah virus prevention and control. For cross-referencing, milestones may be related to one another and the corresponding risks/barriers and mitigations may be the same as, similar to, or related to those for other milestones.

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
CROSS-CUTTING ISSUES			
1. By 20XX, create a living biobank of human and animal reference samples for development of NiV MCMs.	• Lack of dedicated funding (S3, L3)	• Prioritize funding for development of a biobank of NiV reference samples.	
	• Lack of policies for sample collection, storing, and use (S2, L3)	• Facilitate collaborative development of plans for and creation of a biobank, including policies for ongoing sample collection, storage, and use.	
	• Lack of adequate case numbers for sample collection (S2, L3)	• Improve surveillance capabilities (including the ability to transport samples) in areas of NiV spillover to promote case detection. • Ensure that plans are in place to collect samples over multiple years.	
	• Challenges with international regulations for transport of clinical samples and other biologic materials (S2, L3)	• Review and harmonize all applicable international regulations for transport of biological materials. • Develop a process for expediting MTA development to promote transfer of materials.	
2. By 20XX, generate standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains to facilitate	• Lack of dedicated funding (S3, L2)	• Prioritize funding to produce critical research materials.	
	• Limited BSL-4 capacity to produce critical research materials (S3,L2)	• Ensure availability of BSL-4 facilities for production of critical research materials.	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap			
MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
R&D of NiV MCMs, using WHO international standards, when available, as calibrators.	• Lack of clinical samples (including representation of the different NiV strains (S3, L3)	• Create a biobank of reference samples from different areas of NiV spillover. (See Cross-Cutting Issues: Milestone 1.)	
	• Lack of consensus on criteria for standardization and validation (S3, L2)	• Convene an expert working group to create a consensus document on standardization and validation criteria for critical research materials.	
3. By 20XX, standardize animal models for use in preclinical studies of NiV MCMs and for bridging data between animals and humans.	• Lack of dedicated funding, particularly for high-cost NHP studies (S3, L3)	• Prioritize funding for NiV animal studies.	
	• Limited capacity of BSL-4 facilities for animal studies of NiV infection (S3, L2)	• Ensure availability of BSL-4 facilities for animal studies of NiV infection.	
	• Regulations and restrictions applicable to animal research, including ethical considerations regarding use of NHPs (S2, L3)	• Request that the R&D Blueprint SAG consider the establishment of a workgroup or process to address this issue.	
	• Lack of standardized assays for animal study research (S3, L3)	• Prioritize funding to develop standardized immunologic assays for use in animal models. (See Cross-Cutting Issues: Milestone 2.)	
	• Lack of benchmark parameters (i.e., challenge strain, route, timing, and dose) (S3, L3)	• Convene an expert working group to establish benchmark parameters for animal studies of NiV infection.	
4. By 20XX, complete a multi-center epidemiologic or enhanced surveillance study in areas of known or potential NiV spillover to better characterize NiV epidemiology in humans and animals, including improved understanding of reservoir	• Limited availability of funding in endemic and at-risk areas for epidemiologic research and surveillance (S2, L3)	• Target funding for a multi-center epidemiologic study or enhancements of existing surveillance systems.	
	• Limited capacity to conduct a coordinated research study on NiV epidemiology in humans and animals (S2, L3)	• Conduct a capacity assessment in potential study sites. • Provide necessary training and resources to ensure capacity in study/surveillance sites.	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
species.	• Lack of standardized and validated NiV diagnostic assays available for field use in various conditions (S3, L3)	• Prioritize development of standardized and validated diagnostic assays. (See Cross-Cutting Issues: Milestone 2.)	
5. By 20XX, convene an expert working group to explore options for designing clinical efficacy trials for NiV MCMs, (2) determine feasibility of conducting such clinical efficacy trials, and (3) identify the most appropriate regulatory pathways for licensure.	• Need for regulatory involvement and guidance (S2, L2)	• Ensure involvement of regulators in the working group.	
	• Need for better characterization of clinical trial capacity in areas of NiV spillover (S2, L2)	• Conduct a capacity assessment in potential study sites to determine ability to conduct clinical trials.	
DIAGNOSTICS			
1. By 20XX, delineate use cases for NiV diagnostic assays.	• Lack of a process to determine use cases (S2, L2)	• Identify partners and convene an expert working group to define use cases (including testing of at-risk animals and testing in animal reservoirs). • Use cases should clarify the viral targets for diagnostic assays (e.g., NiV only, NiV and HeV, other henipaviruses).	
2. By 20XX, generate a TPP for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.	• Lack of resources for TPP development (e.g., oversight from WHO, subject-matter expertise, and broad stakeholder input) (S3, L1)	• Identify leadership and partners, establish the process, and prioritize development of a TPP for NiV diagnostics.	
3. By 20XX, advance at least 2 promising NiV point-of-care or near-patient-care diagnostic tests through field studies. [4]	• Lack of clinical and laboratory capacity for conducting field studies in different geographic areas of NiV spillover (S2, L3) • The need to ensure proper test performance under field conditions (S2, L3)	• Provide necessary training and resources to ensure clinical and laboratory capacity for conducting field studies and monitoring test performance.	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
	• Insufficient resources for R&D funding to produce promising diagnostics (S3, L3)	• Incentivize and provide funding streams for supporting R&D of diagnostics (including diagnostic testing in at-risk animals). • Consider development of public-private partnerships to foster diagnostic development. • Consider development of multiplexed tests for detection of multiple pathogens, which may have broader commercial appeal (particularly for testing in livestock).	
4. By 20XX, obtain regulatory clearance/market authorization by a relevant regulatory agency or NRA for at least 1 validated NiV point-of-care or near-patient-care diagnostic assay that is aligned with the TPP. [4]	• Lack of clear guidance from regulatory authorities regarding information needed for regulatory clearance/market authorization (S3, L3)	• Ensure regulatory involvement, including appropriate in-country NRAs, to obtain advance guidance on regulatory requirements.	
	• Insufficient regulatory capacity in countries with NiV spillover (S2, L2)	• Strengthen national regulatory capacity in countries with NiV spillover. • Through WHO-led initiatives, encourage collaboration between national and external regulatory agencies.	
	• The cost to developers of preparing regulatory dossiers (S3, L3)	• Incentivize and provide funding streams to support preparation and submission of regulatory dossiers. • Streamline the regulatory approval process as much as possible.	
THERAPEUTICS			
1. By 20XX, generate a TPP for NiV therapeutics identifying optimal and desirable characteristics.	• Lack of resources for TPP development (e.g., oversight from WHO, subject-matter expertise, and broad stakeholder input) (S3, L1)	• Identify leadership and partners, establish the process, and prioritize development of a TPP for Lassa fever therapeutics.	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
2. By 20XX, conduct clinical trials on m102.4 for safety, tolerability, and efficacy.	• Lack of funding to conduct clinical trials for m102.4 (S3, L3)	• Develop a funding stream for m102.4 clinical trials.	
	• Hesitancy or opposition to clinical trials being conducted in at-risk populations (S2, L3)	• Conduct social science research with at-risk populations to elucidate appropriate social outreach regarding participation in clinical trials. • Work with social science experts and local partners to gain trust in at-risk communities, particularly regarding sensitive sociocultural issues surrounding clinical trials. • Develop locally appropriate consent procedures and ethical review.	
3. By 20XX, identify a company or joint partnerships to advance therapeutic use of m102.4 (including conduct of additional collaborative research) and secure financing for its manufacture, distribution, and post-marketing surveillance.	• Lack of business interest in producing m102.4 (S3, L3)	• Explore incentives for funding m102.4 production or create a sustainable value proposition, possibly through public-private partnerships.	
4. By 20XX, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least 2 new therapies for treatment of NiV infection.	• Lack of dedicated R&D funding to conduct preclinical evaluation of novel NiV therapeutic agents (S3, L3)	• Prioritize R&D funding to promote preclinical evaluation of novel NiV therapeutics. • Consider development of public-private partnerships to foster R&D of novel NiV therapeutics.	
	• Limited capacity of BSL-4 facilities for conducting preclinical research on potential	• Ensure availability of BSL-4 facilities for conducting preclinical research on NiV	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES	PRIMARY RISKS/BARRIERS - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	POTENTIAL MITIGATION STEPS	CRITICAL IMPLEMENTATION PARTNERS
	NiV therapeutics (S3, L2)	therapeutics.	
	• Lack of promising NiV therapeutics in the pipeline (S3, L3)	• Strengthen the pipeline for novel NiV therapeutics.	
	• Limited understanding regarding whether or not genetic diversity of NiV strains will impact therapeutic response to new treatment options (S1, L2)	• Develop a system to monitor antigenic changes and characterize genetic diversity of NiV strains over time. • Ensure that researchers have access to more recent NiV strains and opportunities to evaluate therapeutic responses to diverse strains.	
5. By 20XX, complete phase 1 and 2 clinical trials for at least 1 additional promising therapeutic agent for treatment of NiV infection. [4]	• Lack of capacity to conduct clinical trials (S3, L2)	• Provide necessary training and resources to ensure capacity in clinical trial sites.	
	• Hesitancy or opposition to clinical trials being conducted in at-risk populations (S1, L3)	• Conduct social science research with at-risk populations to elucidate appropriate social outreach regarding participation in clinical trials. • Work with social science experts and local partners to gain trust in at-risk communities, particularly regarding sensitive sociocultural issues surrounding clinical trials. • Develop locally appropriate consent procedures and ethical review.	
	• Difficulty in receiving approval from in-country NRAs to conduct clinical trials (S2, L2)	• Consult with NRAs in advance on preliminary trial designs.	
6. By 20XX, complete phase 3 clinical trials for at least 1 additional promising therapeutic agent that is aligned with the TPP. [4]	• Hesitancy or opposition to clinical trials being conducted in at-risk populations (S1, L3)	• Conduct social science research with at-risk populations to elucidate appropriate social outreach regarding participation in clinical trials. • Work with social science experts and local	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap			
MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
		partners to gain trust in at-risk communities, particularly regarding sensitive sociocultural issues surrounding clinical trials. • Develop locally appropriate consent procedures and ethics agreements.	
	• Difficulty in receiving approval from in-country NRAs to conduct clinical trials (S2, L2)	• Consult in advance with appropriate NRAs on preliminary trial designs.	
	• Lack of capacity to conduct clinical trials (S3, L2)	• Provide necessary training and resources to ensure capacity in clinical trial sites.	
	• Lack of validated diagnostic assays formore rapid detection of NiV infection (S2, L2)	• Prioritize R&D and validation of NiV diagnostic assays.	
7. By 20XX, obtain regulatory approval for a new NiV therapeutic agent that is aligned with the TPP for treatment of NiV infection. [4]	• National regulatory capacity in endemic and at-risk areas may be insufficient to expedite review and approval of NiV therapeutics (S2, L2)	• Encourage collaboration between national and external regulatory agencies.	
	• Lack of clarity and guidance on regulatory pathways and requirements (S2, L2)	• Review recommendations of the expert panel convened to determine most appropriate pathways for regulatory approval. (See Cross-Cutting Issues: Milestone 5.) • Ensure early regulatory involvement, including appropriate NRAs, to obtain guidance on regulatory pathways and requirements.	
	• The cost to developers of preparing regulatory dossiers (S3, L2)	• Incentivize developers and provide funding streams to support preparation, submission, and management of regulatory dossiers. • Streamline the regulatory approval	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
		process as much as possible.	
VACCINES			
1. By 20XX, identify and standardize correlates and/or surrogates of protection to facilitate NiV vaccine research.	• Limited funding for R&D of NiV vaccines (S3, L3)	• Prioritize R&D funding to promote preclinical research that will inform development of NiV vaccines, including identification of correlates of protection.	
	• Lack of different NiV strain stocks for vaccine research (S1, L2)	• Develop and maintain a biobank of human and animal NiV samples. (See Cross-Cutting Issues: Milestone 1.)	
2. By 20XX, complete preclinical evaluation of the preliminary safety, tolerability, and efficacy of at least 1 new NiV vaccine candidate.	• Insufficient R&D funding to conduct preclinical evaluation of promising NiV vaccines (S3, L3)	• Prioritize R&D funding to complete preclinical evaluation of new NiV vaccines. • Consider development of public-private partnerships to foster R&D of new vaccines for NiV infection.	
	• Limited capacity of BSL-4 facilities for conducting preclinical research on potential NiV vaccines (S3, L2)	• Ensure availability of BSL-4 facilities for conducting preclinical research on NiV vaccines.	
	• Lack of standardized and validated assays to assess immunogenicity of vaccines (S3, L2)	• Prioritize research into development of standardized and validated immunogenicity assays. (See Cross-Cutting Issues: Milestone 2.)	
3. By 20XX, complete phase 1 and phase 2 clinical trials for at least 1 of the most promising NiV vaccines.[4]	• Hesitancy or opposition to clinical trials being conducted in at-risk populations (S2, L2)	• Conduct social science research with at-risk populations to elucidate appropriate social outreach regarding participation in clinical trials. • Work with social science experts and local partners to gain trust in at-risk communities, particularly regarding sensitive sociocultural issues surrounding clinical trials. • Develop locally appropriate consent	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap			
MILESTONES	**PRIMARY RISKS/BARRIERS** - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	**POTENTIAL MITIGATION STEPS**	**CRITICAL IMPLEMENTATION PARTNERS**
		procedures and ethics agreements.	
	• Lack of capacity to conduct clinical trials (S3, L2)	• Provide necessary training and resources to ensure capacity in clinical trial sites.	
	• Difficulty in receiving approval from in-country NRAs to conduct clinical trials (S3, L2)	• Consult with in-country NRAs on preliminary trial designs.	
	• Lack of appropriate validated diagnostic assays for more rapid diagnosis of NiV infection (S2, L2)	• Prioritize R&D and validation of NiV diagnostic assays.	
4. By 20XX, complete phase 3 clinical trials for at least 1 of the most promising candidate NiV vaccines. [4]	• Lack of capacity to conduct clinical trials (S3, L2)	• Provide necessary training and resources to ensure capacity in clinical trial sites.	
	• Hesitancy or opposition to clinical trials being conducted in at-risk populations (S2, L2)	• Conduct a social science project to determine how best to engage at-risk populations for participation in clinical trials. • Work with social science experts to develop specific strategies that will mitigate issues related to clinical trial hesitancy or opposition. • Develop locally appropriate consent procedures and ethical review.	
	• The incidence of NiV infection may be too low to assess vaccine efficacy (S2, L2)	• Refer to recommendations of the expert working group convened to determine feasibility of clinical trials and most appropriate regulatory pathways. (See Cross-Cutting Issues: Milestone 5.)	

Critical Path Analysis of Milestones Identified in the WHO Nipah Virus R&D Roadmap

MILESTONES	PRIMARY RISKS/BARRIERS - Severity Level (S1-3)[1] - Likelihood Level (L1-3)[2]	POTENTIAL MITIGATION STEPS	CRITICAL IMPLEMENTATION PARTNERS
5. By 20XX, obtain regulatory approval for least 1 vaccine for NiV via phase 3 clinical trials or an alternative regulatory pathway. [4]	• The cost to developers of preparing regulatory dossiers (S2, L2)	• Incentivize developers and provide funding streams to support preparation, submission, and management of regulatory dossiers. • Streamline the regulatory approval process as much as possible.	
	• Lack of surveillance capacity and systemic estimates for NiV infection to assess vaccine efficacy and impact over time (S2, L2)	• Target funding to enhance surveillance capacity in areas of known NiV spillover.	

Abbreviations: BSL-4, biosafety level 4 (highest biosafety level); HeV, Hendra virus; MCMs, medical countermeasures; MTA, material transfer agreement; NHP, nonhuman primates; NiV, Nipah virus; NRA, national regulatory authority; R&D, research and development; SAG, scientific advisory group; NRA, national regulatory authority; TPP, target product profile; WHO, World Health Organization.

[1] The scale for seriousness ranges from least serious consequence (S1) to most serious consequence (S3).
[2] The scale for likelihood ranges from least likely to occur (L1) to most likely to occur (L3).
[3] In this table, the term “capacity” refers to infrastructure (including physical facilities and equipment as well as systems and processes), workforce, and/or capability.
[4] These milestones assume that selected MCMs demonstrate adequate performance characteristics to be advanced toward licensure and use. An additional risk to note is that MCMs may not demonstrate adequate performance in field studies or in preclinical or clinical trials; if that occurs, then additional R&D would be needed to develop and advance alternative MCMs and the cycle of milestones would need to be repeated.

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Subject:	Revised Nipah R&D Roadmap and Critical Path Analysis table for your review
Date:	Wednesday, June 27, 2018 2:08:33 PM
Attachments:	Agenda.Nipah R&D TF Meeting.final.062618.docx Nipah Roadmap.with edits from public comment.062618.docx Nipah.CPA table.for taskforce review. June 27.docx
Importance:	High

Dear Nipah taskforce members,

We have revised the Nipah R&D draft roadmap based on comments received during the public comment period; I have attached it here for your review. The new edits are in track changes, so you can see them easily. You will also note that we have included a set of milestones for implementation under each main topic area (these are not in track changes). The milestones don't yet have dates attached to them—we need your input for that.

I have also attached a draft of the Critical Path Analysis (CPA) table that builds on the milestones.

We have structured the upcoming taskforce meeting to be relatively informal. Our primary goal is to walk through the roadmap and the CPA table in detail over the course of our time together. We very much need your input on these documents before we can move them forward.

These materials will form the basis of all of our discussions during the meeting, so please review them carefully before you come to London. I have attached a draft agenda as well, so you can see how the time will flow.

If you can't attend the meeting, we would still very much appreciate your review and hope that you can find time to send us some written comments.

Again, thank you so much for your time on this important project and I look forward to seeing you again in London!

Warm regards,

Mike

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Subject:	2nd TC with Experts to discuss therapeutic protocols for the current Nipah situation; 14:00 Geneva Time, May 25
Attachments:	Nipah_Draft_Roadmap_publiccomment_MAY2018.pdf WHO_NIPAH_baseline_situation_analysis_27Jan2018.pdf

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Dear All,

Thank you very much for your time on the call today. A special thanks to our chair, Dr Ed Cox, and to our country colleagues for their valuable updates.

As agreed, we are inviting you all for a second teleconference tomorrow, Friday 25 May 2018, at the same time (14:00-15:00 CET).
The call will be 60 min long and will mainly focus on the development of a PEP protocol. Dial in details below:

Please join my meeting from your computer, tablet or smartphone.
website).

Many thanks and looking forward to our discussion tomorrow,
Virginia Benassi

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs)—diagnostics, therapeutics, and vaccines—against Nipah virus infection.

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also identified periodically in eastern India; case fatality rates during outbreaks in these countries have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other domestic animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary modes of NiV transmission being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for NiV-infected patients. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, altered mental state or unconsciousness, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that there are multiple strains of NiV. For example, the strain responsible for the outbreak in Malaysia is different from those identified in Bangladesh and India; these strains provoke distinct but overlapping clinical features in both humans and experimentally infected non-human primates.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing zoonotic NiV transmission, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING TOPICS AND ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition for industry and international philanthropic public-private partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. The value proposition would ideally be informed by a robust assessment of the risk of future outbreaks, and will likely require new systematic surveillance studies in humans and susceptible animal hosts in affected areas to strengthen the evidence base.

- Regulatory approval pathways for MCMs can be prohibitively expensive for product developers in the absence of a predictable demand. For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore, licensure of vaccines and therapeutics using alternative regulatory pathways also can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. This raises the cost of MCM development.
- To date, NiV spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh and East India; the healthcare facilities that serve these communities have limited laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition, lack of available laboratory testing, and the occurrence of other diseases that have similar clinical presentations.
- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Conducting phase 1 and phase 2 clinical trials is potentially feasible in endemic regions. However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting phase 3 clinical trials, in terms of achieving a sufficient sample size to estimate MCM efficacy with adequate statistical power. Therefore, alternative regulatory pathways and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time) may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Key needs

- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- Additional prospective serosurveillance data from susceptible animal species and proximate human populations in areas of predicted risk to determine the level of human spillover and to build preparedness for detection of human cases and for limiting exposure. This is particularly important in areas where public health surveillance programs are not feasible or justifiable.
- Standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research. Such criteria should align with desired characteristics outlined in the target product profiles (TPPs) and should address aspects of sustainable MCM production, stockpiling, and access.
- A determination regarding the feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection, which is needed before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities may vary between countries; therefore, early engagement is essential to identify country-specific considerations.
- Outreach and education to clinicians to improve NiV awareness and training, and to ensure availability of diagnostic tools in endemic areas to increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capacity for data sharing and analysis (particularly of NiV sequence data) to support collaborative clinical research, including methods for collecting, standardizing, and sharing clinical data under the authority of local leadership.
- Collaboration between public health authorities in endemic and at-risk areas and international development partners to support NiV surveillance and facilitate effective communication with communities regarding disease prevention activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance (or dedicated prospective research with a surveillance focus) is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, including drivers of infection, in the natural reservoir of Pteropus bats and potentially other bat species.

- Additional research is needed to refine, standardize, and validate relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for development and evaluation of MCMs. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) standardize the challenge strain and dose, and determine the most appropriate lethal NiV dose for MCM development; (3) determine when MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.
- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional studies applying whole genome sequencing of NiV viruses are needed to generate a comprehensive phylogenetic mapping of the global genetic variability among henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage at-risk populations (including vulnerable populations such as children, immunocompromised individuals, and pregnant women) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease. Efforts are needed to: (1) assess potential barriers for conducting clinical trials, (2) assess MCM acceptability in at-risk populations, (3) determine culturally appropriate messaging to enhance MCM acceptance, and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.

2. Undertake surveillance activities (including research studies) to estimate the relative risk and global spread of NiV outbreaks and public value of MCM development
3. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.
4. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.
5. Engage NRAs (particularly in endemic and at-risk areas) to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** key differences in pathogenesis for different NiV strains that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate, and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research studies to enable a more comprehensive mapping of genetic variability henipaviruses in order to improve understanding of their global distribution.
- **Determine** the feasibility of conducting phase 3 clinical trials or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting phase 3 clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** active and passive surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in outbreak detection and institution of effective and timely infection control measures and outbreak response activities. Additionally, latent disease can occur months to years after initial infection.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often limited, and etiologic diagnosis is not always pursued; these issues can lead to delays in diagnosis and outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using conventional laboratory methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Antibodies to different henipaviruses are highly cross-reactive, making it difficult to discriminate which henipaviruses are in circulation using serologic assays. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- A TPP for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobank of human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly

specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.

- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of public health concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- If NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals (such as the Differentiating Infected from Vaccinated Animals (DIVA) test) will be needed.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Develop and assess affordable, highly sensitive and specific, point-of-care NiV diagnostic tests for use in humans and animals that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.
3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.
4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.

- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.

- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection may be warranted.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies using different NiV strains and clinical trials in endemic areas are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, and GS-4734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for different NiV strains, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against a strain from Bangladesh/India to be shorter than for a strain from Malaysia.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.

- Additional studies, as needed, of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.
2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ribavirin and m102.4) for treating and preventing NiV infection, including conduct of animal studies and clinical trials as appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against different NiV strains and other pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company to advance therapeutic use of m102.4 and **secure** financing for its manufacture and distribution.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human, veterinary, and public health systems, which could impact acceptance of NiV vaccines for use in humans or animals.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that can protect against different NiV strains in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines (or broader henipavirus vaccines) to include vaccination strategies, potentially in both humans (including special populations such as children, immunocompromised individuals, and pregnant women) and animals, for different epidemiologic scenarios and different vaccine attributes.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines.

- Further research is needed to determine the cross-protection efficacy for NiV of the HeV-sG subunit vaccine (i.e., the recombinant subunit vaccine Equivac® HeV from Zoetis).
- Additional research is needed to determine if vaccine candidates are cross-protective between different NiV strains, including recently identified strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates or surrogates of protection in humans and animals and standardized assays for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Evaluation of vaccine safety in target populations is needed to better understand the risk of adverse incidents associated with vaccine use.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.
- Mathematical modelling may be useful in: (1) assessing whether or not disease incidence is high enough in endemic areas for conducting clinical trials of candidate vaccines, (2) simulating various epidemiologic scenarios for development of vaccination strategies, (3) estimating the potential impact of NiV vaccines (once vaccines become available), and (4) estimating the vaccine quantity that may be necessary to maintain vaccine stockpiles.
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses and available evidence suggests this vaccine is cross-protective against NiV. Ongoing research into developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.
- Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, and deploy NiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection for use in developing and evaluating NiV vaccines.
- **Identify and standardize** correlates and/or surrogates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Further study** cross protection of various vaccine candidates against different NiV strains, and between NiV strains and HeV strains.
- **Perform** clinical trials to assess safety and immunogenicity in phase 1 and 2 trials, and undertake animal studies for immune bridging to facilitate regulatory licensing.
- **Explore** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **License** safe and effective monovalent NiV vaccines for humans and animals.
- **License** safe and effective multivalent vaccines for use in humans that protect against more than one disease for use in human populations (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a sustainable approach.

Key capacities

- **Improve** surveillance capabilities to assess the impact of vaccine use and vaccination strategies (once vaccines become available).
- **Prepare** clinical trial sites and NRAs in affected countries for future clinical trials with NiV vaccines.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

NIPAH Baseline Situation Analysis

M. Denis, consultant

Draft, Jan 27 2018

Contents

List of abbreviations

AAV	Adeno-associated virus
AGM	African green monkey
CNS	central nervous system
ELISA	enzyme-linked immunosorbent serologic assay
HeV	Hendra virus
IFN	interferon
NK	Natural Killer
NiV	Nipah virus
NP	Nucleoprotein
NHP	Non-human primates
Poly-IC	Polyinosinic:polycytidylic acid
R&D	research and development
RT-PCR	real-time polymerase chain reaction
VEE	Equine Venezuelan Encephalitis
VLP	Virus-like particle
VSV	Vesicular Stomatitis Virus

Introduction

Epidemiology overview

Nipah virus (NiV) is a paramyxovirus (genus Henipavirus) that was first identified in 1999 in Malaysia, where it caused an outbreak of respiratory and neurological disease in pigs and encephalitis in people. Three years later, a genetically distinct NiV independently emerged in India as well as in Bangladesh, where human NiV outbreak events have been reported nearly every year since. A putative NiV also caused an outbreak of disease in horses and people in the Philippines in 2014. To date, there is no reported evidence of NiV outbreaks in humans emerging in any other country than Malaysia, Singapore, Bangladesh, India and Philippines. More than 600 cases of NiV human infections have been reported globally. However, given the delay in identification of the Indian outbreak and large distribution of bats that can carry NiV, it is possible that more human cases have occurred where NiV has not been detected. A total of 276 cases were reported with 106 fatalities (38%) in Malaysia, but case fatalities in later outbreaks in India and Bangladesh were associated with significantly higher case fatality rates of 43 to 100%. NiV infection in humans has a range of clinical presentations, from asymptomatic infection to acute respiratory syndrome and fatal encephalitis. The natural reservoir of the virus consists of the widely distributed fruit bats from the Pteropodidae family. Virus transmission from bats to humans occurs through inhalation, contact or consumption of NiV contaminated foods. NiV is transmitted by zoonotic (from bats to humans, or from bats to pigs and then to humans) as well as human-to-human routes. Human-to-human transmission is particularly notable in the outbreaks in India and Bangladesh, where it has been reported to account for 75% and 51% of cases, respectively. At present no vaccines or antiviral drugs are available for NiV disease and the treatment is just supportive. Current prevention strategies focus on raising disease awareness in affected areas.

The virus

NiV was first isolated by Chua et al. in 1999 after a severe outbreak of viral encephalitis among pig-farmers in Malaysia. The virus, cultured from the cerebrospinal fluid of two patients, was causing syncytial formation of Vero cells after 5 days, and it was found to be a previously undescribed paramyxovirus related to the Hendra virus (HeV). The Henipavirus genus in the subfamily Paramyxovirinae (family Paramyxoviridae) was then created for these two pathogenic viruses, HeV and NiV (Wang 2001). Subsequently, other viruses were added to this genus.

NIV is an enveloped, negative-sense, single-stranded RNA virus. The genome is unusually large, comprising more than 18 000 nucleotides. Its 6 genes code for the nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion glycoprotein (F), attachment glycoprotein (G) and the large polymerase. The viral G protein attaches to the host cell ephrin B2 and/or B3 receptor, and activates the F protein to initiate viral envelope and host membrane fusion and viral entry (reviewed by Ong 2015). Of note, membrane fusion is not only essential for viral entry but also for cell-cell fusion and the process of syncytia formation, which makes it an attractive target for therapeutic development. Numerous studies have been conducted to decipher the mechanisms of NiV and other paramyxoviruses replication.

Relatively few isolates of NiV have been obtained from human cases across different infection outbreaks

The nucleotide sequence of a fair number of NiV isolates has been determined. A relative heterogeneity has been observed among nucleotide sequences obtained from Bangladesh/India as compared with sequences from samples obtained during the initial Malaysian outbreak. This observation led to the classification of two distinct lineages of NiV. Currently available sequences obtained from Malaysia and Cambodia were designated genotype M, while sequences obtained from Bangladesh and India were designated genotype B (Lo 2012, reviewed by Angeletti 2016). A 729 nucleotide region of the N protein gene of NiV has been proposed that can be used for such genotyping. Predicted amino acid identities between NiV-M and NiV-B range from 92% to 100% (reviewed by Rockx 2012).

The variation of nucleotide and amino acid sequences within the Malaysia genotype (NiV-M) has been reported to range, respectively, between 0.19 and 2.21% and 0.18 and 3.67%; and within the Bangladesh genotype (NiV-B) between 0.28 and 1.06% and 0.28 and 0.56%. In Malaysia, very limited variation was observed between human NiV isolates and isolates obtained from bats years later. Interestingly, molecular evidence suggests that at least two major strains of NiV were circulating in pigs during the 1998 outbreak in Malaysia. No data are available to indicate whether these 2 strains represent 2 independent introductions of NiV into the pig population, or if the latter strain evolved from the initial NiV strain. Interestingly, the NiV diversity observed among isolates from bats in Malaysia and Thailand was associated with co-circulation of multiple strains within populations rather than co-evolutionary patterns (Angeletti 2016).

Sequence homology at the level of G and F, the glycoproteins involved in virus binding and entry, is sufficient to allow for antigenic cross-reactivity between HeV and NiV, but NiV-B and NiV-M G and F share a much higher homology

In Bangladesh, genetic heterogeneity in human isolates suggests multiple introductions of NiV in the human population from different colonies of fruit bats.

The NiV attachment glycoprotein G and fusion protein F are essential for virus binding and entry and as such are the primary targets for protective antibody responses. The 83% and 88% amino acid identity between HeV and NiV G and F, respectively, results in antigenic cross-reactivity between these viruses (Rockx 2012). The NiV-M and NiV-B strains share 95% amino acid homology in the G protein and 98% homology in the F protein.

Other henipaviruses

HeV was first isolated in 1994 in Australia from fatal cases of severe respiratory disease in horses. Since its first identification in 1994, it has caused 7 confirmed cases in humans and 4 deaths in Australia, all acquired from contact with infected horses (Broder 2013). Infection of horses results from spill-over events from the natural host reservoir, which has been identified as pteropid bats (Black flying-fox, Pteropus Alecto, and Spectacled flying-fox, Pteropus conspicillatus). In humans, HeV begins as an influenza-like illness, but the involvement of the lung and brain, as with Nipah, can also manifest as an acute severe respiratory syndrome, encephalitis or a combination of both.

Other henipaviruses are not known to cause human disease, except for Mojiang paramyxovirus (MojPV), a henipavirus-like virus, which has been implicated in the death of three miners in China in 2012, following potential zoonotic transmission from rats (Wu 2014).

Immune Responses to NiV Infection

Innate immunity

(adapted from Prescott 2012)

Innate immunity generally refers to the ability of cells to detect the invasion of a microbe by the engagement of pathogen-associated molecular patterns (PAMPs) with host encoded pattern recognition receptors (PRRs). This interaction leads to the expression of several antiviral proteins, as well as type I and type III interferons (IFNs), which activate additional antiviral responses via the Jak/STAT pathway. The NiV genomic RNA is recognized by cellular cytoplasmic RNA helicases. In vitro, endothelial cells (an important cell type targeted in vivo) infected with NiV produce IFNβ as well as innate chemokines and cytokines, including IP-10 and IL-6. IP-10 is a chemokine that attracts activated T lymphocytes, whereas IL-6 is a cytokine that stimulates acute-phase proteins and acts as an inflammatory molecule. These chemokines have the ability to functionally recruit T cells. This mechanism likely contributes to the extensive vasculitis reported in virtually all histopathology studies. In vivo, little is known about the activation of the innate immune system. The expression of innate immune genes has been documented as a response to NiV infection in animal models of disease, including the upregulation of IP-10 and IL-6.

Nevertheless, this innate response is balanced by the ability of NiV (as other RNA viruses causing disease in humans) to stimulate antagonising mechanisms. NiV has been extensively characterized in vitro for this ability to subvert innate immunity and several mechanisms have been identified, as presented below.

Mechanisms of inhibition of innate immunity

The P gene of NiV is transcriptionally edited to produce not only the phosphoprotein but also two alternate V and W proteins, and an alternate ORF expresses a C protein. These proteins possess multiple capabilities to inhibit IFN production, as well as downstream signalling. In addition, the expression of these antagonistic genes is temporally regulated during infection. Both STAT1 and STAT2, which are required for IFNβ signalling, are antagonized by P gene products. The antagonistic function of the P, V, W and C proteins has been assessed in vivo. Hamsters inoculated with recombinant virus lacking the ability to produce either the C or the V protein displayed no pathology, and the viral genome was almost undetectable in these animals, suggesting that the antagonistic properties for these proteins are responsible for pathogenesis. The results were subsequently confirmed by Satterfield (2015). A V-deficient virus was found strongly attenuated, behaving as a replicating non-lethal virus in ferrets.

Bharaj (2016) also reported that the matrix protein (M), which is important for virus assembly and budding, can also inhibit IFN-I responses by targeting TRIM6, IKKε and unanchored polyubiquitin chains.

Finally, the nucleoprotein (N) was shown to impact host innate immune responses by preventing the nucleocytoplasmic trafficking of STAT1 and STAT2, resulting in an antagonistic activity against the JAK/STAT signalling pathway (Sugai 2017).

Adaptive immunity

Adaptive immune responses to NiV infection in humans have been very poorly characterized

Little is known about the adaptive immune response to NiV infection in humans. Studies are lacking to identify protective responses. And the immunological mechanisms that may explain the different patterns of disease severity have not been better elucidated.

Sera from infected patients contain measurable NiV-specific IgM antibodies as early as four days after exposure, persisting for at least 3 months (reviewed by Ong 2015, Mathieu 2015, Prescott 2012). Of note, no difference has been observed in clinical features or mortality between seropositive or seronegative patients. Specific IgG can also be detected by day 25 following infection and were shown to persist for several years. The IgG3 and IgG1 subclass distribution observed in NiV infection is similar to the IgG subclass responses seen in measles (Wong 2001).

The presence of antibodies suggests that both B-cell and CD4+ T-cell responses are elicited in response to virus infection, but the cellular arm of the immune response has not been studied in convalescent subjects, and the role it may play in protection following natural infection or vaccination remains unknown.

The NiV N protein is considered as a highly immunogenic protein in human infections and therefore has been proposed as a target for serological diagnosis (Yu 2006). Of note, using immunoblot assays, the serum from one patient with a relapsing NiV encephalitis complication has recently been shown to not react as robustly with the NiV proteins as compared to the other positive patients'sera, and to bind mainly to the NiV G protein (Tiong 2017).

NiV-neutralizing antibodies have been detected in patients' sera. Animal studies suggest that they are highly protective when elicited/administered before or shortly after infection.

Complement has been reported to significantly enhance neutralization by antibodies specific for the NiV F and G glycoproteins (Johnson 2011).

Pathophysiology and clinical disease, case definition

Clinical disease

(adapted from the reviews by Ong 2015, Kulkarni 2013, Wong 2012 and Rockx 2012)

In the majority of cases, the incubation period of Nipah has been reported to be 5 days to 2 weeks; however, a maximum delay of 2 months between exposure and the onset of illness has also been observed during the outbreak in Malaysia (Goh 2000). The majority of patients initially develop influenza-like signs and symptoms, including fever, headache, myalgia and vomiting. In general, the more severe clinical features manifest as either an acute encephalitic syndrome or less frequently a pulmonary syndrome. It is also very likely that a considerable number of infections remain asymptomatic but the exact prevalence is unknown.

Goh (2000) described the clinical features of the largest cohort of 94 patients with NiV encephalitis who were admitted to a single hospital during the outbreak in Malaysia. Clinical signs such as areflexia/hyporeflexia with hypotonia, pinpoint pupils with variable reactivity, tachycardia, hypertension and abnormal doll's eye reflex were more frequently seen in patients with reduced level of consciousness. Segmental myoclonus characterized by focal, rhythmic jerking of the diaphragm and muscles in the limbs, neck and face was present in 32% of patients, and may be unique to acute NiV encephalitis. Other clinical features (e.g. meningism, generalized tonic-clonic convulsions, nystagmus and cerebellar signs) were also observed. A pulmonary syndrome has been described in some patients who presented with cough, atypical pneumonia, acute respiratory distress syndrome and abnormal chest X-ray findings.

Interestingly, a higher prevalence of respiratory disease was observed during the Bangladesh outbreaks with cases experiencing atypical pneumonia and developing an acute respiratory distress syndrome (Hossain et al., 2008). In Bangladesh, altered mental status and/or unconsciousness was reported in more than 70% of patients, severe weakness in 67% and areflexia/hyporeflexia in 65% of cases, consistent with a high prevalence of acute encephalitis.

In Bangladesh, based on a review of 196 cases, the median duration from onset of illness to death was 6 days (ranging from 1 to 47 days) (Rahman 2012). Overall, mortality was high, ranging from about 40% to 100%. In a large cohort of patients who survived, the majority had no or few sequelae. However approximately 20% of patients were reported to have neurological deficits, neuropsychiatric sequelae and gait/movement disorders. The most intriguing complication of Nipah is probably relapsing encephalitis which may occur weeks to years after symptomatic infection and even after asymptomatic NiV infection. So far, more than 20 cases of relapsing NiV encephalitis have been reported, one of which occurred 11 years after an asymptomatic infection. Clinical and radiological findings suggest that relapsing NiV encephalitis is distinct from acute NiV encephalitis. The brain MRI in relapsing encephalitis shows more extensive and confluent hyperintense cortical lesions. Virus could not be cultured from samples collected in a series of NiV relapsing encephalitis. There are currently no known risk factors for relapsed encephalitis, and the mechanisms for persistent CNS infection and disease recrudescence have not been elucidated.

Risk factors and mechanisms for persistent CNS infection and disease recrudescence are unknown

Pathological features are characterized by disseminated, multi-organ vasculopathy comprising endothelial infection/ulceration, vasculitis, vasculitis-induced thrombosis/occlusion, parenchymal ischemia/microinfarction, and parenchymal cell infection in the central nervous system (CNS), lung, kidney and other major organs. This unique dual pathogenic mechanism of vasculitis-induced microinfarction and neuronal infection causes severe tissue damage in the CNS.

Case definition

To our best knowledge, there is no standard case definition of Nipah disease. Epidemiological investigations have used various definitions. At least a temporal association with a disease outbreak and the need for laboratory confirmation were shared across definitions. For instance, the definition used during the 2004 outbreak in Bangladesh is presented below (WHO 2004)

Can a standard case definition of Nipah disease be developed and used widely?

*A **suspect case** was any person with fever AND one or more of the following isolated neurological signs: altered mental state, confusion, convulsions, unconsciousness or neck stiffness, focal weakness/paralysis after 15 December 2003 OR an individual with fever and vomiting or respiratory symptoms or headache AND having had physical contact or shared daily activities with or lived near to a probable or laboratory-confirmed case after 15 December2003.*
*A **probable case** was any patient, dead or alive, who developed fever AND one or more of the following isolated neurological signs: altered mental state, confusion, convulsions, unconsciousness or neck stiffness, focal weakness/paralysis after 15 December 2003 AND who was living in the same district as a laboratory-confirmed case after 15 December 2003.*
*A **laboratory-confirmed case** was any case positive for Nipah IgM antibody after 15 December 2003.*

Pathophysiology of Nipah

Viral tropism

Infections caused by henipaviruses are characterized by their ability to infect multiple organ systems, with a predominant neurological or respiratory tropism. In human cases of NiV infection, NiV can be detected in bronchiolar epithelial cells and is shed mainly by nasopharyngeal and tracheal secretions in the early phase of the illness (reviewed by Escaffre 2013). During the late stages of disease, virus replication spreads from the respiratory epithelium to the endothelium in the lungs. The infection can sometimes trigger a prominent vasculitis in small vessels and capillaries as characterized by endothelial syncytium and mural necrosis. Without being infected themselves, circulating leukocytes could transfer the virus to endothelial vascular cells (Mathieu 2015). NiV was shown indeed to not infect but to efficiently bind to human lymphocytes, and co-culturing with permissive endothelial cells resulted in virus amplification and infection of these target cells. Moreover, mononuclear leukocytes isolated from infected hamsters transferred lethal infection to naive animals. Leukocytes would thus be used as a vehicle to spread the virus to other organs by the haematogenous route. In addition to the lungs, important target organs are the brain, spleen and kidneys, and viremia following respiratory infection can lead to multi-organ failure.

Entry into the brain may actually occur through two distinct pathways: i.e. via the hematogenous route through the choroid plexus and cerebral blood vessels, or anterogradely via the olfactory nerve. In animal models of infection, early virus replication was indeed observed in the olfactory epithelium (Baseler 2016). In addition, Borisevich (2017) showed that human olfactory neurons are highly susceptible to infection with henipaviruses. Nevertheless, it is currently unknown whether the route of virus transmission seen in animal models is relevant in human infections, since the olfactory epithelial surface is relatively large in these species compared to man (Escaffre 2013).

Determinants of pathogenicity

The determinants of NiV pathogenicity are poorly understood. Clinical observations as well as data generated in non-human primates (NHPs) indicate pathogenic differences between NiV-M and NiV-B isolates. For instance, in African green monkeys (AGM), while NiV-B was uniformly lethal, only 50% of NiV-M-infected animals succumbed to infection. Histopathology of lungs and spleens from NiV-B-infected AGMs was significantly more severe than NiV-M-infected animals. These data showed that NiV-B is more pathogenic than NiV-M in AGMs, as observed in human subjects (Mire 2016). However, the mechanisms underlying these differences remain unknown.

Similarly experimental infections in ferrets suggest that NiV-B is shed at higher levels than NiV-M, which may be sufficient to enable the former virus to transmit more efficiently between humans (Hayman 2014).

As presented above, several gene products of NiV have the capacity to inhibit the early proinflammatory response at sites of infection, thereby preventing control of the infection by the immune system. Immunopathology is another possible mechanism. For instance, it was shown that NiV infection resulted in overexpression of CXCL10 (IP-10) in endothelial cells and perivascular infiltrating cells in the brain of patients, as well as Syrian hamsters. CXCL10 is an important chemoattractant involved in the generation of inflammatory immune response and neurotoxicity. Its expression likely plays a role in the accumulation of inflammatory cells in infected areas of the brain (reviewed by de Wit 2015).

Case detection

The epidemic of Nipah virus in Malaysia in 1998/1999 required more than six months before being effectively controlled, as a consequence of the misdiagnosis of the etiologic agent and the resulting implementation of incorrect control measures (Chua 2013). National systems for surveillance of infectious disease in countries such as Malaysia subsequently recognised Nipah as a zoonotic disease of interest, and included it in their list of notifiable diseases. Hospital-based surveillance was developed, as it is considered necessary to quickly detect NiV outbreaks and initiate appropriate control measures (Rahman 2011). However, surveillance in rural areas of the countries where NiV is endemic in bats can be expected to be of moderate quality, so that many cases likely go undetected (Satterfield 2016b). Veterinary surveillance of NiV encephalitis in pigs is organised as well, as the disease appears among the “OIE-Listed diseases, infections and infestations” in force in 2017.

Case management

Guidelines for the clinical management of Nipah patients have been made available in various countries, including affected countries such as Bangladesh (Rahman 2011) and Malaysia. Such management is based on general, supportive treatment (ensuring fluid and electrolyte balance, oxygen inhalation if required…etc.), symptomatic treatment (fever, convulsions, shock…etc), and procedures to prevent further spread of the disease (isolation, barrier nursing, safe handling of deceased bodies…etc.).

Special populations

To our knowledge, there has been no evidence of increased incidence or increased severity of Nipah in special populations such pregnant women, infants or immunocompromised subjects. However, in the case series reported by Goh (2000) older patients, especially those having diabetes mellitus and those with severe brain-stem involvement, carried a poorer prognosis than other Nipah patients.

Animal models

Small animal models

Mice

Standard laboratory mouse strains such as Balb/c and C57BL/6 do not develop disease upon intraperitoneal or intranasal inoculation with NiV (reviewed by de Wit 2015). However, intracerebral inoculation of these animals results in a lethal infection. In aged mice, intranasal NiV inoculation did not result in clinical disease, but most animals seroconverted and vRNA and infectious virus could be detected between 2 and 15 days after inoculation in the lungs of most animals. In IFNAR-KO mice lacking the type I IFN receptor, intraperitoneal inoculation resulted in neurological disease. Histologically, these animals developed vasculitis, perivascular cuffing and meningeal inflammation; the lungs exhibited bronchointerstitial pneumonia.

Of note, a human lung xenograft model has also been developed to study the pathogenesis of NiV infection in human lung in vivo (Valbuena 2014). The model uses the severely immunodeficient NOD/SCID/γcnull (NSG) mice as hosts to support the successful engraftment of human lung xenografts.

Guinea pigs

NiV infection in guinea pigs has been reported to be often mild and variable, ranging from an inapparent infection following challenge to sudden death (reviewed by Geisbert 2012). One study demonstrated that intraperitoneal inoculation with high doses of NiV resulted only a transient fever with minor weight loss after 5–7 days. Guinea pigs are thus not considered a suitable animal model of NiV infection.

Hamster

The Golden Syrian Hamster is a commonly used model to study henipaviruses pathogenesis as the clinical signs and pathological lesions observed following inoculation highly resemble those observed in humans, even though the disease course seems to be affected by the route of inoculation and the inoculum dose. The virus used for infection also influences the disease course, as NiV-M-infected Syrian hamsters show accelerated virus replication, pathology and death when compared to NiV-B-infected animals (DeBuysscher 2013).

Like human patients, the main histopathological finding in Syrian hamsters infected with NiV is systemic vasculitis. Moreover, animals develop encephalitis, with infection of neurons in the CNS. In hamsters that develop severe respiratory disease, usually upon intranasal inoculation with a high inoculum dose, rhinitis can be observed, with virus replicating in the respiratory as well as olfactory epithelium; bronchointerstitial pneumonia develops in the lungs of these animals (reviewed by Dhondt 2013 and de Wit 2015).

Ferrets

Ferrets inoculated oronasally with NiV develop respiratory as well as neurological signs of disease. Systemic vasculitis is present in infected ferrets. Histologically, the upper respiratory tract, with rhinitis, tonsillitis and nasopharyngitis, as well as the lower respiratory tract, with necrotizing bronchointerstitial pneumonia, are affected; focal necrosis has been detected in the spleen. Encephalitis has not been detected, but non-suppurative meningitis occurred in a subset of animals and viral antigen was detected in brain endothelial cells and meninges, and occasionally in neurons and glial cells close to infected endothelium (de Wit 2015).

Non-Human Primates

Preclinical tests of vaccines and therapeutic products are ideally conducted in an animal species that not only develops similar symptoms of the disease as observed in humans, but also shares similar pathophysiological and immunological mechanisms. Non-human primates typically best meet these requirements. The first NHP model described for NiV infection was the Squirrel monkey. However, more recent studies favoured AGMs.

African Green Monkey

A consistent lethal infection and disease is observed in AGMs with low dose NiV challenge. Infection by intratracheal inoculation results in a rapid spread of the virus within 3–4 days and the establishment of infection in multiple organ systems (reviewed by Dhondt 2013, Geisbert 2012, Geisbert 2010). Severe respiratory pathology, neurological disease, endothelial syncytia, and generalized vasculitis all occur in AGMs infected with NiV, providing an accurate reflection of what is observed in infected humans. This conclusion was further confirmed by a comprehensive study reported in 2015 (Johnston), that captured physiological data together with clinical pathology information and virological testing in 4 AGMs inoculated by intra-tracheal route. Increases in heart rate and respiratory rate seen in most animals during acute phase suggested responses to fever, to a developing hypoxic state, and/or to a state of compensatory shock. Animals that succumbed appeared to enter a state of irreversible decompensated shock with hypothermia, azotemia, hyperglycemia, and signs of liver disease. Similar to NiV disease in humans, pneumonitis, systemic vasculitis, and coagulopathy were prominent findings in these animals, and significant histopathologic lesions were present in most tissues assessed, including lymphoid tissues and the lungs. Virus antigen was often associated with areas of necrosis and/or inflammation. Interestingly, in all animals that survived beyond the acute disease phase, relapse encephalitis was identified.

Other animals

Cats

Cats inoculated with NiV subcutaneously or intranasally and orally develop signs of respiratory disease. Histologically, cats develop bronchointerstitial pneumonia and, in some animals, meningitis (reviewed by de Wit 2015). The virus can be found in the lungs, but also in kidneys, spleen and lymph nodes. The model may be used for evaluation of vaccines or therapies, but its interest in limited by the poor availability of reagents and limited characterization of the pathogenic mechanisms in this species.

Pigs

Efforts have been undertaken in view of the development of a NiV veterinary vaccine for pigs, which explains the large number of studies conducted in this animal species. Oronasal and ocular routes of NiV infection mimic quite well natural infection and most infected animals remain asymptomatic even if viral shedding is observed following challenge (reviewed by Dhondt 2013). Sick animals present fever, depression, cough, shivering, and rarely, neurological signs including abnormal posture and seizures. A subcutaneous route of inoculation was also tested, causing symptomatic infections in pigs. The disease shares a number of features with human infection. However, a number of species-specific differences of NiV replication in porcine and human cells have been identified. Sauerhering (2016) found that NiV growth substantially differs in primary airway epithelial cells between pigs and humans, with a more rapid spread of infection in human airway epithelia. Increased replication in human cells correlated with higher endogenous expression levels of the main NiV entry receptor ephrin-B2. On the other hand, porcine monocytes, natural killer cells, and CD6+/CD8+ T cells have been found to support NiV replication, which may facilitate dissemination of the virus (Prescott 2012).

Comparison of models

The advantages and disadvantages of the various animal models described in the literature are presented in Table 1 below.

Table 1. Summary of advantages / disavantages of the different animal models of NiV infection

Model	Advantages	Disadvantages
Mice	Low cost	Intracranial challenge route not relevant to human infection
IFNAR-/- mice	Low cost Some resemblance with pathology in humans (vasculitis, bronchointerstitial pneumonia, meningeal inflammation but no encephalitis)	Not suited to vaccine studies Intraperitoneal route of infection
Syrian hamsters	Low cost Good resemblance with infection in humans (extensive lesions, respiratory and neurological disease; systemic vasculitis)	Limited availability of reagents
Ferrets	Some resemblance with infection in humans (respiratory disease, systemic vasculitis, but encephalitis not detected)	Limited availability of reagents
AGM	Good resemblance with disease in humans (infection in multiple organ systems, neurological disease and severe respiratory pathology along with generalized vasculitis) Resemblance with immune response in humans	High cost Small sample size
Cats	Intranasal or oral route of challenge Very limited resemblance with disease in humans (respiratory disease, but no neurological signs)	Limited availability of reagents, limited characterization of the model
Pigs	Some resemblance with disease in humans (respiratory disease, encephalitis, as well as congestion and haemorrhagic lesions)	Various species-specific differences of NiV replication identified

More detailed tables describing the disease and histopathological lesions in each animal model can be found in the reviews by Dhondt (2013) and Geisbert (2012). While there is today no ideal animal model to study novel vaccines or therapies against NiV infections, various options are available for preclinical testing of such candidates.

Preclinical models are available for evaluation of NiV vaccine and therapy candidates

Epidemiology, Prevention and Control

Transmission and maintenance

The widely distributed fruit bats from Pteropodidae family are considered to form the natural reservoir of NiV. Serological studies demonstrated evidences of NiV infection in multiple bat species, including frugivorous and insectivorous bats. As summarized by Wacharapluesadee (2016), NiV-B has been found in Pteropus giganteus (India), and Pteropus lylei (Thailand). NiV-M has been found Pteropus hypomelanus (Malaysia and Thailand), Pteropus vampyrus (Malaysia and Indonesia), Pteropus lylei (Cambodia and Thailand), Hipposideros larvatus (Thailand), Taphozous species (Thailand) and Rousettus amplexicaudatus (East Timor). However, viral isolation and molecular characterization was usually only successful in Pteropus species (Simons 2014). Henipavirus infection in bats seems to be asymptomatic. Experimental infection by subcutaneous route has been followed by seroconversion in the majority of animals in the absence of visible clinical disease, while a range of histopathological changes was observed within the tissues. Nipah virus was excreted in urine. As suggested by Middleton (2007), this intermittent, low-level excretion may be sufficient to sustain the net reproductive value of the virus in a species where there is regular urine contamination of the fur, mutual grooming, and where urine droplets are a feature of the environment.

Introduction of NiV infection into the human population occurs by two mechanisms of spill-over from bats: transmission via an intermediate animal host, which precipitated the outbreak in Malaysia; and bat-to-human transmission, which has occurred in Bangladesh and India (Clayton 2017).

In Malaysia, the highest risk of human NiV infection was associated with activities involving close contact with and handling of pigs, and transmission to people is thought to have occurred through direct contact with infectious secretions or excretions of pigs. Importation of infected pigs into Singapore from Malaysia precipitated a cluster of infections in abattoir workers in Singapore, whose exposure risks were also associated with close contact with pigs or their excretions. In Malaysia, NiV was highly infectious for pigs, with all classes of pigs susceptible. The pattern of on-farm infection was consistent with respiratory transmission; between-farm spread was generally associated with the movement of pigs. An extensive post-outbreak surveillance program in Malaysia showed that farms that did not receive pigs at the time of the outbreak generally remained uninfected, even when neighbouring farms were infected (Breed 2006). Human infections were predominantly attributed to contact with live pigs. A small number of infected people had no history of contact with or proximity to pigs, and human-to-human transmission is suspected in a few cases. No human case was attributed to contact with bats.

In Bangladesh, a most common risk factor for human NiV infection is drinking contaminated date palm sap or its fermented product. Date palm sap is harvested from December through to March by cutting into the tree trunk and allowing the sap to flow overnight into an open clay pot. Infrared camera studies have demonstrated that Pteropus giganteus bats frequently visit date palm sap trees and lick the sap during collection, potentially contaminating it with NiV from saliva and/or urine (Simons 2014). The other common risk factor for human infection is contact with a patient with Nipah encephalitis. Overall, these two risk factors reflect the two main mechanisms of human infection observed in Bangladesh as well as in India: bat-to-human and human-to-human transmission. In a small number of instances, direct contact with infectious material left by foraging

Possible NiV outbreak in the Philippines in 2014

(adapted from Ching 2015)

In April 2014, 2 human deaths were reported in the municipality of Senator Ninoy Aquino, island of Mindanao in the Philippines. An outbreak investigation identified additional human deaths and non-fatal infections as well as concurrent neurologic disease and sudden deaths in several horses. Subsequently, a human case was defined as illness in any person with an epidemiologic link to the municipality of Senator Ninoy Aquino and who had experienced acute encephalitis syndrome, severe influenza-like illness, or meningitis during the period from March 3 to May 24, 2014. This case definition was met by 17 persons (11 acute encephalitis syndrome, 5 influenza-like illness, 1 meningitis).

Of the 17 case-patients, a total of 7 (41%) had participated in horse slaughtering and horse meat consumption, and 3 (18%) had only consumed horse. Five (29%) case-patients had been exposed to other human case-patients, but not to any horses.

Neutralizing antibodies against NiV and correspondingly lower neutralizing antibody titers against HeV were found for 3 patients. A serum sample from a patient was positive by real-time PCR for NiV, and a 71 nucleotides long sequence of the P gene of NiV was detected from another patient. This short segment had 99% nucleotide identity with NiV-M and 94%-96% identity with NiV-B. Further attempts to amplify additional genome and isolate the virus were unsuccessful.

This outbreak led to the conclusion that ongoing surveillance is needed to help with prompt response to future potential additional outbreaks of Nipah, including not only diagnosis, but also investigation of risk factors associated with spill-over and virus transmission, implementation of control measures, and further characterization of the virus involved. Another important lesson from this outbreak is the fact that a NiV isolate not identical to the one currently circulating in Bangladesh has the potential to emerge and cause disease in human.

bats in trees or on fruit may have also played a role in bat-to-human transmission, and domesticated animals such as cows, goats and pigs may also pose infection risks to people.

Person-to-person transmission has been identified as a pathway for infection of people in India and Bangladesh. In Siliguri, India, 75% of the patients had a history of hospital exposure, i.e. they were members of the hospital staff or had attended to or visited patients in the hospital (Chadha 2006). Similarly, a study of human NiV infections between 2001 and 2007 in Bangladesh attributed 51% of all cases to onward transmission from an infected person, although only 7% of people were identified as having transmitted their infection. In these instances, close contact between infected and naïve individuals appears to drive transmission, and specific risk factors for exposure are associated with direct contact with a clinically unwell patient or their secretions, in particular patients with severe illness and who later died as a result of their infection. Several studies evaluated the risk factors associated with transmission and suggested that the main mode of human-to-human transmission is via direct contact with the infectious secretions of a patient with clinical disease; this is further supported by isolation of NiV from respiratory secretions and urine of patients (Clayton 2017). So far, the basic reproductive number (R_0) of the strains of NiV that have spilled over in Bangladesh has remained very low, averaging 0.48 (Luby 2013).

What is the potential for NiV and other hendraviruses to cause a pandemic ?
(adapted from Luby 2013)

Human-to-human transmission of NiV remains very limited in current outbreaks. However, humans are susceptible to the virus; and as an RNA virus, NiV is characterized by a high rate of mutation, which has the potential to lead to the emergence of a strain better adapted to spread from human to human. Moreover, if such adapted virus were to infect communities in South Asia, high population densities and global interconnectedness could be expected to rapidly spread the infection.

At risk populations

As demonstrated by the Ebola epidemic in 2013-2016, there is potential for highly pathogenic emerging infections that normally cause only small, isolated and containable outbreaks to occasionally result in large epidemics that inflict a significant mortality and morbidity burden. In this regard, the number of people at risk of acquiring NiV in Bangladesh and the neighboring West Bengal state of India (regions that experience more frequent outbreaks) exceeds 250 million. The total number of people at risk, including all countries that experience NiV outbreaks and in which the Pteropus bats occur naturally, exceeds two billion (Satterfield 2015).

Due to the different mode of virus transmission during the outbreaks in Malaysia and Singapore compared to more recent outbreaks, the definition of at risk populations has evolved. Following the NiV outbreak in March 1999 in Singapore, a serological survey screened individuals potentially exposed to NiV, and found that all of the antibody positive individuals had direct contact with pigs (Chan 2002). Subsequent observations in Bangladesh and India led to very different results.

The risk of NiV transmission is underpinned by social and environmental factors, which should be taken into account for control measures

A review of 196 cases in Bangladesh indicated that cases were distributed in all age groups, with a median age of 25 years (range: 6 months to 75 years) and 124 (63%) cases were observed in males (Rahman 2012).

Human cases of Nipah were found to generally occur in areas near inland water, predominantly the Ganges, which provides a suitable habitat for date palm trees (Simons 2014). The disease was also characterized by seasonality in Bangladesh, with cases occurring only between December and April, roughly coinciding with the time that palm sap is collected (Simons 2014, Stone 2011). The central role for palm sap consumption as a risk factor was identified by a number of studies. For instance, Hedge (2016) analysed a large dataset of 157 cases and 632 controls over the period 2004–2012 in Bangladesh, and found that Nipah cases were 4.9 times more likely to consume raw date palm sap.

Human-to-human NiV transmission is the source of additional risk factors, as illustrated by the study by Hedge (2016), who observed that cases in Bangladesh were 7.3 times more likely to have contact with a Nipah case than controls. Specific cultural practices may underlie the fact that transmission commonly occurs between infected patients and their caregivers. In Bangladesh, societal norms dictate that such care is provided by patients' family members even in hospital settings, where trained health-care professionals adopt a "hands off" approach to patient management. A number of cases of human infection in Bangladesh were attributed to corpse-to-human transmission, through embracing the body of a loved one soon after their death, or through ritual preparation of a corpse for religious burial (Clayton 2017).

Distribution

Table 2 below presents the human cases of NiV that have been confirmed since first identification of the virus.

Table 2. Morbidity and mortality due to Nipah or Nipah-like virus encephalitis

(adapted from http://www.searo.who.int/entity/emerging_diseases/links/nipah_virus_outbreaks_sear/en/)

Year(s)	Country	Reported number of human cases	Reported number (%) of deaths among cases
1998-1999	Malaysia Singapore	276	106 (38%)
2001	India	66	45 (68%)
2001	Bangladesh	13	9 (69%)
2003	Bangladesh	12	8 (67%)
2004	Bangladesh	67	50 (75%)
2005	Bangladesh	12	11 (92%)
2007	Bangladesh	18	9 (50%)
2007	India	5	5 (100%)
2008	Bangladesh	11	9 (82%))
2009	Bangladesh	4	1 (25%)
2010	Bangladesh	16	14 (88%)
2011	Bangladesh	44	40 (91%)
2012	Bangladesh	12	10 (83%)
2013	Bangladesh	24	21 (88%)
2014	*Philippines*	*17**	*9 (53%)*
2014	Bangladesh	18	9 (50%)
2015	Bangladesh	9	6 (67%)

* possible NiV outbreak

Since 2001, outbreaks of Nipah have occurred almost annually in Bangladesh, affecting from 4 to more than 60 subjects (reviewed by Clayton 2017).

NiV has been shown to circulate in Pteropus bats in the countries where outbreaks of human disease have occurred. A 3-year longitudinal study of Pteropus hypomelanus bats (Rahman 2013) in Malaysia indicated an overall NiV seroprevalence of 9.8%, with nonseasonal temporal variation. Viral circulation was demonstrated within the study period.

Evidence of hendravirus or hendravirus-like infection was demonstrated by serological and/or molecular testing in bat samples collected from numerous countries (reviewed by Breed 2013), such as Bangladesh, India, Thailand, Cambodia, Vietnam, Indonesia or Madagascar where samples were found positive for NiV; China with a NiV-like virus; or Australia, Papua New Guinea and Indonesia with HeV. Bat infection was even detected in West African countries, with samples from Ghana and Equatorial Guinea found henipavirus-positive. These bat viruses may have the potential to spill-over into other mammalian hosts, including human. Actually, Pernet (2014) showed evidence of henipavirus infection in human samples from Cameroon.

Current prevention and control strategies

Current strategies for the management of henipaviruses are generally directed at minimising direct or indirect contact with the natural host (e.g. with palm sap), monitoring intermediate hosts, improving biosecurity on farms, and better disease recognition and diagnosis (Breed 2006). Prevention efforts also focus on interventions to interrupt transmission of NiV through person-to-person contact.

In Malaysia, the central strategy has been the implementation of sound farm management practices, such as monitoring herd health and early recognition of disease syndromes (Breed 2006). Several simple on-farm measures can also be taken to reduce the likelihood of spill-over events occurring. They include removal of fruit tree plantations from the immediate vicinity of pig sheds, wire screening of open-sided pig sheds, or ensuring roof run-off does not enter pig pens to avoid contact with bat urine or faeces or partially eaten fruits.

In Bangladesh, interrupting bat-to-human transmission is a main objective. People's knowledge of NiV was found low in a study conducted in randomly selected villages in Bangladesh (Nahar 2015), indicating a need for implementing strategies to increase awareness about the risks associated with this virus. Research was also conducted on methods to protect palm sap from bats. Studies compared methods to protect sap (Khan 2012). Bamboo, dhoincha, jute stick and polythene skirts covering the sap producing areas of a tree effectively prevented bat-sap contact. Pasteurizing date palm sap is another option. Moreover, the Government of Bangladesh issued a recommendation to abstain from drinking raw sap. However, such prevention methods rely on behaviour modifications. While these approaches appear to be effective strategies of prevention, their implementation is often problematic due to cultural factors (Satterfield 2015). Research was then conducted on behaviour change communication interventions. Nahar (2017) reported for instance that a 'do not drink raw sap' message is less effective than an encouragement to only drinking sap if it has been protected from bat contamination, 'safe palm only'. In addition, Parveen (2016) found that during outbreaks, one-way behaviour change communication without meaningful causal explanations is unlikely to be effective. Based on the cultural context, interactive communication strategies in lay language with supporting evidence may help make prevention messages credible in affected communities.

Similar communication is also necessary during outbreaks to interrupt person-to-person transmission. The National Guideline for Management, Prevention and Control of NiV Infection developed in Bangladesh identified a variety of prevention messages and levels of communication, to address risks associated with patient to care giver/contact, patient to health care worker, touching objects used by patients or handling deceased bodies (Rahman 2011). Specific measures are also taken in the hospital setting to avoid further spread of the disease, including isolation of patients, using personal protective equipment and applying standard precautions such as environmental decontamination and safe waste disposal. Another precaution is the safe handling of corpses of suspected Nipah patients.

Summary of epidemiology, prevention and control

Because of their wide distribution and flying range that can cover huge areas of human habitat, pteropid bats are highly effective in NiV dissemination. Moreover, the changing climate can be expected to affect the distribution of this virus reservoir in the future. Hence, the likelihood of additional henipavirus outbreaks in new areas/countries can be considered very high (Ong 2015). In view of the very high case fatality associated to the disease and the lack of safe and effective vaccine and therapies, this is a serious source of concern.

To date, outbreaks have resulted from two main transmission modes, consumption of contaminated palm sap (Bangladesh, India) or direct contact with infected pigs (Malaysia, Singapore). Since 2001, outbreaks of Nipah have been observed almost annually in Bangladesh. Current prevention of disease essentially relies on behaviour modifications to prevent spill-over from bats, even though the implementation of such measures is often challenging.

Human-to-human transmission has contributed significantly to the spread of NiV in Bangladesh and India, as well as during the first recognised (putative) outbreak in the Philippines. Human-to-human transmission is driven by close contact with patients who are clinically unwell, and factors such as patient care and basic infection control measures will be critical considerations for the mitigation of onward transmission of NiV infection in future outbreaks in people (Clayton 2017).

Diagnostics

Reliable detection of markers of NiV infection remains a key to establishing diagnosis both in animals and humans because the diagnosis may not be suspected initially, and disease manifestations can be rather nonspecific (Ong 2015).

Diagnostic Technologies

Various assays have been developed for laboratory confirmation of NiV infections. ELISA and RT-PCR are preferred methods. Virus isolation in cell culture is complicated by the requirement for high containment facilities.

Serological methods

ELISA

Several ELISA systems have been developed for serological testing after the initial investigations of NiV outbreaks in Malaysia and Singapore. Subsequently in Bangladesh, the first NiV outbreaks were only suspected after these tests were found to be positive in patients. These tests have also been used widely for investigative field studies in bats and other animals. ELISAs have been used both for detection of the viral antigen and evaluation of antibody responses (see Table 3 below). Chiang (2010), for instance, reported the development of monoclonal antibody-based antigen capture ELISAs for virus detection and for differentiation between NiV and HeV. Such viral antigen capture ELISAs provide a high throughput format at relatively low cost for screening of samples, and thereby could serve as an alternative to PCR for rapid diagnostic and virus detection (Kulkarni 2013). ELISAs developed for serology testing (IgM or IgG) may use infected cell lysate antigens for coating the plates. However, their use is limited to BSL4 laboratories. To overcome this problem, NiV recombinant proteins, and the N protein in particular, have been produced as an alternative antigen for serological detection of infection.

The specificity and sensitivity of available ELISAs has not been often reported. One such validation study concluded in 92% assay sensitivity and specificity of a NiV-N protein-based IgM capture ELISA, but used as a reference method the ELISA developed at CDC (Yu 2006), which performance documentation had not been disclosed.

Luminex

Two different Luminex assay formats were developed for detection of NiV-specific antibodies, a binding assay and a blocking or inhibition assay (reviewed by Wang 2012). The inhibition assay measures antibodies that block ephrin-B2 receptor binding. Such assay can be considered as a surrogate neutralization test. Similar Luminex assays have been used successfully for HeV diagnosis in human as well as veterinary settings.

Rapid test

From an outbreak investigation/control point of view, it is most useful in some situations to have access to a rapid (point-of-care) test for both human and animal applications. Rapid tests, usually consisting of lateral flow immunochromatographic assays, have been developed for detection of antigen and/or antibody corresponding to a variety of infectious diseases. However, as of today, no such test has been made available for Nipah serology.

Virus neutralization

Serum neutralization tests are considered as the reference for serology testing, and NiV neutralization tests were developed soon after the first outbreak in Malaysia. In a conventional NiV neutralization test, sera are incubated with virus in the wells of 96-cell microtiter plates prior to the addition of Vero cells. Those sera that completely block development of a cytopathic effect are designated as positive. A more rapid plaque assay neutralization procedure has also been developed to detect NiV-neutralizing antibodies, in which a specific number of plaque-forming units of the virus are incubated with dilutions of test serum prior to adsorption to the cell monolayers, and foci of infection detected immunologically after fixation of the cells with methanol (reviewed by Wang 2012, Daniels 2001).

Neutralization testing may not be performed in many laboratories because these tests involve handling live viruses, which requires high containment facilities. Several pseudotyped viruses bearing the NiV F and G proteins have been developed to obviate this requirement, and shown to be more sensitive than conventional serum neutralization assays (Wang 2012). However, further validation of the pseudotype assays, at large scale, would be required to corroborate their high sensitivity and specificity before considering them as a reliable alternative to other serological assays.

Molecular methods

Specific and reliable PCR-based methods and sequencing to identify henipaviruses are now more widely available for outbreak investigations and diagnosis, and have begun to replace serological testing (Ong 2015). A real time RT-PCR TaqMan assay for NiV was first developed in 2004, based on the N gene sequence. The sensitivity of the test was close to 1 plaque forming unit, and it was demonstrated that the assay was able to detect NiV RNA in blood specimens from infected hamsters. The assay was also shown NiV-specific since it failed to detect HeV RNA. A commercial kit based on this technology has obtained CE marking and been made available, for the detection of NiV in serum, plasma, infected animal tissue or secretion by using real time PCR systems.

Subsequently, in another study, several consensus quantitative PCRs were developed for detection of both HeV and NiV. In this study, the N gene SYBR Green assay was the most useful assay for investigation of potentially unknown henipaviruses and the P TaqMan assay was preferred when high sensitivity was required to detect infection by know strains of NiV (reviewed by Wang 2012).

Two versions of a Taqman array card have also been recently developed for simultaneous detection of more than 20 pathogens (including NiV) in cerebrospinal fluid or blood samples (Onyango 2017, Liu 2016). The assays showed good performance when compared to standard PCR, with better specificity (96-99% overall) than sensitivity (< 90% overall). The TaqMan array card could be used in field settings with different objectives: as a rapid screen in case of suspected neurological disease, for outbreak investigations or for the surveillance of specific pathogens including NiV.

Tissue culture method

Virus culture can be used for diagnosing NiV infections. Particularly in any new case or outbreak, isolation of the virus is highly desirable. Brain, lung, kidney and spleen samples should be tested. A cytopathic effect usually develops within 3 days of culture in Vero cells. Initially after low multiplicity infection of cell monolayers, the cytopathic effect is manifested by the formation of syncytia that may contain up to 20 or more nuclei. Subsequently syncytia lift from the substrate, leaving punctate holes in the cell monolayer. Identification methodologies for virus isolates include immunostaining of fixed infected cells, neutralization with specific antisera, PCR of culture supernatants, and electron microscopy (Daniels 2001).

Immunohistochemistry

Where infected animal or human tissues are available, immunohistochemistry using specific anti-henipavirus antibodies is sometimes useful to confirm the diagnosis (Ong 2015). In NiV infections there is a wide range of tissues in which viral antigen can be detected (Daniels 2001), since the primary pathology occurs in the vascular endothelium. However, this method has been more frequently reported for detection of HeV than NiV infections.

Summary and challenges

Table 3 provides an overview of the advantages and disadvantages of the different technologies used for diagnosis of NiV, and table 4 provides a summary of the techniques available in various laboratories for diagnosis of NiV infections. Despite the very narrow geographical distribution of the disease, a variety of diagnostic tools have been made available by various laboratories.

Table 3. Summary of advantages / disavantages of the main NiV assays (for diagnosing human infections)

Technique	Advantages	Disadvantages
ELISA (IgM)	May provide Nipah diagnosis if performed between 4 days and 3 months after exposure	
ELISA (IgG)	May provide Nipah diagnosis	Requires a second serum sample (convalescent serum)
Nucleic acid-based assays	Becoming the clinical diagnostic standard Performed rapidly	
Viral culture	Virus isolation important when a new case or outbreak occurs	Requires too much time to be of use in clinical setting Requires BSL4 precautions

Table 4. Overview of diagnostic tests developed in various laboratories
(adapted from Kulkarni 2013)

Technique	Assay	Laboratory
ELISA (detection of viral antigen)	Antigen-capture ELISA using antibodies produced by DNA immunization	CDC, USA
	Antigen-capture ELISA using antibodies produced by DNA immunization	Nat. Inst. Inf. Dis., Japan
	Monoclonal antibody-based antigen-capture ELISA	CDC, USA
ELISA (detection of IgM and IgG)	Solid-phase blocking ELISA	DVS, Malaysia
	Indirect ELISA based on E coli-expressed N	Chinese Nat. Diagn. Center for Exotic Animal Dis.
	Indirect IgG ELISA based on recombinant N	Institute Trop. Med., Japan
	IgM capture ELISA based on recomb. N	Institute Trop. Med., Japan
Luminex	Binding assay	CSIRO, Australia
	Receptor inhibition (blocking) assay	CSIRO, Australia
Neutralization	Plaque assay	CSIRO, Australia
Pseudotype neutralization	VSV pseudotypes expressing NiV F /G	CDC, USA
	VSV pseudotypes expressing NiV F /G	Nat. Inst. Inf. Dis., Japan
	VSV expressing secreted alkaline phosphatase pseudotyped with NiV F/G	Nat. Inst. Inf. Dis., Japan
Immunohistochemistry	Monoclonal antibody-based immunohistochemistry	Nat. Inst. of Animal Health, Japan
PCR	Real-time RT-PCR (Taqman) using primers in N gene	Institut Pasteur, France
	Real-time RT-PCR (Taqman) using primers in P gene	Inst. of Zoology, UK
	SYBR Green assay using primers in N gene	Inst. of Zoology, UK
	Duplex nested RT-PCR (bat urine specimen)	Chulalongkorn Uni. Hosp., Thailand
	Taqman array card (multiplex)	CDC, USA
Virus culture	Plaque assay	CSIRO, Australia

Many countries in the South-East Asia region did not have adequate facilities for diagnosing the virus when the first outbreaks occurred. However, Bangladesh, India and Thailand developed laboratory capacity and addressed this issue. For instance, a Nipah laboratory was set up in Bangladesh (IEDCR) for safe specimen handling and testing by ELISA to identify IgM and IgG antibodies against NiV (Rahman 2012). In India, a BSL4 laboratory at National Institute of Virology (ICMR), Pune, has prepared to diagnose NiV in the country. The OIE Reference Laboratory for Henipaviruses in Asia–Pacific region is located at Australian Animal Health Laboratory, Geelong, and the High Security Animal Disease Laboratory, in Bhopal, with BSL3 + facility caters the need for exotic animal disease diagnosis (Kulkarni 2013).

Of note, NiV diagnostic tests have to be deployed under a range of circumstances: they are involved in studies of the natural history of infections, diagnosis of the disease in pigs; in special circumstances they may facilitate international trade in known susceptible species and they are a key to the diagnosis of human infection and disease. An additional constraint is that diagnostic technologies should be appropriate to resource-limited environments (Wang 2012).

NiV diagnostic validation studies are still lacking to fully characterize the performance of available assays. In particular, assay cross-reactivity appears as a largely unaddressed topic. The antibody cross-reactivity between HeV and NiV can be expected to decrease NiV assay specificity. However, in some instances, assays providing wide coverage, across all henipaviruses, may be desirable. Surveillance studies indicated the presence of a diverse group of henipavirus or henipa-like viruses in bats at different geographic locations around the world, from Australia, Southeast Asia to China, Madagascar, and West Africa. It is highly possible that any of these bat viruses have the potential to spill-over into other mammalian hosts, including human, and cause diseases. These viruses may induce cross-reactive, but not cross-neutralizing, antibody responses against known henipaviruses (Wang 2012).

Gap analysis: Diagnostics

What are the sensitivity and specificity of current ELISA IgM assays?

Due to the high case fatality rate associated with NIV infections, and the risk of human-to-human transmission, accurate tests, with very high levels of both sensitivity and specificity are needed. The consequences of false negative or false positive assay results are indeed difficult to manage. Available data remain insufficient to determine whether available tests have adequate performance for optimal diagnosis of human NiV infections. Detection of IgM by ELISA has been most widely applied in the context of the latest outbreaks in Bangladesh. However, very limited information has been disclosed about the performance of these assays. Laboratories seem to be more concerned by a possible lack of assay specificity than sensitivity, as IgM ELISA positive results are usually reported to be confirmed by another method (Sazzad 2015, Homaira 2010, Gurley 2007).

Laboratories offering henipavirus testing pay detailed attention to quality assurance issues, but NiV disease may occur in remote locations without ready access to sophisticated laboratory facilities, and therefore reproducible diagnostic test accuracy can sometimes be challenging to deliver at the point of care (Wang 2012). Continued efforts are therefore needed to ensure better access to suitable diagnostics in all areas where NiV outbreaks are likely to occur.

Future directions for research in NiV diagnostics should include assay validation vs. international reference standards (RNA, antibody), point-of-care diagnostic development, and expansion of multiplex assays to distinguish Nipah from other diseases with similar clinical presentations.

Therapeutic interventions

Antiviral drugs

Ribavirin and chloroquine

Ribavirin is a guanosine analogue and broad spectrum nucleoside antimetabolite antiviral drug which features on the WHO Essential medicines list. An inhalation solution of ribavirin is also indicated for the treatment, in young children, of severe lower respiratory tract infections due to respiratory syncytial virus, another paramyxovirus. During the NiV outbreak in Malaysia in 1998/99, ribavirin was given empirically to treat 140 patients. The trial was not randomized. Patients who were managed prior to the availability of ribavirin or refused ribavirin were taken as controls. There were 45 deaths in the treated group (32%) and 29 in the controls (54%), corresponding to 36% reduction in mortality (Chong 2001). On Cox regression analysis, younger age and use of ribavirin were independently associated with better survival (p = 0.011 and 0.013, respectively). To our knowledge, this is the only available report on the efficacy of ribavirin in patients. In subsequent animal studies, ribavirin was found to only delay NiV disease and death (reviewed by Ong 2015, Broder 2013, Steffen 2012).

Ribavirin was also tested in combination with the antimalarial drug chloroquine. Chloroquine was indeed shown early on to block the critical proteolytic processing needed for the maturation and function of the HeV F glycoprotein (Pager 2004), and later shown to inhibit NiV infection in cell culture (Porotto 2009). However, in vivo data proved disappointing. Chloroquine did not protect hamsters when administered either individually or in combination with ribavirin (Freiberg 2010).

Other antiviral drugs

In view of the questionable efficacy of ribavirin and/or chloroquine and the severity of NiV infections in people, considerable effort has been spent in developing and exploring new therapeutic options against henipaviruses. Such research has primarily focused on targeting the fusion and entry steps of the virus infection process, as done by F glycoprotein-targeted peptide fusion inhibitors. A 36 amino acid **HR2-based fusion inhibitor** (NiV-Fc2), analogous to the approved HIV-specific therapeutic peptide enfuvirtide, has been proposed as a specific therapy against henipaviruses (Steffen 2012).

Subsequent studies focused on peptide optimization, leading to the identification of the cholesterol tagged hPIV3-based HR2-derived peptide as a promising candidate (Porotto 2010). Such cholesterol-tagged peptides could penetrate the CNS and exhibited some effective therapeutic activity against NiV infection in the hamster model. However, relatively large amounts of HR2-cholesterol peptides (2 mg/kg) were needed to achieve 60% or less survival of hamsters infected with NiV, when administered simultaneously or prior to NiV inoculation.

Nearly two decades of research on the molecular mechanisms of membrane fusion have not resulted in the identification of a lead candidate with successful proof of concept data in an animal model of NiV infection

Additional studies were conducted more recently in view of better understanding the properties underlying the peptides' potency (Mathieu 2017). The observation of direct correlation between the length of the linker, the antiviral effect of the molecule and its protease sensitivity (leading to decreased stability in vivo) illustrates the major challenges remaining to be addressed by this type of research.

Small-molecule nucleoside inhibitors have also been evaluated against NiV. Very recently, Hotard (2017) evaluated 4'-Azidocytidine (R1479), an antiviral initially developed for hepatitis C therapy, against a range of paramyxoviruses and reported similar levels of in vitro activity against henipaviruses as against flaviruses.

Other candidate drugs evaluated in vitro for their activity against henipavirus infections include cationic compounds and calcium influx inhibitors (Aguilar 2011).

Biologicals

Convalescent plasma

The recent pathogen outbreaks, such as Ebola viral disease or Middle East respiratory syndrome coronavirus, have renewed attention to convalescent plasma and immunoglobulins. In case of severe disease, when no treatment with a proven record of safety and efficacy is available, they may appear as the only available therapeutic option. However, convalescent plasma has not been investigated clinically during outbreaks of NiV infections, and research efforts are rather focused on monoclonal antibodies.

Monoclonal antibodies

Poly-IC12U has not reached 100% protection in the hamster model of NiV infection. Can it be considered a promising drug candidate?

Data from the phase 1 trial of m102.4 will help estimate its therapeutic window, and the future commercial cost of a treatment

Monoclonal antibodies targeting the surface glycoproteins of HeV have shown efficacy against both HeV and NiV as pre- and post-exposure prophylaxis in animal models, but since these antibodies must be administered before the onset of clinical signs, they are unlikely to be useful for treating symptomatic patients, while probably beneficial for post-exposure prophylaxis in potentially exposed individuals (Satterfield 2015). Presently, the only reported and most promising post-exposure therapy against NIV infection is a human monoclonal antibody known as m102.4, which was isolated from a recombinant human phage-displayed Fab library (reviewed by Broder 2013).
The m102.4 mAb has exceptionally potent neutralizing and cross-neutralizing activity against both NiV and HeV viruses and its epitope maps to the ephrin receptor binding site. Testing of m102.4 has confirmed its neutralization activity against several isolates including NiV-M and NiV-B. Effective post-exposure efficacy with m102.4 has been demonstrated in both ferrets and NHPs (AGMs) infected with either HeV or NiV. Of note, a study conducted in AGMs confirmed the higher pathogenicity and lethality of NiV-B compared to NIV-M, and showed that the therapeutic window for m102.4 was much shorter with NiV-B than NiV-M, with all animals succumbing to NiV-B infection if treated later than on days 3 and 5 post inoculation (Mire 2016).

Over the past years, eleven human subjects have been reported to receive high-dose m102.4 therapy on an emergency use basis because of high-risk exposure to HeV in Australia (10 people) or NiV in the United States (one person), and all have remained well with no associated adverse events (Broder 2013). A phase 1 clinical trial has been initiated for this product in April 2015 in Australia in a total of 40 subjects (5 groups of 8 subjects receiving 1 or 2 intravenous infusion(s) of different doses of m102.4) (https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=368110&isReview=true). Assessments include safety/tolerability, pharmacokinetics and immunogenicity parameters. To our knowledge, the outcome of this trial has not been disclosed yet, but the results will be essential for determining the feasibility of using such monoclonal antibody for pre- or post-exposure prophylaxis.

Host directed interventions

Immunomodulators

The innate immune response to NiV infection is thought to alter the pathogenic process that is induced, offering the option of a therapeutic approach based on immunomodulation. A derivative of synthetic polyinosinic:polycytidylic acid (poly-IC12U or Rintatolimod), an analogue of double-stranded RNA which strongly activates IFN production, has been shown effective in limiting disease and increasing survival of NiV-infected hamsters. When administered at 3 mg/kg of body weight daily from the day of infection to 10 days post-infection, prevented mortality in 5 of 6 infected animals (Georges-Courbot 2006). This drug candidate is not new. It was evaluated clinically as early as in the 1980s, and at that time associated to significant toxicity (with adverse events such as fever, arthritis, liver toxicity, thrombocytopenia, or neurotoxicity). Nevertheless, poly-IC12U underwent further clinical development for chronic fatigue syndrome, with more than 90 000 doses administered, and these trials concluded in an acceptable safety profile (Mitchell 2016). Poly-IC12U may thus hold promise for Nipah therapy.

Another strategy, targeting the activation of IRF3-dependent signalling mechanisms with small molecule agonists, has been proposed more recently by Pattabhi (2016), but so far it has not advanced beyond a very early stage of development.

Adjunctive therapies

As for other severe diseases of viral origin, aggressive supportive care may help improve patient survival. NiV infections, especially as seen in Bangladesh, are associated to respiratory disease and respiratory failure. Oxygen supplementation and eventually transfer to ICU are part of the management guidelines of this infection. Ensuring patient access to the best medical practices in this area should remain a priority.

Gap analysis: Therapeutics

Without any other currently available therapeutic options, ribavirin is still considered today as an option for treatment of NiV infections in emergency settings, but its impact on disease progression is highly questionable. Research on alternative treatments appears therefore as critical. Numerous studies have uncovered determinants important for various steps in the NiV replication cycle. While each step of the replication process represents a potential target for the development of antiviral drugs, most efforts have been focused on the mechanisms leading to productive membrane fusion. Until now, such research has not resulted in the identification of many promising candidates, and none of them has been reported successful in an animal model of NiV infection.

The most advanced alternative treatment under development is a monoclonal antibody, m102.4, which was identified by panning a large nonimmune antibody library against the HeV glycoprotein G. This monoclonal antibody was then affinity maturated, converted to IgG1 and proved highly neutralizing against NiV (Zhu 2008). It has now reached clinical phase 1 evaluation. Following high-risk NiV exposure or cases of infection in India or Bangladesh in recent years, there has been an interest in compassionate use of the m102.4 antibody, which was found difficult to orchestrate as the product was still at a preclinical stage of development. A possible strategy for access to this therapy would be to further develop, manufacture and stockpile the antibody so as to deploy it rapidly when and where another outbreak occurs (Broder 2013). However, more data from clinical studies will be required to determine what could be the indication and usage of this monoclonal antibody.

Another therapeutic approach may be based on immunomodulation, as suggested by the efficacy of poly-IC12U in the hamster model of NiV infection. However, for a better understanding of the probability of success of this approach, poly-IC12U should be evaluated in the same AGM model as used for the m102.4 antibody.

Overall, the development of therapies against NiV infections remains at a very early stage. Research efforts in this area should be encouraged, especially when considering the high case fatality rate associated to NiV infections and the risk of a large outbreak occurring in the future. One may also consider that antiviral drugs usually have the potential for broad virus coverage, so that progress made on NiV research can be expected to bring additional benefits for the management of other henipa- or paramyxovirus infections.

Vaccines

All R&D activities for NiV vaccines are in the pre-clinical stage. Many vaccine candidates including different live recombinant vaccine and subunit vaccines have been produced and tested in various animal models including hamsters, pigs, cats, ferrets and AGMs. These vaccines are based on the F and/or G glycoproteins, and essentially target the induction of NiV neutralizing antibodies, as done with other human paramyxovirus vaccines such as mumps or measles vaccines.

Despite the high level of identity between NiV-M and NiV-B, shouldn't a vaccine be based on Niv-B sequences rather than NiV-M?

Live-attenuated vaccine candidates (reassortant/recombinant)

Due to the extreme lethal nature of NiV, producing a safe, live attenuated vaccine with no potential of reversion is generally considered a difficult approach, although recombinant-derived NiV mutants have been produced that are attenuated in hamster and ferret models. A live-vectored vaccine approach has been preferred.

Vaccinia virus-derived vectors

The first vaccination and challenge experiments were carried out with NiV in the hamster model using recombinant vaccinia viruses (Guillaume 2004). NiV F and G encoding recombinant vaccinia viruses (highly attenuated NYVAC strain) were examined individually and in combination. All animals were completely protected following intraperitoneal NiV challenge, regardless of whether they were immunized with the G or F or both vaccinia virus recombinants. Of note a humoral anamnestic response was observed following virus challenge suggesting that vaccine-induced immune responses did not prevent virus replication, i.e. sterile immunity was not induced. As passive transfer of antisera from vaccinated animals also demonstrated protection, a major role of specific neutralizing antibody in protection was inferred. Subsequently, a recombinant canarypox (Alvac)-based candidate, another highly attenuated vaccinia virus vector suited to veterinary applications, was also tested successfully in a pig model of NiV infection (Weingartl 2006).

Vesicular Stomatitis Virus Vectors

The most studied vectored vaccine candidate is the VSV vector, which uses a similar strategy to that being employed in the Ebola virus vaccine candidate in current clinical trials. However, the VSV vector used has not been identical among studies (reviewed by Satterfield 2016). Three types of vaccines can be distinguished : replication-incompetent VSV pseudotypes expressing NiV G or F proteins (Lo 2013), VSV virions with F and G that can undergo a single round of replication (produced by co-infection of two VSV pseudotypes, one expressing F and one expressing G, which generates a chimeric virus pair that expresses both VSV F and G on the virus surface) (Mire 2013, Chattopadhyay 2011), and replication competent VSV recombinants, where the VSV-G is replaced in the vaccine vector by the Ebola GP so as to overcome the lack of a functional surface protein for virus entry (DeBuysscher 2014).

A study tested the protective efficacy of the replication competent VSVs expressing either N, F or G of the Malaysian strain of NiV. Following a single dose administration, the vaccine vectors expressing G and F fully protected Syrian hamsters from lethal NiV challenge, whereas the N expressing vector conferred only partial protection (DeBuysscher 2014). Another study in AGMs confirmed the protective capacity of the same recombinant virus expressing the G protein of NiV (rVSV-EBOV-GP-NiV-G) (Prescott 2015). Interestingly, in the hamster model, this candidate, when used for single-dose vaccination 1 day before challenge, reduced viral load, limited pathology and fully protected hamsters from NiV infection. The vaccine was even partially protective when administered at early time points following challenge with NiV, suggesting the potential of the vaccine in an outbreak setting or for post-exposure prophylaxis. Moreover, induction of cross-protection between NiV-B and NiV-M has been demonstrated for another VSV-vectored vaccine (single round replication) in AGMs (Mire 2013).

Hence, VSV recombinant vaccines may appear as the most promising vaccine candidates for Nipah. However, a recent toxicity study conducted in mice (Van Den Pol 2017) pointed to a significant safety concern of neurotropism for such recombinant vaccine. A combination of VSV recombinant viruses expressing both Nipah F and G (as described by Mire 2013 and Chattopadhyay 2011) was indeed found even more neurotropic than wild-type VSV after intracranial injection, evoking a rapid lethal response in the adult brain. The observation is in contrast with an earlier study that showed that both the VSV-Ebola and VSV-Lassa viruses were devoid of neurotropic actions in mice and rats, and suggests that a vaccine expressing a single NiV glycoprotein should be preferred over a combination of F and G.

Measles Virus vector

Live-attenuated measles vaccines have been used since the 1960’s worldwide, and have appeared as attractive candidates for development of vectored vaccines. In particular, measles virus induces strong cellular immunity and durable responses. A concern related to the use of a measles vector is due to the possible negative effect of pre-existing immunity in human populations on the immunogenicity of such vector vaccine. Nevertheless, the vaccine has been considered to offer the potential to elicit long-term immunity against both MV and NiV, and has been proposed as a way to reach general childhood vaccination against NiV. The pathway of clinical development for such combination vaccine remains largely unclear, and most likely would require too significant investments to appear as a feasible strategy. In any case, protection data in AGMs for this candidate have appeared suboptimal, as after 2 injections of the vaccine, animals were not protected against the brain lesions induced by NiV challenge (Yoneda 2013).

The neurotropic nature of a VSV vaccine expressing both F and G is a critical safety concern

Venezuelan Equine Encephalitis virus vector

A replicon vaccine vector system was developed from an attenuated strain of Venezuelan equine encephalitis virus (VEE). Alphavirus replicon vectors offer several potential advantages as vaccine delivery systems. They typically express heterologous genes to high levels and induce self-limiting infection. Replicons expressing HIV or cancer target antigens have been evaluated in human subjects, and some promising data generated. A VEE-vectored NiV vaccine candidate has been evaluated in mice and found to induce NiV neutralizing antibodies following a 3-dose vaccination schedule (Defang 2010). To date, the protective potential of this vaccine candidate remains unknown.

Adeno-Associated Virus

Adeno-associated viruses are probably more popular as gene-therapy vectors than vaccine platforms. Nevertheless, a number of AAV-based vaccine candidates have been shown to induce promising data in animal models. Ploquin (2013) demonstrated that a single injection of the AAV8-NiV.G vector is sufficient to protect the hamsters against lethal challenge with NiV.

Subunit vaccines

A subunit vaccine incorporating recombinant glycoprotein G has been approved as a veterinary vaccine for HeV in Australia in 2015. This vaccine is formulated using glycoprotein G with a proprietary immunostimulatory complex adjuvant. Currently, the vaccine is administered to horses by the intramuscular route as two immunizations, three to six weeks apart followed by boosting six months later. The effectiveness of this vaccine in horses suggests this approach may also be of interest in humans (Satterfield 2015). Various formulations of an experimental subunit G vaccine against Nipah have been evaluated successfully in animal models, including AGMs, confirming the potential of this type of vaccine. Some of these studies, with challenge performed up to more than a year after vaccination, demonstrated the durability of protection induced by these vaccine.

HeV is a zoonotic infection affecting horses and humans in Australia. A vaccine has been made available for horses in 2012 (Broder 2013). Horse owners in Australia are encouraged to vaccinate their horses to both reduce the risk of HeV infection, and to prevent potential transmission to humans. Vaccine uptake has been limited though, with an estimated 11-17% of horses in Australia vaccinated (Manyweathers 2017). Spill-over events of HeV in horses continue to occur.

NiV virus-like particles (VLPs) have also been obtained by co-expression of the G, F and M proteins under optimized conditions. They appeared as an alternative vaccine candidate, especially in view of their ability to induce a neutralizing antibody response in mice (Walpita 2011).

A vaccine candidate requiring multiple injections does not seem very compatible with the targeted indication for at-risk persons in the area of an ongoing outbreak

Vaccine summary

Table 5 below presents the various vaccine candidates evaluated in preclinical models.

Table 5. Nipah vaccine candidates with preclinical data (adapted from Satterfield 2015)

Candidate name/ identifier & institution	Efficacy or immunogenicity data in animal model	Developer	Reference
VV-NiV.F and/or G	100% protection in hamster model, following 2 vaccinations (1 month apart) starting 4 months before challenge	INSERM	Guillaume 2004
ALVAC-F/G*	100% protection in pig model, no evidence of clinical illness, following 2 vaccinations (14 days apart) starting 28 days before challenge	CFIA-NCFAD	Weingartl 2006
VSV-NiVM F and G	High neutralizing antibody titers induced in mice following single intranasal vaccination	Yale University	Chattopadhyay 2011
VSV-NiVB F and/or G	100% protection in ferret model, no evidence of clinical illness, following single vaccination 28 days before challenge	UTMB	Mire 2013
VSV-NiVM F or G	100% protection in hamster model, no evidence of clinical illness, following single vaccination 32 days before challenge	CDC	Lo 2014
rVSV-EBOV-GP-NiV-G	100% protection in hamster model following single-dose vaccination 1 day before challenge	RML	DeBuysscher 2014
	100% protection in AGM model following single-dose vaccination, 29 day before challenge		Prescott 2015
rMV-Ed-G	100% protection in hamster model, no evidence of clinical illness, following 2 vaccinations (21 days apart) starting 28 days before challenge	UoT	Yoneda 2013
	100% protection in AGM model, but no protection against infection (pathological changes observed in brain), following 2 vaccinations (28 days apart) starting 35 days before challenge		
V-NiVG (VEE)	High neutralizing antibody titers induced in mice following 3 vaccinations at weeks 0, 5 and 18	USU	Defang 2010
AAV-NiVM G	100% protection in hamster model, no evidence of clinical illness, following single vaccination 32 days before challenge	INSERM	Ploquin 2013
rLa-NiVG and/or rLa-NiVF**	High neutralizing antibody titers induced in pigs following 2 vaccinations (4 weeks apart)	CAAS-SKLVB	Kong 2012
HeV sG (subunit) + CpG	100% protection in ferret model, no evidence of clinical illness, following 2 vaccinations (20 days apart) 20 or 434 days before challenge	Zoetis, Inc. /USU	Pallister 2013
HeV sG (subunit) + Alum + CpG	100% protection in AGM model, no evidence of clinical illness, following 2 vaccinations (21 days apart) starting 42 days before challenge		Bossart 2012
	100% protection in cat model, no evidence of clinical illness, following 2 vaccinations (21 days apart) starting 42 days before challenge		McEachern 2008
HeV sG (subunit) + Quil A + DEAE-dextran + Montanide	100% protection in cat model, no evidence of clinical illness, following 3 vaccinations (14 days apart) starting 104 days before challenge		Mungall 2006
VLPs (F/G/M)	High neutralizing antibody titers induced in mice following 3 vaccinations at weeks 0, 2 and 4	UTMB	Walpita 2011

** Canarypox vector, suited to veterinary applications*

*** Newcastle Disease Virus vector, suited to veterinary applications*

Abbreviations: USU (Uniformed Services University of the Health Sciences); UTMB (University of Texas Medical Branch); CDC (Centers for Disease Control and Prevention); RML (Rocky Mountain Laboratories); TJU (Thomas Jefferson University); CFIA-NCFAD (Canadian Food Inspection Agency – Centre for Foreign Animal Diseases); Institut national de la santé et de la recherché médicale (INSERM); UoT (University of Tokyo); CAAS-SKLVB (Chinese Academy of Agricultural Sciences (CAAS) – State Key Laboratory of Veterinary Biotechnology (SKLVB).

Another summary of the present status of knowledge associated to the different vaccine candidates under evaluation for Nipah considers the advantages and disadvantages of these vaccines platforms, as shown in Table 6 below.

Table 6. Advantages and disadvantages of the different vaccine platforms under evaluation for Nipah (human vaccination)

Platform	Advantages	Disadvantages
Vaccinia-based vectors	Promising data in animal models of protection MVA strain well known in humans	2-dose schedule Concerns linked to anti-vector immunity
VSV-vectored vaccines	Promising data in animal models of protection Efficacy in animals vaccinated 1 day prior to challenge Platform used successfully in the context of an Ebola outbreak Single dose schedule	Neurotropism issue identified with vector expressing a combination of F and G
Measles-vectored vaccine	Promising data in animal models of protection Vector consisting of the measles vaccine strain	2-dose schedule Suboptimal protection observed in AGM model Pre-existing immunity against the vector in human populations
VEE replicon	Self-limiting infection Phase 1 clinical data available for VEE combined with HIV or prostate antigen	No protection data in NiV challenge model available 3-dose schedule
Subunit vaccine	Promising data in animal models of protection Expected safety	Need for an adjuvant, impacting perception of safety and potentially complicating clinical development Need for several injections less compatible with use in emergency setting

Gap analysis: Vaccines

As of today, no NiV vaccine candidate has advanced to a clinical phase of development. Consequently, it remains difficult today to assess the likelihood that any of these candidates will demonstrate characteristics compatible with the Target Product Profile developed by WHO in June 2017 for a Nipah vaccine (http://www.who.int/blueprint/priority-diseases/key-action/nipah/en/). Nevertheless, the probability of success of a vaccine against Nipah is expected to be very high. Other related paramyxoviruses, such as measles and mumps viruses, yield long-term immunity after natural infection, and live attenuated vaccines have been developed that have been used safely and effectively against these pathogens for decades. It has also been suggested that the likelihood of success of viral vaccines targeting viruses with average incubation periods of at least 5–7 days, as seen with NiV, is higher than that of vaccines for viruses with shorter incubation periods. In addition, various candidate vaccine platforms have demonstrated the feasibility of using one or both of the NiV glycoprotein (G) and fusion (F) protein as the antigen(s) to stimulate a protective immune response in various preclinical challenge models including hamsters, cats, ferrets, AGMs and pigs. Little or no clinical signs of disease were observed in vaccinated animals after NiV challenge, and protection against mortality often reached 100% (Satterfield 2016).

One of the most promising vaccine candidates is a VSV-G recombinant. In the hamster model, this vaccine was found protective following single-dose vaccination 1 day before challenge, suggesting the potential of this product for use in an emergency setting. However, despite encouraging efficacy data in animals, the proof of concept of such vaccine is still far from being established, as evidenced by the recent observation of a neurotropic effect with a VSV vector system expressing both NiV F and G. Another candidate of interest is a subunit vaccine containing adjuvanted recombinant G glycoprotein. The success of such vaccine candidate in experimental animal models of NiV infection and the use of Equivac HeV® as an effective subunit vaccine in horses suggest this approach may also be efficacious in humans (Satterfield 2016). However, the requirement for a multiple-dose schedule of immunization with this type of vaccine does not appear very compatible with the targeted indication for immunization of at-risk persons in the area of an ongoing NiV outbreak.

In any case, the epidemiology and sporadic nature of NiV outbreaks will make large scale clinical efficacy trials very difficult to organise, so that the pathway towards licensure for a Nipah vaccine will most likely require creative approaches. Specific opportunities, such as the procedure for Emergency Use Assessment and Listing (WHO EUAL), to facilitate access to critically needed vaccines in case of emergency are likely to be of help. In the USA, the Food And Drug Administration's Animal Rule pathway is intended to enable approval of drugs against highly lethal infections in situations where definitive human efficacy studies cannot be conducted (Satterfield 2016). In this instance, efficacy is to be demonstrated via protection in one or two experimental animal models that replicate key characteristics of the human disease and are predictive of the mechanism of protection in humans. The AGM, ferret, and hamster models are well established and accurately model human disease. However, no vaccines have been approved through this mechanism to date. And even with the Animal Rule, human clinical trials are still required for demonstrating vaccine safety.

The key issues with vaccines are delivery and costs (Hayman 2014). The question of how to finance such product development has to be raised, as a NiV vaccine would likely find use primarily in the low and middle income countries where the virus is currently endemic. In view of the current low disease incidence, a reasonable objective may be to advance the development of a vaccine candidate to early phases of clinical development, which would aim at better preparing for a potential epidemic of larger size. In parallel, it would be important to clarify expectations for a NiV vaccine. Answering questions such as "what role could a NiV vaccine play in controlling the disease? with what cost/effectiveness?" would help define the best way forward in terms of vaccine research and development.

Vector Control

The NiV outbreaks in Malaysia in 1998-1999 have been associated with infection of pigs with subsequent spread to human populations, while the outbreaks in Bangladesh resulted from direct contamination from infected fruit bats and human-to-human transmission. Theoretically, vector control efforts may target these 2 species. In any case, a transdisciplinary approach will be required to develop appropriate host management strategies that both maximise the conservation of bat populations as well as minimise the risk of disease outbreaks in domestic animals and humans (Breed 2006).

Pigs

NiV was first described following the 1998-1999 outbreaks of disease in pigs and humans in peninsular Malaysia. Over one million pigs were culled to contain the outbreak (Breed 2006), which had an enormous economic and social impact. To date, no other disease outbreak has been observed in this animal species, but following an outbreak of NiV observed in horses in 2014, the potential for new spill-over into swine can be considered as threatening. Vaccinating pigs has been proposed as a means to prevent spread to human populations, but it is also a veterinary option, aimed at protecting swine herds in the event of a new outbreak. As of today, a swine vaccine against NiV is not available, but promising results have been obtained with the canarypox vectored vaccine candidate and R&D efforts targeting the development of a veterinary vaccine against Nipah are still ongoing.

Bats

(adapted from Breed 2006)

Management of a bat-borne zoonotic disease has been previously attempted for rabies virus. Historically, population reduction was used as a control strategy for vampire bats in Latin America. The methods used at that time are now generally considered inhumane, unethical and ineffective and some of the targeted species have become endangered in their natural environment. Trap, vaccination and release programs using parenteral vaccination have been employed for rabies control of a few terrestrial wildlife species, but this approach is considered prohibitively expensive for large populations and logistically impossible for non-terrestrial species. An oral vaccine has been shown capable of inducing a protective immune response to rabies in vampire bats following oral vaccine delivery, and hence an oral vaccination approach may be plausible for other bat species. However, such program would be very ambitious. A specific vaccine would have to be developed, including a biomarker for discriminating between vaccinated and naturally infected individuals (Mackenzie 2003). Moreover, various aspects of bat ecology and behaviour would require further study before an oral vaccine, suitable bait and vaccination strategy can be deployed. The feasibility of using a NiV vaccine in wild bats for controlling outbreaks in human populations is still far from being established.

General conclusion and recommendations

This document gathers available data pertaining to NiV epidemiology, diagnostics, as well as vaccines, therapeutics and other methods for prevention and control. It identifies research and knowledge gaps which are meant to be used as a basis for discussion on the development of a NiV R&D roadmap.

Nipah is one of eleven diseases considered as in urgent need of R&D attention in the revised priority list of pathogens issued by WHO in January 2017 (http://www.who.int/blueprint/priority-diseases/en/). Since 2001, it has caused almost annual outbreaks in Bangladesh, and the infection has been associated to a case fatality rate of up to 100%. Nipah is a zoonotic disease. As its reservoir, the Pteropus bat, has a flying range that can cover huge areas, the risk of further spread of the disease is perceived as very high.

Since the first identification of the virus in 1999, significant efforts have already been devoted to development of diagnostic methods, outbreak preparedness and response as well as epidemiological surveillance. The outcome of research has been impressive as well, in terms of characterizing the virus, elucidating its mechanisms of replication, identifying strategies for development of novel therapies and conducting preclinical studies for an array of vaccine candidates.

However, more progress is still needed to offer the most adequate tools for controlling Nipah. In terms of diagnostics, validation of methods and standardization across laboratories would improve currently available tests. Moreover, it would be useful to further invest in strategies that can facilitate access of patients to suitable diagnostics in all areas where NiV outbreaks are likely to occur.

In terms of therapies, animal models have suggested that the m102.4 human monoclonal antibody can prevent NiV infection and/or disease. The antibody is being developed for human use as both a NiV and HeV.countermeasure. Clinical data are still required to define how m120.4 would be used in patients (pre- or post-exposure), and what would be the cost of a treatment. It has been suggested that pre-positioning of the product in NiV endemic areas would facilitate access to this therapy in the event of another outbreak. However, the feasibility of such stockpiling would largely depend on international cooperation and financial support (Broder 2013).

Research on alternative therapeutic candidates should also be encouraged. An advantage of antivirals and immunomodulators compared to biologicals is their expected breadth of coverage. While NiV infections are the focus of this report, therapies that would also prove efficient against other henipaviruses appear as highly desirable.

Among the different vaccine candidates tested in preclinical models, VSV recombinant viruses appear as the most promising. However, the neurotropic potential of such vaccine should be investigated in detail before the product can be advanced to a clinical phase of development. Alternative vaccine approaches may also built on the experience gained with the subunit vaccine againt HeV.

Finally, an essential strategy for controlling Nipah should focus on preventing virus transmission from bats to humans. Controlling the virus in its wild reservoir does not seem a feasible approach. However, establishing or reinforcing surveillance systems is of utmost importance to ensure that NiV outbreaks can be detected quickly and appropriate control measures promptly initiated. Also essential are the efforts on behaviour change communication interventions, so as to increase awareness on the risks associated with this virus.

REFERENCES

Aguilar HC, Lee B. Emerging paramyxoviruses: molecular mechanisms and antiviral strategies. Expert Rev Mol Med. 2011 Feb 24;13:e6.

Angeletti S, Lo Presti A, Cella E, Ciccozzi M. Molecular epidemiology and phylogeny of Nipah virus infection: A mini review. Asian Pac J Trop Med. 2016 Jul;9(7):630-4.

Baseler L, Scott DP, Saturday G, et al. Identifying Early Target Cells of Nipah Virus Infection in Syrian Hamsters. PLoS Negl Trop Dis. 2016 Nov 3;10(11):e0005120.

Bharaj P, Wang YE, Dawes BE, et al. The Matrix Protein of Nipah Virus Targets the E3-Ubiquitin Ligase TRIM6 to Inhibit the IKKε Kinase-Mediated Type-I IFN Antiviral Response. PLoS Pathog. 2016 Sep 13;12(9):e1005880.

Borisevich V, Ozdener MH, Malik B, Rockx B. Hendra and Nipah Virus Infection in Cultured Human Olfactory Epithelial Cells. mSphere. 2017 Jun 28;2(3).

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med. 2012 Aug 8;4(146):146ra107.

Breed A, Field H, Epstein JH, Daszak P. Emerging henipaviruses and flying foxes – Conservation and management perspectives. Biol Conservation 2006;131:211-220.

Breed AC, Meers J, Sendow I, et al. The distribution of henipaviruses in Southeast Asia and Australasia: is Wallace's line a barrier to Nipah virus? PLoS One. 2013 Apr 24;8(4):e61316.

Broder CC, Xu K, Nikolov DB, Zhu Z, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antiviral Res. 2013 Oct;100(1):8-13.

Chadha MS, Comer JA, Lowe L, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. Emerg Infect Dis. 2006 Feb;12(2):235-40.

Chan KP, Rollin PE, Ksiazek TG, et al. A survey of Nipah virus infection among various risk groups in Singapore. Epidemiol Infect. 2002 Feb;128(1):93-8.

Chattopadhyay A, Rose JK. Complementing defective viruses that express separate paramyxovirus glycoproteins provide a new vaccine vector approach. J Virol. 2011 Mar;85(5):2004-11.

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J. 2010 Jun 3;7:115.

Ching PK, de los Reyes VC, Sucaldito MN, et al. Outbreak of henipavirus infection, Philippines, 2014. Emerg Infect Dis. 2015 Feb;21(2):328-31.

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann Neurol. 2001 Jun;49(6):810-3.

Chua KB, Goh KJ, Wong KT, et al. Fatal encephalitis due to Nipah virus among pig-farmers in Malaysia. Lancet. 1999 Oct 9;354(9186):1257-9.

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect. 2013 Jun;2(6):e37.

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus. Curr Opin Virol. 2017 Feb;22:97-104.

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect. 2001 Apr;3(4):289-95.

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis. 2013;7(1):e2024.

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine. 2014 May 7;32(22):2637-44.

Defang GN, Khetawat D, Broder CC, Quinnan GV Jr. Induction of neutralizing antibodies to Hendra and Nipah glycoproteins using a Venezuelan equine encephalitis virus in vivo expression system. Vaccine. 2010 Dec 16;29(2):212-20.

de Wit E, Munster VJ. Animal models of disease shed light on Nipah virus pathogenesis and transmission. J Pathol. 2015 Jan;235(2):196-205.

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens. 2013 Apr 9;2(2):264-87.

Escaffre O, Borisevich V, Rockx B. Pathogenesis of Hendra and Nipah virus infection in humans. J Infect Dev Ctries. 2013 Apr 17;7(4):308-11.

Freiberg AN, Worthy MN, Lee B, Holbrook MR. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol. 2010 Mar;91(Pt 3):765-72.

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS One. 2010 May 18;5(5):e10690.

Geisbert TW, Feldmann H, Broder CC. Animal challenge models of henipavirus infection and pathogenesis. Curr Top Microbiol Immunol. 2012;359:153-77.

Georges-Courbot MC, Contamin H, Faure C, Let al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother. 2006 May;50(5):1768-72.

Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. N Engl J Med. 2000 Apr 27;342(17):1229-35.

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol. 2004 Jan;78(2):834-40.

Gurley ES, Montgomery JM, Hossain MJ, et al. Person-to-person transmission of Nipah virus in a Bangladeshi community. Emerg Infect Dis. 2007 Jul;13(7):1031-7.

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315.

Hegde ST, Sazzad HM, Hossain MJ, et al. Investigating Rare Risk Factors for Nipah Virus in Bangladesh: 2001-2012. Ecohealth. 2016 Dec;13(4):720-728.

Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. Epidemiol Infect. 2010 Nov;138(11):1630-6.

Hossain MJ, Gurley ES, Montgomery JM, et al. Clinical presentation of nipah virus infection in Bangladesh. Clin Infect Dis. 2008 Apr 1;46(7):977-84.

Hotard AL, He B, Nichol ST, et al. 4'-Azidocytidine (R1479) inhibits henipaviruses and other paramyxoviruses with high potency. Antiviral Res. 2017 Aug;144:147-152.

Johnson JB, Aguilar HC, Lee B, Parks GD. Interactions of human complement with virus particles containing the Nipah virus glycoproteins. J Virol. 2011 Jun;85(12):5940-8.

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One. 2015 Feb 23;10(2):e0117817.

Khan SU, Gurley ES, Hossain MJ, et al. A randomized controlled trial of interventions to impede date palm sap contamination by bats to prevent nipah virus transmission in Bangladesh. PLoS One. 2012;7(8):e42689.

Kong D, Wen Z, Su H, et al. Newcastle disease virus-vectored Nipah encephalitis vaccines induce B and T cell responses in mice and long-lasting neutralizing antibodies in pigs. Virology. 2012 Oct 25;432(2):327-35.

Kulkarni DD, Tosh C, Venkatesh G, Senthil Kumar D. Nipah virus infection: current scenario. Indian J Virol. 2013 Dec;24(3):398-408.

Liu J, Ochieng C, Wiersma S, et al. Development of a TaqMan Array Card for Acute-Febrile-Illness Outbreak Investigation and Surveillance of Emerging Pathogens, Including Ebola Virus. J Clin Microbiol. 2016 Jan;54(1):49-58.

Lo MK, Bird BH, Chattopadhyay A, et al. Single-dose replication-defective VSV-based Nipah virus vaccines provide protection from lethal challenge in Syrian hamsters. Antiviral Res. 2014 Jan;101:26-9.

Lo MK, Rota PA. Molecular virology of the henipaviruses. Curr Top Microbiol Immunol. 2012;359:41-58.

Luby SP. The pandemic potential of Nipah virus. Antiviral Res. 2013 Oct;100(1):38-43.

Mackenzie JS, Field HE, Guyatt KJ. Managing emerging diseases borne by fruit bats (flying foxes), with particular reference to henipaviruses and Australian bat lyssavirus. J Appl Microbiol. 2003;94 Suppl:59S-69S.

Manyweathers J, Field H, Longnecker N, et al. "Why won't they just vaccinate?" Horse owner risk perception and uptake of the Hendra virus vaccine. BMC Vet Res. 2017 Apr 13;13(1):103.

Mathieu C, Augusto MT, Niewiesk S, et al. Broad spectrum antiviral activity for paramyxoviruses is modulated by biophysical properties of fusion inhibitory peptides. Sci Rep. 2017 Mar 8;7:43610.

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther. 2015 Mar;13(3):343-54.

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine. 2008 Jul 23;26(31):3842-52.

Middleton DJ, Morrissy CJ, van der Heide BM, et al. Experimental Nipah virus infection in pteropid bats (Pteropus poliocephalus). J Comp Pathol. 2007 May;136(4):266-72.

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. Sci Rep. 2016 Aug 3;6:309-16.

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J. 2013 Dec 13;10:353.

Mitchell WM. Efficacy of rintatolimod in the treatment of chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME). Expert Rev Clin Pharmacol. 2016 Jun;9(6):755-70.

Mungall BA, Middleton D, Crameri G, et al. Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. J Virol. 2006 Dec;80(24):12293-302.

Nahar N, Paul RC, Sultana R, et al. Raw Sap Consumption Habits and Its Association with Knowledge of Nipah Virus in Two Endemic Districts in Bangladesh. PLoS One. 2015 Nov 9;10(11):e0142292.

Nahar N, Paul RC, Sultana R, et al. A Controlled Trial to Reduce the Risk of Human Nipah Virus Exposure in Bangladesh. Ecohealth. 2017 Sep 13.

Ong KC, Wong KT. Henipavirus Encephalitis: Recent Developments and Advances. Brain Pathol. 2015 Sep;25(5):605-13.

Onyango CO, Loparev V, Lidechi S, et al. Evaluation of a TaqMan Array Card for Detection of Central Nervous System Infections. J Clin Microbiol. 2017 Jul;55(7):2035-2044.

Pager CT, Wurth MA, Dutch RE. Subcellular localization and calcium and pH requirements for proteolytic processing of the Hendra virus fusion protein. J Virol. 2004 Sep;78(17):9154-63.

Pallister JA, Klein R, Arkinstall R, et al. Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. Virol J. 2013 Jul 16;10:237.

Parveen S, Islam MS, Begum M, et al. It's not only what you say, it's also how you say it: communicating nipah virus prevention messages during an outbreak in Bangladesh. BMC Public Health. 2016 Aug 5;16:726.

Pattabhi S, Wilkins CR, Dong R, et al. Targeting Innate Immunity for Antiviral Therapy through Small Molecule Agonists of the RLR Pathway. J Virol. 2015 Dec 16;90(5):2372-87.

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342.

Ploquin A, Szécsi J, Mathieu C, Guillaume V, Barateau V, Ong KC, Wong KT, Cosset FL, Horvat B, Salvetti A. Protection against henipavirus infection by use of recombinant adeno-associated virus-vector vaccines. J Infect Dis. 2013 Feb 1;207(3):469-78.

Porotto M, Orefice G, Yokoyama CC, et al. Simulating henipavirus multicycle replication in a screening assay leads to identification of a promising candidate for therapy. J Virol. 2009 May;83(10):5148-55.

Prescott J, de Wit E, Feldmann H, Munster VJ. The immune response to Nipah virus infection. Arch Virol. 2012 Sep;157(9):1635-41.

Prescott J, DeBuysscher BL, Feldmann F, et al. Single-dose live-attenuated vesicular stomatitis virus-based vaccine protects African green monkeys from Nipah virus disease. Vaccine. 2015 Jun 4;33(24):2823-9.

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis http://www.iedcr.gov.bd/pdf/files/nipah/National_Nipah.pdf. 2011

Rahman M, Chakraborty A. Nipah virus outbreaks in Bangladesh: a deadly infectious disease. WHO South East Asia J Public Health. 2012 Apr-Jun;1(2):208-212.

Rahman SA, Hassan L, Epstein JH, et al. Risk Factors for Nipah virus infection among pteropid bats, Peninsular Malaysia. Emerg Infect Dis. 2013 Jan;19(1):51-60.

Rockx B, Winegar R, Freiberg AN. Recent progress in henipavirus research: molecular biology, genetic diversity, animal models. Antiviral Res. 2012 Aug;95(2):135-49.

Satterfield BA, Cross RW, Fenton KA, et al. The immunomodulating V and W proteins of Nipah virus determine disease course. Nat Commun. 2015 Jun 24;6:7483.

Satterfield BA, Dawes BE, Milligan GN. Status of vaccine research and development of vaccines for Nipah virus. Vaccine. 2016 Jun 3;34(26):2971-2975.

Sauerhering L, Zickler M, Elvert M, et al. Species-specific and individual differences in Nipah virus replication in porcine and human airway epithelial cells. J Gen Virol. 2016 Jul;97(7):1511-9.

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis. 2015 Feb;21(2):349-51.

Simons RR, Gale P, Horigan V, et al. Potential for introduction of bat-borne zoonotic viruses into the EU: a review. Viruses. 2014 May 16;6(5):2084-121.

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses. 2012 Feb;4(2):280-308.

Sugai A, Sato H, Takayama I, Yoneda M, Kai C. Nipah and Hendra Virus Nucleoproteins Inhibit Nuclear Accumulation of STAT1 and STAT2 by Interfering with Their Complex Formation. J Virol. 2017 Aug 23. pii: JVI.01136-17.

Tiong V, Lam CW, Phoon WH, et al. Serum from Nipah Virus Patients Recognises Recombinant Viral Proteins Produced in Escherichia coli. Jpn J Infect Dis. 2017 Jan 24;70(1):26-31.

Valbuena G, Halliday H, Borisevich V, Goez Y, Rockx B. A human lung xenograft mouse model of Nipah virus infection. PLoS Pathog. 2014 Apr 3;10(4):e1004063.

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric Viruses with Vesicular Stomatitis Virus: Actions in the Brain. J Virol. 2017 Feb 28;91(6).

Wacharapluesadee S, Samseeneam P, Phermpool M, et al. Molecular characterization of Nipah virus from Pteropus hypomelanus in Southern Thailand. Virol J. 2016 Mar 25;13:53.

Walpita P, Barr J, Sherman M, Basler CF, Wang L. Vaccine potential of Nipah virus-like particles. PLoS One. 2011 Apr 6;6(4):e18437.

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol. 2012;359:179-96.

Wang L, Harcourt BH, Yu M, et al. Molecular biology of Hendra and Nipah viruses. Microbes Infect. 2001 Apr;3(4):279-87.

Weingartl HM, Berhane Y, Caswell JL, et al. Recombinant nipah virus vaccines protect pigs against challenge. J Virol. 2006 Aug;80(16):7929-38.

Wong SC, Ooi MH, Wong MN, et al. Late presentation of Nipah virus encephalitis and kinetics of the humoral immune response. J Neurol Neurosurg Psychiatry. 2001 Oct;71(4):552-4.

Wong KT, Tan CT. Clinical and pathological manifestations of human henipavirus infection. Curr Top Microbiol Immunol. 2012;359:95-104.

World Health Organization. NiV outbreak(s) in Bangladesh, January-April 2004. Wkly Epidemiol Rec 2004;79:168-71.

Wu Z, Yang L, Yang F, et al. Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. Emerg Infect Dis. 2014 Jun;20(6):1064-6.

Yoneda M, Georges-Courbot MC, Ikeda F, et al. Recombinant measles virus vaccine expressing the Nipah virus glycoprotein protects against lethal Nipah virus challenge. PLoS One. 2013;8(3):e58414.

Yu F, Khairullah NS, Inoue S, et al. Serodiagnosis using recombinant nipah virus nucleocapsid protein expressed in Escherichia coli. J Clin Microbiol. 2006 Sep;44(9):3134-8.

Zhu Z, Bossart KN, Bishop KA, et al. Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. J Infect Dis. 2008 Mar 15;197(6):846-53.

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus infection.

*(**Note:** This draft roadmap is organized into the three main categories of MCMs—diagnostics, therapeutics, and vaccines—to allow reviewers an opportunity to examine the development of the roadmap components for each countermeasure. Some of the topics and issues apply to more than one MCM; these are highlighted as cross-cutting. This document is relatively detailed to ensure that it includes the most important topics and issues. As this draft is refined, the content may be reorganized and certain items may be "rolled up" into a smaller number of broader topics and issues, strategic goals, and priority areas and activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India; associated case fatality rates in these countries have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected human-to-human NiV transmission. Additionally, several other animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary NiV sources being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B).

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING TOPICS AND ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition for industry and international philanthropic public-private

partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs.

- Regulatory approval pathways for MCMs can be prohibitively expensive for product developers. For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore, licensure of vaccines and therapeutics using alternative regulatory pathways also can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions.
- To date, NiV spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh and East India; the healthcare facilities that serve these communities have very rudimentary laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.
- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Conducting phase 1 clinical trials, and possibly phase 2 clinical trials, is likely feasible in endemic regions. However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting phase 3 clinical trials for demonstrating efficacy. Therefore, alternative regulatory pathways and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time) may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Key needs

- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- Standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research.
- A determination regarding the feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection, which is needed before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities may vary between countries; therefore, early engagement is essential to identify country-specific considerations.
- Outreach and education to clinicians in endemic and at-risk areas to improve NiV awareness and training, and increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capabilities for data reporting in resource-limited settings to promote collaborative sharing of NiV sequence and strain data, which can be used to support MCM development.
- Collaboration with local government authorities in endemic and at-risk areas to support NiV surveillance and facilitate effective communication with communities regarding disease prevention activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for development and evaluation of MCMs. For example, efforts are needed to: (1)

determine the appropriate animal model(s) for screening assay development; (2) determine the most appropriate lethal NiV dose for MCM development; (3) determine when MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.

- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between NiV_B and NiV_M infection, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Some evidence suggests that there are differences in NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies. More information on this issue is needed.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional research is needed to develop and assess next generation sequencing (NGS) for whole gene analysis, which can be used to enhance understanding of the global distribution of henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage at-risk populations (including vulnerable populations such as pregnant women, children, and immunocompromised persons) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease. Efforts are needed to: (1) assess potential barriers for conducting clinical trials; (2) assess MCM acceptability in at-risk populations; (3) determine culturally appropriate messaging to enhance MCM acceptance; and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.
2. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.
3. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.

4. Engage NRAs (particularly in endemic and at-risk areas) to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate, and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research on the uses of NGS for understanding henipavirus distribution.
- **Determine** the feasibility of conducting phase 3 clinical trials or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in instituting effective and timely infection control measures. Additionally, latent disease can occur long after initial infection.

- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using PCR and ELISA methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobank of human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.

- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the Differentiating Infected from Vaccinated Animals (DIVA) test.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Develop and assess affordable, highly sensitive and specific, point-of-care NiV diagnostic tests for use in humans and animals that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.
3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.

4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.
- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials using both NiV_M and NiV_B are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, and GS-4734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV_B and NiV_M, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV_B to be shorter than for NiV_M.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- Additional studies of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.
2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ribavirin and m102.4) for treating and preventing NiV infection, including conduct of animal studies and clinical trials as appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company to advance therapeutic use of m102.4 and **secure** financing for its manufacture and distribution.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human, veterinary, and public health systems, which could impact acceptance of NiV vaccines for use in humans or animals.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that can protect against both NiV$_M$ and NiV$_B$ in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks.

Commented [MGN1]: Perhaps needs to be changed to be inclusive of all NiV strains

- Guidance on use of NiV vaccines (or broader henipavirus vaccines) to include vaccination strategies, potentially in both humans and animals, for different epidemiologic scenarios and different vaccine attributes.

Commented [MGN2]: Perhaps add, "including special populations such as elderly, immune-compromised, and pregnant individuals"

- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines, such as the soluble G glycoprotein subunit (sG) and recombinant vesicular stomatitis (rVSV) vaccine.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoetis).
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV$_M$ and NiV$_B$, including recent strains; only a few studies demonstrating cross-protection have been performed to date.

Commented [MGN3]: All NiV strains

- The identification of specific correlates of protection in humans and animals and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Evaluation of vaccine safety in target populations is needed to better understand the risk of adverse incidents associated with vaccine use.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than

one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines, estimating the vaccine quantity for stockpiles, and in simulating various epidemiologic scenarios and possible strategies for vaccine use.
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. While one HeV vaccine is available for horses, antibodies against HeV have not been found to be cross-reactive with NiV. Ongoing research into developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.
- Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, and deploy NiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection for use in developing and evaluating NiV vaccines.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Determine** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and animals.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines for use in humans that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a worthwhile approach.

Key capacities

- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the impact of vaccination strategies, once vaccines become available.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus infection.

*(**Note:** This draft roadmap is organized into the three main categories of MCMs—diagnostics, therapeutics, and vaccines—to allow reviewers an opportunity to examine the development of the roadmap components for each countermeasure. Some of the topics and issues apply to more than one MCM; these are highlighted as cross-cutting. This document is relatively detailed to ensure that it includes the most important topics and issues. As this draft is refined, the content may be reorganized and certain items may be "rolled up" into a smaller number of broader topics and issues, strategic goals, and priority areas and activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India; associated case fatality rates in these countries have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected human-to-human NiV transmission. Additionally, several other animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary NiV sources being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B).

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING TOPICS AND ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition for industry and international philanthropic public-private

partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs.

- Regulatory approval pathways for MCMs can be prohibitively expensive for product developers. For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore, licensure of vaccines and therapeutics using alternative regulatory pathways also can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions.
- To date, NiV spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh and East India; the healthcare facilities that serve these communities have very rudimentary laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.
- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Conducting phase 1 clinical trials, and possibly phase 2 clinical trials, is likely feasible in endemic regions. However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting phase 3 clinical trials for demonstrating efficacy. Therefore, alternative regulatory pathways and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time) may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Key needs

- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- Standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research.
- A determination regarding the feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection, which is needed before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities may vary between countries; therefore, early engagement is essential to identify country-specific considerations.
- Outreach and education to clinicians in endemic and at-risk areas to improve NiV awareness and training, and increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capabilities for data reporting in resource-limited settings to promote collaborative sharing of NiV sequence and strain data, which can be used to support MCM development.
- Collaboration with local government authorities in endemic and at-risk areas to support NiV surveillance and facilitate effective communication with communities regarding disease prevention activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Knowledge gaps

- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for development and evaluation of MCMs. For example, efforts are needed to: (1)

determine the appropriate animal model(s) for screening assay development; (2) determine the most appropriate lethal NiV dose for MCM development; (3) determine when MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.

- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between NiV_B and NiV_M infection, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Some evidence suggests that there are differences in NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies. More information on this issue is needed.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional research is needed to develop and assess next generation sequencing (NGS) for whole gene analysis, which can be used to enhance understanding of the global distribution of henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage at-risk populations (including vulnerable populations such as pregnant women, children, and immunocompromised persons) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease. Efforts are needed to: (1) assess potential barriers for conducting clinical trials; (2) assess MCM acceptability in at-risk populations; (3) determine culturally appropriate messaging to enhance MCM acceptance; and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.
2. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.
3. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.

4. Engage NRAs (particularly in endemic and at-risk areas) to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate, and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research on the uses of NGS for understanding henipavirus distribution.
- **Determine** the feasibility of conducting phase 3 clinical trials or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in instituting effective and timely infection control measures. Additionally, latent disease can occur long after initial infection.

- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using PCR and ELISA methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobank of human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.

- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the Differentiating Infected from Vaccinated Animals (DIVA) test.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Develop and assess affordable, highly sensitive and specific, point-of-care NiV diagnostic tests for use in humans and animals that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.
3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.

4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.
- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials using both NiV_M and NiV_B are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, and GS-4734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV_B and NiV_M, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV_B to be shorter than for NiV_M.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- Additional studies of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.
2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ribavirin and m102.4) for treating and preventing NiV infection, including conduct of animal studies and clinical trials as appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company to advance therapeutic use of m102.4 and **secure** financing for its manufacture and distribution.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human, veterinary, and public health systems, which could impact acceptance of NiV vaccines for use in humans or animals.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that can protect against both NiV_M and NiV_B in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines (or broader henipavirus vaccines) to include vaccination strategies, potentially in both humans and animals, for different epidemiologic scenarios and different vaccine attributes.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines, such as the soluble G glycoprotein subunit (sG) and recombinant vesicular stomatitis (rVSV) vaccine.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoetis).
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B, including recent strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates of protection in humans and animals and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Evaluation of vaccine safety in target populations is needed to better understand the risk of adverse incidents associated with vaccine use.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than

one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.

- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines, estimating the vaccine quantity for stockpiles, and in simulating various epidemiologic scenarios and possible strategies for vaccine use.
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. While one HeV vaccine is available for horses, antibodies against HeV have not been found to be cross-reactive with NiV. Ongoing research into developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.
- Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, and deploy NiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection for use in developing and evaluating NiV vaccines.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Determine** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and animals.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines for use in humans that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a worthwhile approach.

Key capacities

- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the impact of vaccination strategies, once vaccines become available.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

From: Milligan, Gregg N. on behalf of Milligan, Gregg N. <gnmillig@utmb.edu>
To: Kris Moore; christopher.broder@usuhs.edu; chuakb@tll.org.sg; egurley1@jhu.edu; sluby@stanford.edu; mahmudur57@gmail.com; pyr3@cdc.gov; linfa.wang@duke-nus.edu.sg
Cc: K.Cook@wellcome.ac.uk; benassiv@who.int; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; "Josie Golding"; "PREZIOSI, Marie-pierre"; mto@umn.edu
Subject: RE: Revised Nipah virus R&D roadmap for taskforce review and comment--please send written comments by April 25
Date: Wednesday, April 25, 2018 4:30:47 PM
Attachments: Nipah Draft Roadmap.revised for taskforce review.April 2018 gnm edits.docx

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Hello Kris,
I found the document very inclusive of the ideas discussed at the meeting. I don't have any substantive changes to suggest for the majority of the document but had a few suggestions for the vaccine section. The document is very nicely done.
Gregg

From: Kris Moore [mailto:kamoore@umn.edu]
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Subject: Revised Nipah virus R&D roadmap for taskforce review and comment--please send written comments by April 25
Importance: High

Dear Nipah Virus R&D Roadmap Taskforce members:

Attached please find a revised version of the Nipah virus R&D roadmap for your review. This version incorporates comments made during the in-person consultation in early March, comments we received after the consultation, and recent literature. We also consolidated all of the cross-cutting issues near the beginning of the roadmap.

We would very much appreciate your written feedback/comments on this version **no later than Wednesday April 25.**

We're sending this to you as a Word doc to make editing and commenting easier. We suggest that you cc the whole group when you provide edits/comments—to ensure that you all see each other's perspectives.

Once we incorporate your additional feedback, the next step will be to post the document for public comment. We also plan to begin working on a set of measurable and achievable milestones and will start considering ways to approach the critical path analysis.

Again, we very much appreciate your ongoing support and involvement with this important project.

Warm regards,

Kris

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Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus infection.

*(**Note:** This draft roadmap is organized into the three main categories of MCMs—diagnostics, therapeutics, and vaccines—to allow reviewers an opportunity to examine the development of the roadmap components for each countermeasure. Some of the topics and issues apply to more than one MCM; these are highlighted as cross-cutting. This document is relatively detailed to ensure that it includes the most important topics and issues. As this draft is refined, the content may be reorganized and certain items may be "rolled up" into a smaller number of broader topics and issues, strategic goals, and priority areas and activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized, however, in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also ~~occurring~~ identified periodically in eastern India; associated case fatality rates during outbreaks in these countries have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropus bat species) and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected, although limited, human-to-human NiV transmission. Additionally, several other animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary routes of human NiV ~~sources~~ infection being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely

related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, dizziness, cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_{M}) and in Bangladesh/India (NiV_{B}).

Commented [EG1]: Nearly all cases in Bangladesh have altered mental status or unconsciousness.

Commented [EG2]: Would be good to provide the year here since there could also be variation within South Asia- which paper is this specifically?

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV infection (and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING TOPICS AND ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition for industry and international philanthropic public-private

partnerships are needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs.

- Regulatory approval pathways for MCMs can be prohibitively expensive for product developers. For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore, licensure of vaccines and therapeutics using alternative regulatory pathways also can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions.
- To date, NiV spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh and East India; the healthcare facilities that serve these communities have very rudimentary laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.

Commented [EG3]: True, but it is not typically clinically distinguishable from other causes of encephalitis.

- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses for livestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Conducting phase 1 clinical trials, and possibly phase 2 clinical trials, is likely feasible in endemic regions. However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting phase 3 clinical trials for demonstrating efficacy. Therefore, alternative regulatory pathways and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time) may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Commented [EG4]: In Bangladesh, the greater concern from local authorities would be in conducting the phase 1 rather than the phase 2.

Key needs

- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- Standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research.
- A determination regarding the feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection, which is needed before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities may vary between countries; therefore, early engagement is essential to identify country-specific considerations.
- Outreach and education to clinicians in endemic and at-risk areas to improve NiV awareness and training, and increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capabilities for data reporting in resource-limited settings to promote collaborative sharing of NiV sequence and strain data, which can be used to support MCM development.
- Collaboration with local government authorities in endemic and at-risk areas to support NiV surveillance and facilitate effective communication with communities regarding disease prevention activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturing to endemic and at-risk areas.

Commented [EG5]: This one is tricky. Nipah is rare. Heightened awareness about the dangers of Nipah lead to patients being turned away, particularly if hospital staff do not feel well trained and equipped to perform required PPE. Education itself will only create fear, unless we can also ensure supplies and ongoing training and support. Local clinics cannot currently diagnose anyway, but they could be trained on how to collect specimens.

Commented [EG6]: Not exactly sure what this means.

Commented [EG7]: Collaboration between local authorities and who? International public health stakeholders? NiV experts?

Knowledge gaps

- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses, and drivers of infection in the natural reservoir of Pteropus bats and potentially other fruit bats.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models (e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is

essential for development and evaluation of MCMs. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) determine the most appropriate lethal NiV dose for MCM development; (3) determine when MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.

- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between NiV$_{B}$ and NiV$_{M}$ infection, as well as differences between strains in Bangladesh, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Some evidence suggests that there are differences in NiV$_{B}$ and NiV$_{M}$ pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies. More information on this issue is needed.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hosts and pathogenicity.
- Additional research is needed to develop and assess next generation sequencing (NGS) for whole gene analysis, which can be used to enhance understanding of the global distribution of henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage at-risk populations (including vulnerable populations such as pregnant women, children, and immunocompromised persons) for participation in clinical trials and to ensure acceptance of new NiV MCMs, especially if therapeutics and vaccines do not consistently prevent disease. Efforts are needed to: (1) assess potential barriers for conducting clinical trials; (2) assess MCM acceptability in at-risk populations; (3) determine culturally appropriate messaging to enhance MCM acceptance; and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.
2. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.

3. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.
4. Engage NRAs (particularly in endemic and at-risk areas) to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate, and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research on the uses of NGS for understanding henipavirus distribution.
- **Determine** the feasibility of conducting phase 3 clinical trials or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote** early communication between developers and appropriate NRAs for clarity and guidance on the regulatory aspects of MCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** surveillance capacity to: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiV surveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorization from appropriate NRAs for NiV MCMs. This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Clarify** the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in instituting effective and timely infection control measures and outbreak detection and response. Additionally, latent disease can occur long after initial infection.

- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis, outbreak investigation and response.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using PCR and ELISA methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species) appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Commented [EG8]: There are now PCR methods that take a few hours on hand held devices. They are expensive, but quick. I think that PCR based techniques could be useful, even given the realities of timeline, but they are expensive in terms of infrastructure and are not currently available in the facilities where patients seek care. Of course, these aren't as easy as rapid tests could be.

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobank of human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.

- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of public health concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the Differentiating Infected from Vaccinated Animals (DIVA) test.

Commented [EG9]: And, perhaps, and estimate of how many would be sufficient to make this case. Ballpark?

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Develop and assess affordable, highly sensitive and specific, point-of-care NiV diagnostic tests for use in humans and animals that are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.

3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.
4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiV infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.
- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.

- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.
- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials using both NiV_M and NiV_B are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides, antifusion peptides, and GS-4734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV_B and NiV_M, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV_B to be shorter than for NiV_M.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- Additional studies of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer. This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.
2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ribavirin and m102.4) for treating and preventing NiV infection, including conduct of animal studies and clinical trials as appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company to advance therapeutic use of m102.4 and **secure** financing for its manufacture and distribution.
- **Develop** guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human, veterinary, and public health systems, which could impact acceptance of NiV vaccines for use in humans or animals.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that can protect against both NiV$_M$ and NiV$_B$ in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiV vaccines (or broader henipavirus vaccines) to include vaccination strategies, potentially in both humans and animals, for different epidemiologic scenarios and different vaccine attributes.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategies over time.

Commented [EG10]: Enhanced surveillance may be needed to test such vaccines, to have enough cases to power trials.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines, such as the soluble G glycoprotein subunit (sG) and recombinant vesicular stomatitis (rVSV) vaccine.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoetis).
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV$_M$ and NiV$_B$, including recent strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates of protection in humans and animals and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Evaluation of vaccine safety in target populations is needed to better understand the risk of adverse incidents associated with vaccine use.

- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines, estimating the vaccine quantity for stockpiles, and in simulating various epidemiologic scenarios and possible strategies for vaccine use.

Commented [EG11]: And for vaccine trials.

- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. While one HeV vaccine is available for horses, antibodies against HeV have not been found to be cross-reactive with NiV. Ongoing research into developing NiV/HeV vaccines for livestock (or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.
- Additional research is needed to determine if development of multivalent vaccines for animals (that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, and deploy NiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection for use in developing and evaluating NiV vaccines.
- **Further study** cross protection of various vaccine candidates against NiV$_B$ and NiV$_M$ strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.

- **Determine** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and animals.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines for use in humans that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a worthwhile approach.

Key capacities

- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the impact of vaccination strategies, once vaccines become available.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Commented [EG12]: And, improve our chances of being able to test vaccines.

Policy and commercialization

- **Provide** guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. Curr Opin Virol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

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To: Kris Moore; christopher.broder@usuhs.edu; chuakb@tll.org.sg; sluby@stanford.edu; gnmillig@utmb.edu; mahmudur57@gmail.com; pyr3@cdc.gov; linfa.wang@duke-nus.edu.sg
Cc: K.Cook@wellcome.ac.uk; benassiv@who.int; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; "Josie Golding"; "PREZIOSI, Marie-pierre"; mto@umn.edu
Subject: RE: Revised Nipah virus R&D roadmap for taskforce review and comment--please send written comments by April 25
Date: Wednesday, April 25, 2018 3:49:09 PM
Attachments: Nipah Draft Roadmap.revised for taskforce review.April 2018_eg.docx

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Kris,

Many thanks for your efforts to put this draft together. I think the draft does a great job of summarizing our conversations in London. I've included some comments and suggestions, attached.

All the best,
Emily

From: Kris Moore [mailto:kamoore@umn.edu]
Sent: Friday, April 13, 2018 6:19 PM
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Subject: Revised Nipah virus R&D roadmap for taskforce review and comment--please send written comments by April 25
Importance: High

Dear Nipah Virus R&D Roadmap Taskforce members:

Attached please find a revised version of the Nipah virus R&D roadmap for your review. This version incorporates comments made during the in-person consultation in early March, comments we received after the consultation, and recent literature. We also consolidated all of the cross-cutting issues near the beginning of the roadmap.

We would very much appreciate your written feedback/comments on this version **no later than Wednesday April 25.**

We're sending this to you as a Word doc to make editing and commenting easier. We suggest that you cc the whole group when you provide edits/comments—to ensure that you all see each other's perspectives.

Once we incorporate your additional feedback, the next step will be to post the document for public comment. We also plan to begin working on a set of measurable and achievable milestones and will start considering ways to approach the critical path analysis.

Again, we very much appreciate your ongoing support and involvement with this important project.

Warm regards,

Kris

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NipahResearch and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus infection.

*(**Note:**This draft roadmap is organized into the three main categories of MCMs—diagnostics, therapeutics, and vaccines—to allow reviewers an opportunity to examine the development of the roadmap components for each countermeasure. Some of the topics and issues apply to more than one MCM; these are highlighted as cross-cutting.This document is relatively detailed to ensure that it includes the most important topics and issues. As this draft is refined, the content may be reorganized and certain items may be "rolled up" into a smaller number of broader topics and issues, strategic goals, and priority areas and activities.)*

INTRODUCTION

Nipah virus (NiV)is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illness in pigs and encephalitic disease in humans occurred in Malaysia and Singapore in 1998and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported inthese countries since May 1999.NiV infection was subsequently recognized, however,in Bangladeshin 2001 and nearly annual outbreaks have occurred in that country since, with disease also~~ occurring~~ being recognized periodically in eastern India; associated case fatality rates in these countries have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropusbatspecies)and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected human-to-human NiV transmission. Additionally, several other animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In theoutbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary NiV sources being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely

related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat and in healthcare workers who cared for cases. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, dizziness,cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated ~~that two distinct~~multiple strains of NiV. The strain responsible for the outbreak in Malaysia is different from the strains seen in ~~were responsible for the outbreaks in Southeast Asia (NiV$_M$) and in~~ Bangladesh/India (NiV$_B$).

Commented [SPL1]: We have isolated multiple different strains in Bangladesh.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease.

Commented [SPL2]: I recommend spelling out this acronym, so that readers don't need to learn a new acronym to understand the article.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, respond, prevent, and control outbreaks of NiV infection(and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING TOPICS AND ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value propositionfor industry and international philanthropic public-private partnershipsare needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs.

- Regulatory approval pathways for MCMs can be prohibitively expensive for product developers. For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore, licensure of vaccines and therapeutics using alternative regulatory pathways also can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions.
- To date, NiV spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh and East India; the healthcare facilities that serve these communities ~~have very rudimentary~~ are primary in nature in terms of laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.
- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses forlivestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Conducting phase 1 clinical trials, and possibly phase 2 clinical trials,is likely feasible in endemic regions. However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting phase 3 clinical trials for demonstrating efficacy. Therefore, alternative regulatory pathways and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time) may need to be

considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Key needs

- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- Standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research.
- A determination regardingthe feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection, which is needed before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities may vary between countries; therefore, early engagement is essential to identify country-specific considerations.
- Outreach and education to clinicians in endemic and at-risk areas to improve NiV awareness and training, and increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capabilities for data reporting in resource-limited settings to promote collaborative sharing of NiV sequence and strain data, which can be used to support MCM development.
- Collaboration with ~~local~~ government authorities in endemic ~~and at-risk areas~~countries to support NiV surveillance and facilitate effective communication with communities regarding disease prevention activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturingto endemic and at-risk areas.

Commented [Mahmudur3]: It will be necessary to involve the development partners who support the countries

Knowledge gaps

- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats.
- Additional research is needed torefine, standardize, and validate relevant animal challenge models(e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in

supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for development and evaluation of MCMs. For example, efforts are needed to: (1) determine the appropriate animal model(s) for screening assay development; (2) determine the most appropriate lethal NiV dose for MCM development; (3)determine when MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.

- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between ~~NiV$_B$ and NiV$_M$ infection~~different NiV strains, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Some evidence suggests that there are differences in ~~NiV$_B$ and NiV$_M$~~ pathogenesis among different strains of NiV that may have implications for the development of safe and effective NiV vaccines or therapies.More information on this issue is needed.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hostsand pathogenicity.
- Additional research is needed to develop and assess next generation sequencing (NGS) for whole gene analysis, which can be used to enhance understanding of the global distribution of henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage at-risk populations (including vulnerable populations such as pregnant women, children, and immunocompromised persons) for participation in clinical trials and to ensure acceptance of new NiVMCMs, especially if therapeutics and vaccines do not consistently prevent disease. Efforts are needed to: (1) assess potential barriers for conducting clinical trials; (2) assess MCM acceptability in at-risk populations; (3) determine culturally appropriate messaging to enhance MCM acceptance; and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.
2. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.

3. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.
4. Engage NRAs (particularly in endemic and at-risk areas) to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

~~4.~~5.

Commented [Mahmudur4]: Consider adding "Enhance disease detection capabilities through active and passive surveillance"

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform**phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** the key differences between NiV$_B$ and NiV$_M$ pathogenesisthat may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate, and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research on the uses of NGS for understanding henipavirus distribution.
- **Determine** the feasibility of conducting phase 3 clinical trials or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Commented [SPL5]: As noted above, I do not consider this dichotomous framing appropriate. It has been used in the literature, but I see this as erroneous. There is not a single strain of NiV$_B$, but rather multiple.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote**early communication between developers and appropriate NRAs forclarity and guidance on the regulatory aspects ofMCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** surveillance capacityto: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiVsurveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorizationfrom appropriate NRAs for NiVMCMs.This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Clarify**the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Commented [SPL6]: This is a key component. The general description of research needs and capacity development, seem to me to be fairly complete, but a thoughtful consideration of priority in sequence could help direct development.

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection arenonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in instituting effective and timely infection control measures. Additionally, latent disease can occur long after initial infection.

- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using PCR and ELISA methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species)appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge forensuring diagnostic preparednessto respond tofuture outbreaks.

Commented [SPL7]: A related problem is that in many of the contexts where these patients present, etiologic diagnosis is often not pursued. We need more than an inexpensive sensitive and specific Nipah diagnostic. For this to be used clinicians need to see value in conducting the tests. Having a specific therapeutic treatment could help motivate this. A multiplex diagnostic tool that provided insight on other pathogens (especially if they were treatable) would also encourage more frequent testing.

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobankof human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course,are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- International reference standards to calibrate diagnostic assays.

- Validation of promising diagnostics in endemic and at-risk geographic regions.
- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the Differentiating Infected from Vaccinated Animals (DIVA) test.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detectionat various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Develop and assess affordable, highly sensitive and specific, point-of-care NiVdiagnostic tests for use in humans and animalsthat are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.
3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.

4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiVinfectionat different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.
- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protectionagainst lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials using both NiV$_{M}$ and NiV$_{B}$are needed to assess the safety, tolerability, and efficacyof m102.4 (and possibly other mAbs) for PEPand potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the mostpromising novel treatments (such as fusion inhibitory peptides, antifusion peptides, and GS-4734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV$_{B}$ and NiV$_{M}$, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV$_{B}$ to be shorter than for NiV$_{M}$.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- Additional studies of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer.This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies,diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines.

Commented [SPL8]: I suspect there is differences of opinion on this. On the one hand there is observational data from the Malaysian outbreak that NiV patients who received ribavirin had lower case fatality than NIV patients who did not. The unanswered question is whether this improved survival was a result of the ribavirin or whether it was primarily a result of ribavirin being used later in the outbreak once clinicians recognized the disease and possibly implemented better supportive care. The absence of impact on animal models suggests (but does not prove) that the improvement in human survival was due to confounding rather than biological effect. Given a fixed amount of resources and the opportunity cost of identifying patients who might benefit, I personally would not be particularly enthusiastic to conduct a phase III trial of ribavirin in Nipah patients in Bangladesh.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.
2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ribavirin and m102.4) for treating and preventing NiV infection, including conduct of animal studies andclinical trialsas appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infectionthat are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV$_B$ and NiV$_M$and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company to advance therapeutic use of m102.4 and **secure** financing for its manufacture and distribution.
- **Develop**guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human, veterinary, and public health systems, which could impact acceptance of NiV vaccinesfor use in humans or animals.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that can protect against both NiV_M and NiV_Bin humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiVvaccines (or broader henipavirus vaccines)to include vaccination strategies, potentially in both humans and animals, for different epidemiologic scenarios and different vaccine attributes.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategiesover time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines, such as the soluble G glycoprotein subunit (sG) and recombinant vesicular stomatitis (rVSV) vaccine.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoetis).
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B, including recent strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates of protection in humans and animals and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Evaluation of vaccine safety in target populations is needed to better understand the risk of adverse incidents associated with vaccine use.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than

one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.

- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines, estimating the vaccine quantity for stockpiles, and in simulating various epidemiologic scenarios and possiblestrategies for vaccine use.
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. While one HeV vaccine is available for horses, antibodies against HeV have not been found to be cross-reactive with NiV. Ongoing research into developing NiV/HeV vaccines for livestock(or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.
- Additional research is needed to determine if development of multivalent vaccines for animals(that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, and deployNiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection for use in developing and evaluatingNiV vaccines.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains,and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Determine** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effectivemonovalent NiV vaccines for humans and animals.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines for use in humans that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a worthwhile approach.

Key capacities

- **Improve**surveillance capabilities,particularly in rural areas of resource-limited settings, to assess the impact of vaccination strategies, once vaccines become available.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Policy and commercialization

- **Provide**guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoSNegl Trop Dis2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95[Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoSNegl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al.Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295.[Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE.A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B.Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE.Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. CurrOpinVirol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

From: Steve Luby on behalf of Steve Luby <sluby@stanford.edu>
To: Kris Moore
Cc: christopher.broder@usuhs.edu; chuakb@tll.org.sg; Emily Gurley; gnmillig@utmb.edu; pyr3@cdc.gov; Linfa Wang; K.Cook@wellcome.ac.uk; benassiv@who.int; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; Josie Golding; PREZIOSI, Marie-pierre; Michael Osterholm; Mahmudur Rahman
Subject: Re: Revised Nipah virus R&D roadmap for taskforce review and comment--please send written comments by April 25
Date: Wednesday, April 25, 2018 1:48:58 PM
Attachments: Nipah Draft Roadmap.revised for taskforce review.April 2018MR sl.docx

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Hi Kris,

Attached are a few additional comments.

Steve

Mahmudur Rahman Wednesday, April 25, 2018 8:17 AM

Dear Kris,

Greetings from Bangladesh !!!

Hope you all are doing fine.

Please find attached herewith the "Nipah Research and Development (R&D) Roadmap" with my comments in track change mode.

Best regards,

Mahmud

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Kris Moore Friday, April 13, 2018 3:19 PM

Dear Nipah Virus R&D Roadmap Taskforce members:

Attached please find a revised version of the Nipah virus R&D roadmap for your review. This version incorporates comments made during the in-person consultation in early March, comments we received after the consultation, and recent literature. We also consolidated all of the cross-cutting issues near the beginning of the roadmap.

We would very much appreciate your written feedback/comments on this version **no later than Wednesday April 25.**

We're sending this to you as a Word doc to make editing and commenting easier. We suggest that you cc the whole group when you provide edits/comments—to ensure that you all see each other's perspectives.

Once we incorporate your additional feedback, the next step will be to post the document for public comment. We also plan to begin working on a set of measurable and achievable milestones and will start considering ways to approach the critical path analysis.

Again, we very much appreciate your ongoing support and involvement with this important project.

Warm regards,

Kris

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NipahResearch and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, underpinning strategic goals, and prioritizing areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus infection.

*(**Note:**This draft roadmap is organized into the three main categories of MCMs—diagnostics, therapeutics, and vaccines—to allow reviewers an opportunity to examine the development of the roadmap components for each countermeasure. Some of the topics and issues apply to more than one MCM; these are highlighted as cross-cutting.This document is relatively detailed to ensure that it includes the most important topics and issues. As this draft is refined, the content may be reorganized and certain items may be "rolled up" into a smaller number of broader topics and issues, strategic goals, and priority areas and activities.)*

INTRODUCTION

Nipah virus (NiV)is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak involving severe respiratory illnessin pigs andencephalitic disease in humans occurred in Malaysia and Singapore in 1998and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs, with an overall case fatality rate of 40%. No new outbreaks have been reported inthese countries since May 1999.NiV infection was subsequently recognized, however,in Bangladeshin 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India; associated case fatality rates in these countries have ranged from 75% to 100%. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the known natural reservoir (Pteropusbatspecies)and several other bat species in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, Ghana, and the Philippines. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and suspected human-to-human NiV transmission. Additionally, several other animal species were found to be infected with NiV on the farms involved in the outbreak, including horses, cats, and dogs. In theoutbreaks in Bangladesh, intermediary hosts between bat and human have not played a major role, with the primary NiV sources being human consumption of bat-contaminated raw date palm sap and subsequent person-to-person transmission.

The zoonotic potential of NiV is significant, particularly because of its ability to amplify in livestock, which can serve as a source of exposure to humans. NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). HeV was initially recognized in 1994, following an outbreak of fatal cases of severe respiratory disease in horses and humans in the Brisbane suburb of Hendra in Queensland, Australia. To date, confirmed HeV disease has been confined to Australia. An outbreak of an unidentified henipavirus (possibly NiV or a closely

related virus) occurred among horses and humans in the Philippines in 2014. This outbreak likely involved spillover of NiV into horses and subsequent disease in humans following consumption of contaminated horsemeat. Detailed genomic information for this virus is limited.

In humans, NiV infection results in neurologic and respiratory syndromes, with fever, headache, dizziness,cough, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and relapsing encephalitis, and survivors may experience long-term neurological sequelae. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B).

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no MCMs exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and effective vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of MCMs to prevent and control NiV outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment (PPE), effective community engagement, adequate infection prevention and control practices, and workforce development and training in endemic and at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, respond, prevent, and control outbreaks of NiV infection(and other closely related henipaviruses) that are readily available and accessible for use in areas of known or potential NiV spillover. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

CROSS-CUTTING TOPICS AND ISSUES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value propositionfor industry and international philanthropic public-private partnershipsare needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs.

- Regulatory approval pathways for MCMs can be prohibitively expensive for product developers. For example, obtaining regulatory approval for diagnostic tests through the premarket approval (PMA) process is costly, but may be necessary when an Emergency Use Authorization (EUA), which is associated with lower approval costs, is not applicable. Furthermore, licensure of vaccines and therapeutics using alternative regulatory pathways also can be very costly, given the regulatory requirements for such approval.
- High-level biocontainment requirements may pose an impediment to research on NiV pathogenesis and development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions.
- To date, NiV spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh and East India; the healthcare facilities that serve these communities ~~have very rudimentary~~ are primary in nature in terms of laboratory and clinical infrastructure for diagnosis and treatment.
- The natural reservoir for NiV is fruit bats of the Pteropus genus; these bats have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover events from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis, disease recognition, and treatment more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.
- Because NiV infection results in low mortality rates in livestock (e.g., approximately 1% to 5% in pigs), infection in animal herds may not be recognized until after human cases are identified. This delay in diagnosis may lead to an entire herd being infected before livestock are tested for NiV, which could cause large financial losses forlivestock owners and increases the likelihood of NiV infection in exposed animal husbandry workers.
- While ferrets, Syrian hamsters, and IFNAR-KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure of MCMs via alternative regulatory pathways. Costs, space requirements (particularly in BSL-4 containment facilities), and ethical concerns constrain the use of AGMs.
- Conducting phase 1 clinical trials, and possibly phase 2 clinical trials,is likely feasible in endemic regions. However, because NiV infection occurs as relatively small, focal outbreaks, the low disease incidence poses a major challenge for conducting phase 3 clinical trials for demonstrating efficacy. Therefore, alternative regulatory pathways and/or innovative study designs (e.g., including combining clinical trial data across outbreaks over time) may need to be considered for licensure of NiV vaccines or therapeutics, if classic clinical trial designs (e.g., randomized controlled trials [RCTs]) are not applicable.

Key needs

- Enhanced clinical, laboratory, and public health infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- Standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains for R&D of MCMs for NiV infection. (Assays that can be used at lower biosafety levels are an important priority.)
- Clear criteria for down-selection and prioritization of candidate MCMs to move forward into clinical trials versus those that need additional preclinical research.
- A determination regardingthe feasibility of conducting clinical trials of therapeutics and vaccines for NiV infection, which is needed before considering alternative regulatory pathways for licensure (such as the United States Food and Drug Administration's [FDA's] Animal Rule).
- Early and recurrent communications between product developers and the appropriate national regulatory authorities (NRAs) to obtain clarity and guidance on clinical trial requirements, regulatory pathways and requirements, and other considerations for NiV MCMs during the pre-licensure and post-licensure periods. Regulatory pathways and NRA capabilities may vary between countries; therefore, early engagement is essential to identify country-specific considerations.
- Outreach and education to clinicians in endemic and at-risk areas to improve NiV awareness and training, and increase the likelihood of accurate and timely diagnosis and treatment of NiV infection.
- Enhanced capabilities for data reporting in resource-limited settings to promote collaborative sharing of NiV sequence and strain data, which can be used to support MCM development.
- Collaboration with ~~local~~ government authorities in endemic ~~and at-risk areas~~countries to support NiV surveillance and facilitate effective communication with communities regarding disease prevention activities. Human health, animal health, and wildlife officials should be engaged as part of a long-term collaborative effort.
- Clarification regarding the potential for and possible strategies to promote technology transfer for NiV MCM development and manufacturingto endemic and at-risk areas.

Commented [Mahmudur1]: It will be necessary to involve the development partners who support the countries

Knowledge gaps

- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new geographic areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats.
- Additional research is needed torefine, standardize, and validate relevant animal challenge models(e.g., ferret, Syrian hamster, IFNAR-KO mouse, and AGM models) to define their role in supporting basic research on the pathogenesis and immunology of NiV infection, which is essential for development and evaluation of MCMs. For example, efforts are needed to: (1)

determine the appropriate animal model(s) for screening assay development; (2) determine the most appropriate lethal NiV dose for MCM development; (3)determine when MCMs should be administered in animal models to best mimic realistic timing of MCM use in humans; (4) bridge NiV MCM data between animal models and humans, such as identifying thresholds of vaccine protection; and (5) identify the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain regulatory approval.

- Additional information is needed on the virology, immunology, and pathogenesis of NiV in humans and animals to inform development of NiV MCMs. This includes evaluating the pathophysiologic differences between NiV_B and NiV_M infection, determining the mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis, identifying factors influencing the development of permanent neurological sequelae, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor viral heterogeneity and antigenic changes over time that may impact the epidemiologic and clinical features of disease, and thereby influence MCM development.
- Some evidence suggests that there are differences in NiV_B and NiV_M pathogenesisthat may have implications for the development of safe and effective NiV vaccines or therapies.More information on this issue is needed.
- Further research is needed to better understand viruses in the Henipavirus genus, including their reservoir hostsand pathogenicity.
- Additional research is needed to develop and assess next generation sequencing (NGS) for whole gene analysis, which can be used to enhance understanding of the global distribution of henipaviruses.
- Sociological and anthropological research is needed to understand how to best engage at-risk populations (including vulnerable populations such as pregnant women, children, and immunocompromised persons) for participation in clinical trials and to ensure acceptance of new NiVMCMs, especially if therapeutics and vaccines do not consistently prevent disease. Efforts are needed to: (1) assess potential barriers for conducting clinical trials; (2) assess MCM acceptability in at-risk populations; (3) determine culturally appropriate messaging to enhance MCM acceptance; and (4) identify public health strategies to promote vaccine use.

Strategic Goals

1. Identify sources of funding and develop appropriate private-sector incentives and competitions to promote R&D of NiV MCMs.
2. Stimulate and support basic science research for better understanding of NiV virology, pathogenesis, and the immune response to infection in humans and animals.
3. Strategically strengthen laboratory, clinical, and public health infrastructure and capacity at the local and national levels in areas of known or potential NiV spillover.

4. Engage NRAs (particularly in endemic and at-risk areas) to gain guidance on requirements for clinical trials, regulatory pathways, and other considerations that will impact MCM development, acceptance, and post-licensure surveillance.

~~4.~~5.

Commented [Mahmudur2]: Consider adding "Enhance disease detection capabilities through active and passive surveillance"

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Expand** research to further understand the ecology and epidemiology of NiV and other pathogenic henipaviruses in human and animal populations (wild and domestic) over time and across geographic areas, using a One Health approach.
- **Continue to perform**phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Conduct** basic science research on the virology, pathogenesis, and immunology of NiV infections to inform development of MCMs.
- **Determine** the key differences between NiV_{B} and NiV_{M} pathogenesisthat may have implications for the development of safe and effective NiV vaccines or therapies.
- **Refine, validate, and standardize** relevant animal models to support the development and evaluation of NiV MCMs.
- **Generate** research tools to promote R&D of MCMs for NiV infection (i.e., standardized and validated assays, reagents, antibodies, nucleic acids, and stocks of NiV challenge strains), particularly those that can be used at lower biosafety levels.
- **Conduct** research on the uses of NGS for understanding henipavirus distribution.
- **Determine** the feasibility of conducting phase 3 clinical trials or identify alternative approaches for assessing efficacy of new NiV vaccines and therapeutics, in coordination with the appropriate NRAs.
- **Establish** a plan for conducting clinical trials in endemic regions in coordination with local government agencies, if clinical trials are considered to be a feasible option for efficacy assessment.
- **Conduct** social science research to determine strategies for engaging communities for participation in clinical trials and to support acceptance of MCMs for NiV infection as they become available.

Product development

- **Define** criteria for down-selection and prioritization of candidate MCMs that should be moved forward.
- **Promote**early communication between developers and appropriate NRAs forclarity and guidance on the regulatory aspects ofMCM development for NiV infection.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure in endemic and at-risk areas to promote early diagnosis, treatment, and implementation of vaccination programs for NiV prevention and control.
- **Improve** surveillance capacityto: (1) better define the incidence of disease in NiV-endemic and at-risk areas and (2) promote targeted research in non-endemic areas to identify evidence of spillover of NiV or other related henipaviruses from the natural reservoir to human or animal populations.
- **Develop** a shared data platform to facilitate sharing of NiV sequence and strain data.
- **Collaborate** with local government authorities (including human health, animal health, and wildlife representatives) to support NiVsurveillance and disease prevention activities in endemic and at-risk areas.
- **Promote** community-based outreach programs that transfer skills and knowledge for the prevention and early recognition of NiV disease in areas of known or potential NiV spillover risk.
- **Strengthen** infrastructure and capacity for post-marketing pharmacovigilance of licensed NiV therapeutics and vaccines.

Policy and commercialization

- **Establish** a sustainable value proposition and **secure** funding to complete development, licensure, manufacture, deployment, and use of affordable MCMs for NiV infection.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostics, therapeutics, and vaccines to endemic and at-risk areas.
- **Ensure** access to regulatory guidance, oversight, review, and authorizationfrom appropriate NRAs for NiVMCMs.This should be done when clinical trials and approaches for regulatory approval are being determined.
- **Clarify**the potential for and possible strategies to promote technology transfer for development and manufacturing of MCMs for NiV infection.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection arenonspecific and the diagnosis often is not suspected at the time of presentation. This can hinder accurate diagnosis and creates challenges in instituting effective and timely infection control measures. Additionally, latent disease can occur long after initial infection.

- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Clinical sample quality, quantity, type, timing of collection, and the time necessary to transfer the sample from the patient to the laboratory can affect the accuracy of laboratory results.
- Various types of test methods and platforms are required to test patients at different phases of NiV infection, which can complicate diagnostic needs and capabilities.
- Owing to the biosafety precautions necessary when working with the NiV virus, diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- The time required to perform diagnostic testing using PCR and ELISA methods poses challenges, given the rapid disease progression of NiV infection.
- Pteropus bat species (and perhaps other bat species)appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge forensuring diagnostic preparednessto respond tofuture outbreaks.

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- A biobankof human and animal clinical samples to assess and validate diagnostic tests and a process for how best to judiciously use the samples. A clear approach is needed to: (1) determine what clinical samples should be collected, based on what would be most useful (e.g., plasma, whole blood, urine, cerebrospinal fluid, etc.); (2) outline the purposes of sample collection; (3) identify who would have access to the samples; and (4) prioritize use of samples and sample distribution.
- Clarification regarding the use cases for different diagnostic assays and what viruses are targeted (i.e., NiV, NiV and HeV, or all henipaviruses), since the corresponding performance, validation, and regulatory approval requirements may differ depending on how and in which population (i.e., human or animal) the test will be used. For example, it may be desirable to have a point-of-care screening test that is highly sensitive and a confirmatory test that is highly specific. In animals, it may be desirable to have a diagnostic test with high sensitivity to screen reservoir populations and a highly specific test for livestock. Diagnostic use cases need to be considered in tandem with the use of therapeutics and other interventions.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course,are robust for use under a variety of conditions (e.g., varying humidity, temperature, etc.), can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Optimal deployment strategies for diagnostics in different geographic areas based on the risk and epidemiology of NiV infection.
- International reference standards to calibrate diagnostic assays.
- Validation of promising diagnostics in endemic and at-risk geographic regions.

- Diagnostic preparedness to detect NiV, HeV, and other emergent henipaviruses in humans and animals as they arise.
- In-country laboratories able to conduct proficiency testing to monitor reproducibility and performance of NiV diagnostic assays in the field.
- A sufficient number of laboratories committed to using the diagnostics on a regular basis to support the business case for Nipah diagnostics, given the costs of regulatory approval.
- If feasible, multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern that may cause similar clinical syndromes in endemic or at-risk areas. Since validation and regulatory approval of multiplex assays can prove challenging, an alternate approach would be the development of multiple single assays that can be run in parallel.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the Differentiating Infected from Vaccinated Animals (DIVA) test.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids (e.g., urine and respiratory secretions), and tissue samples to enhance the ability to diagnose infection at different stages of disease. Additionally, further research on the kinetics of NiV in the animal reservoirs is needed.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, limits of detection, cross-reactivity, and quantitative vs. qualitative data) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs) and tests that are designed to detect more than one henipavirus. Further testing of diagnostics should be conducted in animal models before field trials in humans are pursued.
- A clear understanding is needed of the potential for cross-reactivity of diagnostic tests in animal populations to allow accurate interpretation of test results, since substantive economic consequences (such as trade restriction for livestock) could be triggered by positive results.

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detectionat various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Develop and assess affordable, highly sensitive and specific, point-of-care NiVdiagnostic tests for use in humans and animalsthat are sufficiently robust for the conditions in which they will be used and that have minimal requirements for biosafety precautions and staff training. Consideration also should be given to development of multiplex assays that can detect related henipaviruses, in addition to NiV, or that can detect other pathogens of concern in endemic and at-risk areas.
3. Generate guidance on deployment strategies and use of diagnostic tests for NiV detection in areas of known or potential henipavirus spillover risk.

4. Enhance diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose NiVinfectionat different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and **develop** appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Create** a biobank of clinical human and animal samples for use in researching new diagnostic agents.
- **Continue to research** cross-reactivity of diagnostic tests in animal populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Define** use cases for diagnostic assays.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus and be performed accurately and safely in remote areas under a variety of circumstances.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other pathogens of concern in the geographic region that cause similar clinical syndromes.
- **Develop** diagnostics applicable to mass testing in livestock to identify NiV infection early and to reduce the likelihood of transmission of NiV from livestock to humans.
- **Develop** serologic testing to differentiate vaccinated from infected animals (such as a DIVA test), if NiV or HeV vaccines become widely used (long-term consideration).

Key capacities

- **Generate** international reference standards to calibrate diagnostic assays.
- **Support** in-country laboratories in monitoring performance of NiV diagnostics in the field.
- **Enhance** diagnostic preparedness in areas of known or potential henipavirus spillover risk to promote early detection of NiV, HeV, and other emergent henipaviruses in humans and animals.

Policy and commercialization

- **Develop** guidance on optimal strategies for deployment and use of new NiV diagnostic tests across different geographic areas, as such tests become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Patients typically present late in the clinical course of disease, which decreases the likelihood of successful treatment.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons.
- In the NiV-endemic region of Bangladesh, hundreds of patients are admitted to hospitals annually with a diagnosis of encephalitis, but do not have NiV infection. In the absence of confirmatory testing, treating all patients with encephalitis and their contacts for NiV infection would be costly and labor intensive, with relatively little benefit; therefore accurate and rapid diagnosis is critical.
- Studies in animals often evaluate usefulness of therapeutics when delivered prior to disease onset or early during the disease course. Patients with NiV infection often are detected later in the clinical course, which creates challenges for predicting how well an agent will work in the field.
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier to inhibit viral replication and prevent severe neurologic disease.
- Healthcare systems in endemic countries often do not have adequate infection control systems in place to prevent person-to-person transmission. They also lack the ability to rapidly identify contacts most likely to benefit from PEP therapy.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat acute NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat chronic (relapsing) NiV infection to decrease associated long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock.

- Improved patient care in endemic areas (such as the ability to provide ventilator support for seriously ill patients).

Knowledge gaps

- Ribavirin may be an option for treatment of NiV infection, but animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protectionagainst lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to either HeV or NiV. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials using both NiV$_{M}$ and NiV$_{B}$are needed to assess the safety, tolerability, and efficacyof m102.4 (and possibly other mAbs) for PEPand potentially early treatment of clinical disease.
- Additional research is needed regarding the likelihood of escape mutants with mAb use. While evidence of escape mutants has not been found to date with mAb 102.4, it may be necessary to consider mAb cocktails.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the mostpromising novel treatments (such as fusion inhibitory peptides, antifusion peptides, and GS-4734 [a broad-spectrum agent being used to treat Ebola virus disease survivors]), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV$_{B}$ and NiV$_{M}$, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV$_{B}$ to be shorter than for NiV$_{M}$.
- Further research is needed to broaden the number of novel antiviral candidates for treatment of NiV infection.
- Additional data are needed to establish the pharmacokinetic/pharmacodynamics (PK/PD) relationship of promising therapeutic candidates.
- Additional studies of therapeutic candidates in the AGM model, followed by human clinical trials for safety, feasibility, and efficacy.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the types of exposures that warrant such intervention and the most appropriate agents to administer.This determination should include feasibility for PEP distribution in both endemic and at-risk areas, including Bangladesh, which has hundreds of potentially-exposed persons annually that could be candidates for PEP.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of components of supportive care for NiV, such as optimal fluid and respiration management strategies,diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform best-practice guidelines.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection, and ensure that therapies are readily available, affordable, and accessible in areas of known or potential NiV spillover.
2. Develop guidance for the use of therapeutics for disease treatment and PEP as new therapies become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies (such as ribavirin and m102.4) for treating and preventing NiV infection, including conduct of animal studies andclinical trialsas appropriate and feasible.
- **Continue to identify** new therapeutic options for treating and preventing NiV infection that should undergo further evaluation.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best-practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infectionthat are active against different NiV strains and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both $\mathrm{NiV_B}$ and $\mathrm{NiV_M}$and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Promote** enhancements to the healthcare delivery systems in impacted areas to improve clinical management and supportive care of patients with NiV infection.
- **Ensure** that mechanisms are in place to finance, generate, and maintain stockpiles of NiV therapeutics for outbreak control.

Policy and commercialization

- **Identify** a company to advance therapeutic use of m102.4 and **secure** financing for its manufacture and distribution.
- **Develop**guidance for the use of therapeutics for disease treatment and PEP, as new therapies become available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Sociocultural issues may hinder trust in the formal human, veterinary, and public health systems, which could impact acceptance of NiV vaccinesfor use in humans or animals.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control.

Key needs

- Nipah vaccines that can protect against both NiV_M and NiV_Bin humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks.
- Guidance on use of NiVvaccines (or broader henipavirus vaccines)to include vaccination strategies, potentially in both humans and animals, for different epidemiologic scenarios and different vaccine attributes.
- Once vaccines are available, enhanced surveillance capacity to assess the impact of vaccination programs and to refine vaccination strategiesover time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- Further research is needed to clarify vaccine attributes (such as time from administration to immune protection, duration of immunity, and the need for booster doses) and to determine safety profiles of candidate vaccines, such as the soluble G glycoprotein subunit (sG) and recombinant vesicular stomatitis (rVSV) vaccine.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoetis).
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B, including recent strains; only a few studies demonstrating cross-protection have been performed to date.
- The identification of specific correlates of protection in humans and animals and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Evaluation of vaccine safety in target populations is needed to better understand the risk of adverse incidents associated with vaccine use.
- If evidence at some point supports the need for a broader, population-based vaccination strategy (beyond reactive use for outbreak control in affected communities), additional research may be warranted on the development of multivalent vaccines that protect against more than

one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus [MV]) for use in NiV endemic areas.

- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines, estimating the vaccine quantity for stockpiles, and in simulating various epidemiologic scenarios and possiblestrategies for vaccine use.
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. While one HeV vaccine is available for horses, antibodies against HeV have not been found to be cross-reactive with NiV. Ongoing research into developing NiV/HeV vaccines for livestock(or other animals) and the potential for their use in endemic regions is needed to further assess the merit of this potential control strategy.
- Additional research is needed to determine if development of multivalent vaccines for animals(that protect against more than one disease) would increase the likelihood of vaccine uptake by food animal producers and the broader veterinary community.

Strategic Goals

1. Develop, evaluate, license, and deployNiV vaccines for use in humans and potentially animals (e.g., livestock, companion animals).
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Develop and refine guidance on vaccine use in humans and animals that aligns with current NiV epidemiology and takes into consideration attributes of new vaccines as they become available.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection for use in developing and evaluatingNiV vaccines.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains,and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research into candidate vaccines and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Complete** preclinical evaluation of promising candidate NiV vaccines for safety, immunogenicity, efficacy in animal models, correlates of protection, and durability.
- **Determine** whether multivalent vaccines for animal populations would increase vaccine acceptability and uptake by food-animal producers and the broader veterinary community.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effectivemonovalent NiV vaccines for humans and animals.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines for use in humans that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease for use in animal populations, if this is deemed to be a worthwhile approach.

Key capacities

- **Improve**surveillance capabilities,particularly in rural areas of resource-limited settings, to assess the impact of vaccination strategies, once vaccines become available.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.

Policy and commercialization

- **Provide**guidance on vaccination strategies for various target populations and epidemiologic scenarios that align with vaccine attributes, once vaccines are available.
- **Consider** development of a strategy for vaccine surge capacity to rapidly ramp up the vaccine supply, if NiV is used as a bioterrorism agent, or if an NiV strain emerges with increased capacity for person-to-person transmission, and thus more rapid spread.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis. Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoSNegl Trop Dis2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146):146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6):doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95[Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoSNegl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al.Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295.[Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE.A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B.Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE.Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Weatherman S, Feldman H, de Wit E. Transmission of henipaviruses. CurrOpinVirol 2018 Feb;28:7-11. doi: 10.1016/j.coviro.2017.09.004 [Full text]

From:	Mahmudur Rahman on behalf of Mahmudur Rahman <mahmudur57@gmail.com>
To:	Kris Moore
Cc:	christopher.broder@usuhs.edu; chuakb@tll.org.sg; Emily Gurley; Steve Luby; gnmillig@utmb.edu; pyr3@cdc.gov; Linfa Wang; K.Cook@wellcome.ac.uk; benassiv@who.int; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; Josie Golding; PREZIOSI, Marie-pierre; Michael Osterholm
Subject:	Re: Revised Nipah virus R&D roadmap for taskforce review and comment--please send written comments by April 25
Date:	Wednesday, April 25, 2018 11:17:33 AM
Attachments:	Nipah Draft Roadmap.revised for taskforce review.April 2018 Comments from Prof. Mahmudur Rahman.docx

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Dear Kris,

Greetings from Bangladesh !!!

Hope you all are doing fine.

Please find attached herewith the "Nipah Research and Development (R&D) Roadmap" with my comments in track change mode.

Best regards,

Mahmud

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On Sat, Apr 14, 2018 at 4:19 AM, Kris Moore <kamoore@umn.edu> wrote:

> Dear Nipah Virus R&D Roadmap Taskforce members:
>
> Attached please find a revised version of the Nipah virus R&D roadmap for your review. This version incorporates comments made during the in-person consultation in early March, comments we received after the consultation, and recent literature. We also consolidated all of the cross-cutting issues near the beginning of the roadmap.
>
> We would very much appreciate your written feedback/comments on this version **no later than Wednesday April 25.**
>
> We're sending this to you as a Word doc to make editing and commenting easier. We suggest that you cc the whole group when you provide edits/comments—to ensure that you all see each other's perspectives.
>
> Once we incorporate your additional feedback, the next step will be to post the document for

public comment. We also plan to begin working on a set of measurable and achievable milestones and will start considering ways to approach the critical path analysis.

Again, we very much appreciate your ongoing support and involvement with this important project.

Warm regards,

Kris

Kristine Moore, MD, MPH

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NIPAH R&D ROADMAP DEVELOPMENT TASKFORCE

Call Date: January 9, 2018

Taskforce Members on the Call: Christopher Broder, Kaw Bing Chua, Stephen Luby, Gregg Milligan, Pierre Rollin, and Mahmudur Rahman

Taskforce Members Unable to be on the Call: Emily Gurley and Lin-Fa Wang

R&D Roadmap Technical Team: Michael Osterholm, Kristine Moore, Julie Ostrowsky, Becky Johnson, and Anje Mehr (CIDRAP)

CONFERENCE CALL SUMMARY

Topic	Key Discussion Points
R&D roadmap architecture	• The WHO generic methodology for developing R&D roadmaps for priority pathogens (draft dated Aug 30, 2017) provides the basis for developing R&D roadmaps, as part of the global R&D Blueprint initiative. • Specific resources for guiding R&D roadmap development include the 2013 Malaria vaccine technology roadmap, the 2016 MERS-CoV roadmap report in Nature Medicine, and the 2016 MERS-CoV summary roadmap. • The Nipah R&D roadmap will provide a framework for identifying the vision, underpinning strategic goals, and prioritizing research areas and activities for accelerating the collaborative development of diagnostics, therapeutic agents, and vaccines against Nipah virus (NiV). The scope of R&D for medical countermeasures (MCMs), as put forward in the WHO roadmap generic methodology, includes basic research through to late-stage development, licensure, and early use of products.
Role of taskforce (TF) members	• TF members will be actively involved in reviewing draft roadmap documents and providing feedback; participating in the March 1-2, 2018 consultation at the Wellcome Trust offices in London; and assisting with ongoing review, development, and dissemination of the Nipah R&D roadmap over the next 6 months.
Roadmap development and timeline	• The draft Nipah R&D roadmap will provide the basis for discussion at the consultation in March. • The final roadmap product may include a summarized outline format as well as a text version similar to the MERS-CoV roadmap report in Nature Medicine. • The CIDRAP technical team will distribute a revised version of the draft roadmap in mid-February 2018, prior to the consultation, along with relevant background information. • Following the consultation, the team will further revise the draft roadmap based on information obtained during the consultation and will seek additional feedback from TF members and others before posting a new draft online for public comment. After further review from WHO and other key stakeholders, the final roadmap document will be available for publication by the end of August 2018.
Feedback on draft roadmap document: *General*	• Taskforce members generally agreed with the content in the introductory section, but indicated that the section could be improved by incorporating additional critical Nipah considerations, such as a description of the virus, the zoonotic potential, and the geographic location in which NiV and Hendra virus (HeV) are found. In addition to human health implications, NiV has the potential to impact trade and travel. • Consider subcategorizing Primary Challenges to group topical elements (e.g., clinical aspects, laboratory aspects, financial constraints).
Feedback on draft roadmap document: *Cross Cutting*	• Laboratory biosafety level (BSL) is primarily an issue when working with animal models and/or neutralization assays, which require the use of a BSL4 facility. Much of the NiV work is done at a lower level (e.g., serological tests are conducted in BSL2 conditions in Bangladesh).

	• NiV and HeV have multiple appropriate animal models for human disease. African green monkeys (AGMs) best replicate human disease, and hamsters and ferrets also serve as good animal models. The current animal models may provide sufficient data for the US Food and Drug Administration (FDA) Animal Rule regulatory pathway for approval for diagnostics, therapeutics, and vaccines. However, the US FDA may require additional data on correlates of protection in the animal models as compared to humans when evaluating MCMs under the US FDA Animal Rule. • While animal models for NiV are well-developed, taskforce members expressed concern that they might not be deemed sufficient for evaluation under the US FDA Animal Rule.
Feedback on draft roadmap document: *Diagnostics*	*Primary challenges:* • Strain variability (i.e., NiV$_M$ and NiV$_B$) has not been recognized to cause problems for diagnostics or MCMs. In fact, HeV reagents have been effectively used for NiV. Strain variability should not be emphasized in the Diagnostics or Therapeutics section. *Key needs:* • Point-of-care rapid diagnostic tests (RDTs) applicable under a variety of circumstances are necessary for NiV surveillance and to ensure appropriate therapeutic distribution/use. Different use cases may be necessary, given the variety of circumstances under which NiV diagnostics may be needed. • Improved infrastructure is needed in relation to RDTs to ensure that health professionals responsible for diagnostic testing know that RDTs are available and how to use them accurately and safely. • To increase the likelihood of RDT use, consider combining NiV detection with another more-common disease for the geographic region (e.g., the strategy of developing a combined RDT for malaria and NiV was discussed). *Priority Areas/Activities:* • The ideal sample(s) are those that easy to collect. In the field, saliva and respiratory secretions have been used for diagnostic work. Urine may also be a suitable sample type for diagnostics. Research is needed to better understand the most practical sample type(s) for different situations. *Other:* • The group discussed clinical manifestations of NiV and implications for the sample type that should be collected for making the diagnosis. Of note, unpublished data show low rates of asymptomatic infection, with NiV spread generally occurring via saliva or respiratory secretions. To better direct development of diagnostics, understanding viral load in different sample types (e.g., saliva, respiratory secretions, urine) would be beneficial.
Feedback on draft roadmap document: *Therapeutics*	*Primary challenges:* • Patients typically present late in the clinical course, which decreases the likelihood of successful treatment. • BSL4 laboratories are required for high-throughput screening. *Other:* • One team has explored the effectiveness of therapeutics at point-of-fever in an animal model. • The human monoclonal antibody m102.4 has shown protection in nonhuman primates (NHPs), has been used effectively as a compassionate use therapeutic for 14 humans, and recently underwent a Phase 1 study involving 40 adults. Taskforce members discussed sharing the safety data from the Phase 1 study, which has not yet been published.
Feedback on draft roadmap document: *Vaccines*	*Primary challenges:* • Due to the fact that NiV generally occurs as small, focal outbreaks, conducting Phase 3 studies are probably unrealistic. However, Phase 1 studies are very plausible, and Phase 2 studies may be possible in endemic regions. *Key needs:* • To conduct clinical trials in the endemic regions at risk for NiV infection, additional information is needed regarding what the government (e.g., Ministry of Health)

	requirements for conducting such studies would be. Additionally, a strategy is needed to address vaccine acceptability in the at-risk population. *Other:* • Anti-vaccine sentiments in the general public are currently an issue for HeV vaccine use in horses, and are expected to be a hurdle for NiV vaccine acceptability as well. Incorporating effective communication strategies for communities that would benefit from an NiV vaccine will be paramount to vaccine success.
Other comments	• The CIDRAP Team requested that taskforce members provide written comments on the draft roadmap document by January 19, if possible. • CIDRAP staff may follow-up with certain individual members of the taskforce, as necessary.

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, outlining the underpinning strategic goals, and prioritizing research areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus (NiV) infection.

*(**Note:** The content below is organized into the three main categories for R&D of MCMs [diagnostics, therapeutics, and vaccines] to allow reviewers an opportunity to follow the lines of thinking for each category. Some issues are cross cutting and apply to more than one category. As this draft is refined, the content may be reorganized to create one over all roadmap and to group cross-cutting issues together. In addition, this document is relatively detailed to ensure that the most important issues are identified and discussed. As the document evolves, certain items may be "rolled up" into a smaller number of broader issues, strategic goals, and primary activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak occurred in pigs and humans in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the natural reservoir for NiV (Pteropus bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, and Ghana. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and human-to-human NiV transmission. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a role, with human consumption of bat-contaminated raw date palm sap serving as the primary NiV source. In humans, NiV infection has neurological and respiratory syndromes, with fever, headache, dizziness, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and/or relapsing encephalitis, and survivors may experience long-term neurological deficits. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B). NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). To date, HeV disease has been confined to Australia. An outbreak of an unidentified Henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2015.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and

timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's initial list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no medical countermeasures [MCMs] exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of medical countermeasures to prevent and control LF outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment, effective community engagement, and workforce development and training in at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV (and other closely related henipaviruses) that are available and readily deployable for use when needed. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. Additionally, latent disease can occur long after initial infection. Both of these factors can hinder accurate diagnosis.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- NiV appears to have the potential for strain heterogeneity and antigenic variability, which could potentially influence accuracy of diagnostic testing over time.
- The natural reservoir for NiV is fruit bats of the Pteropus genus, which have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis

more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.

- Pteropus bat species also appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- High-level biocontainment requirements may pose an impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Diagnostic preparedness to detect HeV and other emergent henipaviruses in humans and animals as they arise.
- Proficiency testing, to monitor reproducibility and performance of NiV diagnostic assays in the field.
- Enhanced laboratory infrastructure in at-risk areas, with a particular focus on improving regional and national laboratory capacity for case and outbreak confirmation.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the DIVA test.
- Long-term need: multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or additional high-consequence pathogens.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, and limits of detection) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs).
- Additional validation data are needed to assess performance characteristics of NiV diagnostic assays against the various strains of NiV.

- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor antigenic changes over time that may impact diagnostic testing and the epidemiologic and clinical features of disease.
- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats. *(*cross cutting)*

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Promote the development and assessment of affordable, highly sensitive and specific, point-of-care diagnostic tests for use in humans and animals that have minimal requirements for biosafety precautions and staff training and that can detect NiV and other related henipaviruses.
3. Strengthen laboratory infrastructure and capacity in the endemic and at-risk areas to improve early diagnostic capabilities.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and develop appropriate standards for their use.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Expand research** to further understand the ecologic and epidemiology of NiV and other henipaviruses in human and animal populations over time, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to research** development of serologic tests to distinguish vaccinated from infected animals, if henipavirus vaccines are to be widely used in livestock populations.

Product development

- **Generate** a TPP for NiV diagnostics.

- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other high-consequence pathogens in humans and animals.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure for detection of NiV in endemic and at-risk areas, particularly in support of enhanced national and regional diagnostic capabilities.
- **Improve surveillance capacity** to: (1) better define the incidence of disease in NiV-endemic areas and (2) promote surveillance in non-endemic areas to enhance detection of emergent NiV disease or disease caused by other related henipaviruses.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV point-of-care diagnostics.
- **Ensure** access to regulatory review and authorization of NiV diagnostic assays.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostic products to endemic and at risk areas.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV disease occurs in relatively small outbreak settings, the low incidence of disease poses a major challenge for conducting phase 3 clinical trials; therefore, alternative regulatory pathways will need to be considered for licensure of vaccines or therapeutics. *(*cross cutting)*
- Differences between the NiV_M and NiV_B strains may impact the effectiveness of developed MCMs, yet NiV_M has been used significantly more often than NiV_B in research. *(*cross cutting)*
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier and can access the brain and spinal cord to inhibit viral replication. This is particularly important for preventing late-onset CNS disease.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons. In addition, the healthcare systems in endemic areas are not able to rapidly identify contacts most likely to benefit from PEP. *(*cross cutting)*

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat both acute and chronic (relapsing) NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock. *(*cross cutting)*

Knowledge gaps

- Additional information is needed on the immunology and pathogenesis of NiV in order to develop appropriate treatment options. This includes evaluating the pathophysiologic differences between NiV_B and NiV_M infection, determining the mechanisms of late-onset and relapsing NiV encephalitis, identifying factors influencing the development of permanent neurological sequela, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ribavirin was found to reduce mortality by 36% in the 1998-1999 NiV outbreak in Malaysia and is considered an option for treatment of NiV infection. However, animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to HeV-infected horses. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional

animal studies and clinical trials are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV_B and NiV_M, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV_B to be shorter than for NiV_M.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the type of exposures that warrant such intervention and the most appropriate agents to administer.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of individual components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform supportive care and best-practice guidelines.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), such as determining the most appropriate lethal NiV dose for MCM development and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection and ensure that therapies are prepositioned in endemic areas for use as needed.
2. Stimulate basic research for better understanding of NiV pathogenesis and the immune response to infection.
3. Strengthen clinical and laboratory infrastructure in endemic and at-risk areas to promote early diagnosis of NiV cases so that effective treatment and PEP can be rapidly administered.
4. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Conduct** basic science research on the immunology and pathogenesis of NiV infections to inform development of new therapeutic agents.
- **Determine** the key differences between NiV_B and NiV_M that may have implications for the development of safe and effective NiV therapeutics.

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies for NiV infection, including animal studies and clinical trials as appropriate and feasible.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against both NiV_B and NiV_M and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Refine, standardize, and validate** animal challenge models for future assessment of promising therapeutic candidates.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV therapeutics.
- **Support** plans for licensure/registration, adequate manufacturing, and subsequent distribution of safe and effective therapies to endemic areas and to other at-risk areas on an as-needed basis for outbreak control.
- **Secure** financing for procurement and deployment of NiV therapeutics for outbreak control once treatment agents are available.
- **Develop** treatment and PEP guidance as new therapies demonstrate safety and efficacy.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV occurs as relatively small, focal outbreaks, the low incidence of infection poses a major challenge for conducting phase 3 clinical trials for efficacy demonstration; therefore, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics. *(*cross cutting)*

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- The absence of improved diagnostic assays for timely diagnosis of infection creates a challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control, once vaccines are available for use. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, African green monkeys (AGMs) are regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*

Key needs

- Safe and effective PEP (either vaccines or therapeutic agents) to prevent infection following exposure to NiV and guidance on their use. Such countermeasures are important tools to protect healthcare workers, family caregivers, and livestock workers. *(*cross cutting)*
- Nipah vaccines that can protect against both NiV_M and NiV_B in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks. Research tools, such as standardized neutralization assays, to compare immunogenicity of different vaccines.
- Enhanced surveillance capacity to provide more accurate information on the current incidence and geographic distribution of NiV in endemic and at-risk areas in order to refine potential vaccination strategies, particularly over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- The mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis are unknown. Further research to better understand viral clearance has important implications for development of vaccines and therapeutics.
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B; only a few studies demonstrating cross-protection have been performed to date.
- Further research is needed to determine durability and safety profiles of candidate vaccines, such as VSV recombinants, where concerns regarding potential neurotropism have been raised.

- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoestis).
- The identification of specific correlates of protection and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines and in simulating various epidemiologic scenarios that may impact strategies for vaccine use.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), including determining the most appropriate NiV dose for MCM development (Johnston 2015) and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses. Ongoing research into developing NiV/HeV vaccines for livestock is needed to further assess the merit of this potential control strategy.
- If evidence at some point supports the need for a broader, population-based vaccination strategy, additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.

Strategic Goals

1. Develop, evaluate, and license NiV vaccines for use in humans and potentially livestock, primarily for reactive/outbreak use in endemic and at-risk areas.
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection.
- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.

- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research efforts and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Generate** research tools (e.g., standardized neutralization assays) to compare immunogenicity of different vaccines.
- **Complete preclinical evaluation** of promising candidate NiV vaccines for safety, immunogenicity, efficacy, correlates of protection, and durability.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and livestock, primarily for reactive use during outbreaks.

- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.

Key capacities

- **Refine and validate** relevant animal challenge models for NiV infection.
- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the true burden and incidence of NiV disease, which may in turn, inform vaccination strategies.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV vaccines.
- **Ensure** access to regulatory review and authorization of NiV vaccines, particularly if non-traditional pathways are needed for licensure.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.
- **Provide** ongoing assessment to inform development and maintenance of NiV vaccine stockpiles over time; mathematical modelling may be used to contribute to this assessment.
- **Continue** to provide guidance on vaccination strategies for various target populations and epidemiologic scenarios, once vaccines are available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis, Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

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Subject:	Call notes from the Nipah Roadmap Taskforce Conference Call which was held on Tuesday, January 9
Date:	Thursday, January 18, 2018 8:48:13 PM
Attachments:	Nipah Taskforce Conference Call Summary 20180109.final.doc Nipah Draft Roadmap.V5.docx
Importance:	High

Dear Nipah roadmap taskforce members:

First, I want to thank all of you who were able to participate in our conference call on Tuesday January 9. The discussion was very helpful and we very much appreciate you input.

Second, please send us any written edits or comments on the draft document as soon as possible, if you have a moment. I have attached the draft again here. We plan to revise the draft and send it on to WHO for review soon, in order to meet our current timelines. Any additional written comments you may have would be most appreciated.

Finally, I have attached meeting notes from the conference call for your records.

Again, thank you for all of your support in this important effort. We will be in contact again soon regarding next steps in preparing for the in-person March meeting in London.

Warm regards,

Mike

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DRAFT V5 —NOT FOR DISTRIBUTION— January 1, 2018

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, outlining the underpinning strategic goals, and prioritizing research areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus (NiV) infection.

*(**Note:** The content below is organized into the three main categories for R&D of MCMs [diagnostics, therapeutics, and vaccines] to allow reviewers an opportunity to follow the lines of thinking for each category. Some issues are cross cutting and apply to more than one category. As this draft is refined, the content may be reorganized to create one over all roadmap and to group cross-cutting issues together. In addition, this document is relatively detailed to ensure that the most important issues are identified and discussed. As the document evolves, certain items may be "rolled up" into a smaller number of broader issues, strategic goals, and primary activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak occurred in pigs and humans in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the natural reservoir for NiV (Pteropus bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, and Ghana. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and human-to-human NiV transmission. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a role, with human consumption of bat-contaminated raw date palm sap serving as the primary NiV source. In humans, NiV infection has neurological and respiratory syndromes, with fever, headache, dizziness, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and/or relapsing encephalitis, and survivors may experience long-term neurological deficits. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B). NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). To date, HeV disease has been confined to Australia. An outbreak of an unidentified Henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2015.

Commented [pyr31]: Mostly Bangladesh, not seen in Malaysia. Not occurring in healthcare facilities, only at home, certainly due to lack of basic precaution

Commented [EG2]: There were a couple of suspect cases of this in Malaysia, but this has been a prominent feature of the outbreaks in India and Bangladesh.

Commented [EG3]: Cough has been more common than vomiting in the Bangladesh case series.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and

timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's initial list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no medical countermeasures [MCMs] exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of medical countermeasures to prevent and control LF outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment, effective community engagement, and workforce development and training in at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

Commented [pyr34]: Basic hand washing!

Commented [SPL5]: I consider this paragraph as essential framing and urge that it be retained, even as we work towards a more focused document. It is difficult, because medical countermeasures do not stand alone. They need to interface with a system that constructively deploys them. At a minimum we should strive for countermeasures that are fit for purpose for the contexts where they are needed.

Commented [EG6R5]: Complete agree with Steve on this.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV (and other closely related henipaviruses) that are available and readily deployable for use when needed. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. Additionally, latent disease can occur long after initial infection. Both of these factors can hinder accurate diagnosis.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- NiV appears to have the potential for strain heterogeneity and antigenic variability, which could potentially influence accuracy of diagnostic testing over time.

Commented [pyr37]: Rapid test, lateral flow. Better if multivalent. A single Nipah test is unlikely to be widely used

Commented [EG8]: While this is true, even when testing is nearby, it can still take days to get back a PCR or ELISA result as these are not rapid tests as current used. Within the context of very quick progression to death, we need a bedside rapid diagnostic.

Commented [pyr39]: So far, virus quite stable, no impact on diagnostic tests. Need to be vigilant but "so far so good"

- The natural reservoir for NiV is fruit bats of the Pteropus genus, which have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis

more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.

- Pteropus bat species also appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Commented [pyr310]: What going on in Malaysia? No data since the outbreak in 1999

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- High-level biocontainment requirements may pose an impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*

Commented [pyr311]: Not really a problem for diagnostic, but it is one for pathogenesis and vaccine studies

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.

Commented [pyr312]: Nipah test would be more likely used if combined with something else. In addition to increase the "market, wuld be good if it is Nipah/hendra

- Diagnostic preparedness to detect HeV and other emergent henipaviruses in humans and animals as they arise.
- Proficiency testing, to monitor reproducibility and performance of NiV diagnostic assays in the field.
- Enhanced laboratory infrastructure in at-risk areas, with a particular focus on improving regional and national laboratory capacity for case and outbreak confirmation.

Commented [SPL13]: Given the wide range of Pteropus bats this needs to be defined. Clearly, we need low cost accurate diagnostics, but we also need to consider the system that needs to be in place in order to make use of the diagnostics. We risk mission creep if we include development of global surveillance as part of medical countermeasures, but there is a difference between how we would optimally deploy point of care diagnostics in Bangladesh and West Bengal, India, compared with the Philipines or Cameroon. We should consider the different needs in these different contexts, to assure that we have an approach that is fit for purpose.

Commented [EG14R13]: Agree

- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the DIVA test.
- Long-term need: multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or additional high-consequence pathogens.

Commented [EG15]: Or, other pathogens on a differential diagnosis for humans?

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.

Commented [SPL16]: Salivary pcr is often positive in Bangladesh before antibodies are detectable.

- More information is needed regarding the performance characteristics (including sensitivity, specificity, and limits of detection) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs).
- Additional validation data are needed to assess performance characteristics of NiV diagnostic assays against the various strains of NiV.

Commented [pyr317]: On going for ELISA assays

- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor antigenic changes over time that may impact diagnostic testing and the epidemiologic and clinical features of disease.
- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats. *(*cross cutting)*

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Promote the development and assessment of affordable, highly sensitive and specific, point-of-care diagnostic tests for use in humans and animals that have minimal requirements for biosafety precautions and staff training and that can detect NiV and other related henipaviruses.
3. Strengthen laboratory infrastructure and capacity in the endemic and at-risk areas to improve early diagnostic capabilities.

Commented [SPL18]: Because this is a wildlife shed virus and these creatures live near food sources, spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh. The health care facilities that serve these communities not only have very rudimentary laboratory infrastructure, they also do not have the routine practice of using diagnostic tests to manage patients. Thus, achieving the stated strategic goals requires a much broader, deeper effort, than providing reagents and testing for proficiency at national labs.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and develop appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Expand research** to further understand the ecology~~ic~~ and epidemiology of NiV and other henipaviruses in human and animal populations over time, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to research** development of serologic tests to distinguish vaccinated from infected animals, if henipavirus vaccines are to be widely used in livestock populations.

Commented [pyr319]: For Malaysia, pig surveillance could be an important surveillance project

Commented [SPL20]: Consider whether efforts to promote sharing of sequence and strains would benefit from attention.

Product development

- **Generate** a TPP for NiV diagnostics.

- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other high-consequence pathogens in humans and animals.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure for detection of NiV in endemic and at-risk areas, particularly in support of enhanced national and regional diagnostic capabilities.
- **Improve surveillance capacity** to: (1) better define the incidence of disease in NiV-endemic areas and (2) promote surveillance in non-endemic areas to enhance detection of emergent NiV disease or disease caused by other related henipaviruses.

Commented [EG21]: Perhaps targeted research to identify evidence of spillover and human risk might come before investments in broader surveillance efforts.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV point-of-care diagnostics.
- **Ensure** access to regulatory review and authorization of NiV diagnostic assays.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostic products to endemic and at risk areas.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Commented [pyr322]: Serotherapies studies in Nipah patient are past-dues

Primary challenges

- Because NiV disease occurs in relatively small outbreak settings, the low incidence of disease poses a major challenge for conducting phase 3 clinical trials; therefore, alternative regulatory pathways will need to be considered for licensure of vaccines or therapeutics. *(*cross cutting)*
- Differences between the NiV$_{M}$ and NiV$_{B}$ strains may impact the effectiveness of developed MCMs, yet NiV$_{M}$ has been used significantly more often than NiV$_{B}$ in research. *(*cross cutting)*

Commented [SPL23]: Moreover, if we actually had more strains we may identify more heterogeneity than is currently apparent.

- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier and can access the brain and spinal cord to inhibit viral replication. This is particularly important for preventing late-onset CNS disease.

Commented [EG24]: And can pose a dilemma if therapeutics prolong life and improve survival but are unable to prevent severe neurologic damage.

- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons. In addition, the healthcare systems in endemic areas are not able to rapidly identify contacts most likely to benefit from PEP. *(*cross cutting)*

Commented [EG25]: Or, infection control measures which are not standard practice in many at-risk countries.

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*

Commented [pyr326]: Impact on pathogenesis studies and vaccine trials

Commented [pyr327]:

Commented [EG28]: Studies in animals often test usefulness of therapeutics when delivered prior to disease onset or very early during the disease course. Since humans with Nipah virus are often only detected once they are ill, the findings are less useful for understanding how the drug might work in humans.

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat both acute and chronic (relapsing) NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock. *(*cross cutting)*

Commented [SPL29]: Would be ideal, but, given the biology, may be much more difficult than an acute countermeasure. Might separate these into 2, so that the ideal does not become the enemy of the good.

Commented [EG30]: Patients in Malaysia were much more likely to survive than patients in Bangladesh. One hypothesis is that this is because patients in Malaysia had access to ventilator support, which is typically unavailable in Bangladesh. There may be health systems approaches that could improve survival.

Knowledge gaps

Commented [pyr331]: Anthropological studies to insure that vaccine trials are accepted

- Additional information is needed on the immunology and pathogenesis of NiV in order to develop appropriate treatment options. This includes evaluating the pathophysiologic differences between NiV_B and NiV_M infection, determining the mechanisms of late-onset and relapsing NiV encephalitis, identifying factors influencing the development of permanent neurological sequela, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Patients treated with Ribavirin late in the 1998-1999 NiV outbreak in Malaysia experienced 36% lower mortality than persons from earlier in the outbreak who were not treated with Ribavirin.~~was found to reduce mortality by 36% in the 1998-1999 NiV outbreak in Malaysia and is considered~~Ribaviran may be an option for treatment of NiV infection. However, animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number

of individuals exposed to HeV-infected horses. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV$_B$ and NiV$_M$, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV$_B$ to be shorter than for NiV$_M$.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the type of exposures that warrant such intervention and the most appropriate agents to administer.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of individual components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform supportive care and best-practice guidelines.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), such as determining the most appropriate lethal NiV dose for MCM development and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA’s Animal Rule to obtain licensing. *(*cross cutting)*

Commented [SPL32]: An important related issue is how we protect the health care workers and fellow patients in these settings. Part of this is covered in the vaccine section, but the capacity to protect health care workers and other patients is extremely limited in the contexts where we see recurrant NiV outbreaks. A clear eyed understanding of the context, and piloting approaches within these contexts is essential to develop and refine medical counter measures to that they can reduce pandemic risk. To be effective these countermeasures need to work, not only in idealized trial conditions, but in the everyday setting where high risk patients present.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection and ensure that therapies are prepositioned in endemic areas for use as needed.
2. Stimulate basic research for better understanding of NiV pathogenesis and the immune response to infection.
3. Strengthen clinical and laboratory infrastructure in endemic and at-risk areas to promote early diagnosis of NiV cases so that effective treatment and PEP can be rapidly administered.
4. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Commented [EG33]: Median days from onset to death for patients in Bangladesh who die from Nipah virus is 6 days. Typically, they present unconscious on around day 3. So, there is little time to act but even less when healthcare seeking is delayed, because progression is so fast. This is a worthy goal, but the limited access to healthcare will reduce the number patients we can find in time to treat and will have an impact on field trials.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Conduct** basic science research on the immunology and pathogenesis of NiV infections to inform development of new therapeutic agents.

- **Determine** the key differences between NiV_B and NiV_M that may have implications for the development of safe and effective NiV therapeutics.
- **Continue to research** the safety, tolerability, and efficacy of investigational therapies for NiV infection, including animal studies and clinical trials as appropriate and feasible.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against both NiV_B and NiV_M and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Refine, standardize, and validate** animal challenge models for future assessment of promising therapeutic candidates.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV therapeutics.
- **Support** plans for licensure/registration, adequate manufacturing, and subsequent distribution of safe and effective therapies to endemic areas and to other at-risk areas on an as-needed basis for outbreak control.
- **Secure** financing for procurement and deployment of NiV therapeutics for outbreak control once treatment agents are available.
- **Develop** treatment and PEP guidance as new therapies demonstrate safety and efficacy.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

Commented [pyr334]: Combination vaccine for better acceptability from population and will be more useful

- Because NiV occurs as relatively small, focal outbreaks, the low incidence of infection poses a major challenge for conducting phase 3 clinical trials for efficacy demonstration; therefore,

alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics. *(*cross cutting)*
- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- The absence of improved diagnostic assays for timely diagnosis of infection creates a challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control, once vaccines are available for use. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, African green monkeys (AGMs) are regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*

Key needs

- Safe and effective PEP (either vaccines or therapeutic agents) to prevent infection following exposure to NiV and guidance on their use. Such countermeasures are important tools to protect healthcare workers, family caregivers, and livestock workers. *(*cross cutting)*
- Nipah vaccines that can protect against both NiV_M and NiV_B in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks. Research tools, such as standardized neutralization assays, to compare immunogenicity of different vaccines.
- Enhanced surveillance capacity to provide more accurate information on the current incidence and geographic distribution of NiV in endemic and at-risk areas in order to refine potential vaccination strategies, particularly over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- The mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis are unknown. Further research to better understand viral clearance has important implications for development of vaccines and therapeutics.
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B; only a few studies demonstrating cross-protection have been performed to date.

- Further research is needed to determine durability and safety profiles of candidate vaccines, such as VSV recombinants, where concerns regarding potential neurotropism have been raised.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoestis).
- The identification of specific correlates of protection and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines and in simulating various epidemiologic scenarios that may impact strategies for vaccine use.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), including determining the most appropriate NiV dose for MCM development (Johnston 2015) and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses. Ongoing research into developing NiV/HeV vaccines for livestock is needed to further assess the merit of this potential control strategy.
- If evidence at some point supports the need for a broader, population-based vaccination strategy, additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.

Commented [EG35]: And for the estimation of stockpiles that would be required to effectively respond to outbreaks, under various transmission assumptions.

Commented [SPL36]: I suspect that this is an easier technical problem than it is an economic problem. Only 15% of Australian horses are vaccinated against HeV. Pigs in low income countries are rarely protected against swine cholera or Japanese Encepahlitis. The incentives for livestock raisers are not well aligned for deployment as a human public health tool.

Commented [EG37R36]: Indeed, even when we offered free JE vaccine to pig raisers in northern Bangladesh, many refused due to lack of trust in the formal vet care system.

Commented [SPL38]: Should consider surge capacity to rapidly ramp up vaccine supply in case NiV is used as a bioterror agent, or we face a strain that naturally develops increased capacity for person to person transmission.

Strategic Goals

1. Develop, evaluate, and license NiV vaccines for use in humans and potentially livestock, primarily for reactive/outbreak use in endemic and at-risk areas.
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection.

- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research efforts and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Generate** research tools (e.g., standardized neutralization assays) to compare immunogenicity of different vaccines.
- **Complete preclinical evaluation** of promising candidate NiV vaccines for safety, immunogenicity, efficacy, correlates of protection, and durability.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and livestock, primarily for reactive use during outbreaks.

- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.

Key capacities

- **Refine and validate** relevant animal challenge models for NiV infection.
- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the true burden and incidence of NiV disease, which may in turn, inform vaccination strategies.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV vaccines.
- **Ensure** access to regulatory review and authorization of NiV vaccines, particularly if non-traditional pathways are needed for licensure.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.
- **Provide** ongoing assessment to inform development and maintenance of NiV vaccine stockpiles over time; mathematical modelling may be used to contribute to this assessment.
- **Continue** to provide guidance on vaccination strategies for various target populations and epidemiologic scenarios, once vaccines are available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis, Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

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To:	"Michael Osterholm"; "christopher.broder@usuhs.edu"; "chuakb@tll.org.sg"; "egurley1@jhu.edu"; "sluby@stanford.edu"; "gnmillig@utmb.edu"; "mahmudur57@gmail.com"; "linfa.wang@duke-nus.edu.sg"
Cc:	"K.Cook@wellcome.ac.uk"; "benassiv@who.int"; "kamoore@umn.edu"; "jto@umn.edu"; "johns369@umn.edu"; "mehr0035@umn.edu"; "crcruz@umn.edu"
Subject:	RE: Save the Date - January 9 and 10 (depending on time zone)- WHO R&D roadmap taskforce conference call for Nipah virus
Date:	Monday, January 15, 2018 5:32:45 PM
Attachments:	Nipah Draft Roadmap.V5 sl_eg_per.docx

My comments on the document. See you tomorrow.

Pierre

From: Michael Osterholm [mailto:mto@umn.edu]
Sent: Friday, December 8, 2017 11:47 AM
To: christopher.broder@usuhs.edu; chuakb@tll.org.sg; egurley1@jhu.edu; sluby@stanford.edu; gnmillig@utmb.edu; mahmudur57@gmail.com; Rollin, Pierre (CDC/OID/NCEZID) <pyr3@cdc.gov>; linfa.wang@duke-nus.edu.sg
Cc: K.Cook@wellcome.ac.uk; benassiv@who.int; kamoore@umn.edu; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; crcruz@umn.edu
Subject: Save the Date - January 9 and 10 (depending on time zone)- WHO R&D roadmap taskforce conference call for Nipah virus
Importance: High

Dear Colleagues:

Thank you to those who provided your availability for a Nipah WHO Roadmaps taskforce conference call.

Based on majority availability, the taskforce call will be held on **Tuesday, January 9 at 7 pm CST.** The date and time for this call in the different time zones is as follows:

- PST (US): 5:00 pm (Tuesday January 9)
- MST (US): 6:00 pm (Tuesday January 9)
- CST (US): 7:00 pm (Tuesday January 9)
- EST (US): 8:00 pm (Tuesday January 9)
- GMT (London): 1:00 am (Wednesday January 10)
- CET (Geneva): 2:00 am (Wednesday January 10)
- BST (Bangladesh): 7:00 am (Wednesday January 10)
- SGT (Singapore): 9:00 am (Wednesday January 10)

Call-in information:

- Conference Code for all numbers listed below: 998-3378#
    - USA Toll Free: 866-767-9978
    - UK Toll Free: 0808 1017 535

    - Switzerland Toll Free: 0800 5537 12
    - Singapore Toll Free: 800 101 2395
- For Bangladesh, we do not have a toll free line; we will contact you separately to make logistical arrangements.
- As a BACKUP: International callers may use the following conference call information: Conference Line 205-254-8650 Conference Code: 998-3378#. However, please note that this is NOT a toll-free number.

An agenda and other meeting materials will be shared closer to the conference call date. A meeting summary will be developed and shared with the group after the call. As we anticipated, we were unable to accommodate everyone's schedules, so we apologize if you are not able to participate. If you are unable to participate, we still very much want your input, and we will look at either obtaining input via email or possibly through an individual call, if time allows.

We deeply appreciate your ongoing support and value your expertise and involvement. Please do not hesitate to contact me with any questions.

Mike

Michael T. Osterholm, PhD, MPH
Regents Professor
McKnight Endowed Presidential Chair in Public Health
Director, Center for Infectious Disease Research and Policy
Distinguished University Teaching Professor
Environmental Health Sciences, School of Public Health
Professor, Technological Leadership Institute College of Science and Engineering
Adjunct Professor, Medical School
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RESEARCH STRATEGY

A. BACKGROUND AND SIGNIFICANCE

A1. Henipaviruses are a critical threat to public health.

Over the past 40 years the majority of the major etiological agents of emerged or identified infectious diseases in humans have been viruses[1], and most have been zoonoses caused by RNA viruses[2]. The emergence or reemergence of pathogenic viruses are continuous threats to public health. Among these, the paramyxoviruses, which include many important human and animal pathogens, also include two emerged, zoonotic viral pathogens of importance: the henipaviruses; Hendra virus (HeV) and Nipah virus (NiV)[3,4]. Recently, a third isolate Cedar virus (CedPV) has been added to the *Henipavirus* genus[5]. Pteropid bats appear to be the major reservoir hosts for henipaviruses[6,7]. HeV and NiV have a uniquely broad host tropism capable of infecting at least 18 animal species across 6 orders of mammals, including their natural bats hosts. Among these animals, HeV and NiV can cause a systemic and often fatal respiratory and/or neurological disease in at least 11 mammalian species including humans, with fatality rates ranging from 40-100%[8,9]. There are no vaccines or therapeutics licensed for these viruses, highlighting an important public health unmet need.

Henipaviruses are single-stranded, negative sense, enveloped RNA viruses with two membrane anchored glycoproteins involved in virus entry, one of which mediates host cell receptor attachment (G glycoprotein) and the other, ~~is~~ a Class I fusion (F) glycoprotein, which facilitates virion and host cell membrane fusion. Both HeV and NiV use the cell surface protein ephrin-B2 as a functional entry receptor[5]. The viral G and F glycoproteins are the major antigenic targets of neutralizing Abs and also the main focus of several vaccine strategies[10]. The focus of this proposal is developing human Abs to the G protein.

NiV and HeV remain significant biothreats to humans and economically important livestock in Australia and throughout South East Asia, and there are no vaccines or antivirals approved for human use[9]. Surveillance research has detected henipavirus reactive sera and homologous RNA across a wide geographic area and in a number of bat species. Henipavirus seropositive samples have been reported in Malaysia, Indonesia, Papua New Guinea, Thailand, India, Ghana and Madagascar among both fruit and insectivorous bats and domestic pigs, suggesting that the range of NiV and/or HeV and the risk of potential outbreaks is widespread[11-22].

Further Ab discovery for HeV and NiV has been significantly hampered because of the burdensome requirements of biosafety level-4 (BSL-4) containment. However, whereas HeV and NiV are bat-borne disease-causing zoonoses, CedPV is not known to be zoonotic and has been shown to be nonpathogenic in animals susceptible to HeV and NiV disease, and resides in nature in the same bat host species as HeV[5,23]. Nucleic acid based detection studies have identified related henipavirus species, including complete genomic sequences[20,24], but HeV, NiV, and CedPV are the only virus isolates reported. We have recently rescued recombinant CedPV encoding turboGFP (rCedPV-GFP) using reverse genetics, and this platform is a new system that can be used to study henipavirus cell biology safely under BSL-2 containment. But of further significance, it is an authentic henipavirus infection and replication reporter system that is now suitable for high-throughput screening (HTS) applications for the purposes of drug discovery of potentially pan-anti-henipavirus antiviral molecules. Recently, Marsh and colleagues described the isolation of CedPV from pooled bat urine collected in Queensland, Australia[5]. CedPV is closely related to HeV and NiV, genetically and antigenically, and is the first new *Henipavirus* isolate, but the virus does not cause disease in experimental infections of mice, ferrets or guinea pigs[5].

A2. Human Abs are a feasible strategy for the prevention or treatment of henipavirus infections. There is proof-of-principle for the approach we propose here as post-exposure human monoclonal Ab (mAb) therapy (anti-NiV/HeV G glycoprotein mAb m102.4) has been examined[4,25-28]. Humanized mAb, hu5B3-1, is a potent cross-reactive anti-NiV/HeV F specific neutralizing mAb. The mAb was derived from murine 5B3 [29] which recognizes a conformational epitope in the pre-fusion form of the F glycoprotein, and binding of 5B3 prevents fusion triggering. The mAb hu5B3-1 has been shown to provide protection against NiV and HeV challenge in the ferret model when administered single dose at days 3 and 5 post virus challenge (Mire, Geisbert and Broder, unpublished). In addition, a human mAb, m102.4, recognizes the NiV/HeV G glycoprotein and is a potent cross-reactive neutralizing mAb. MAb m102.4 blocks ephrin receptor binding and the crystal structure of the mAb-G complex has been determined[27,30]. Further, m102.4 has been shown to provide post-exposure protection against NiV and HeV challenge in both the ferret[28] and nonhuman primate models[4,25,26]. m102.4 has been successfully administered to 14 individuals on emergency protocol because of HeV or NiV exposure[31], and has already completed Phase I randomized, double blind, placebo controlled study, dose escalation trial in Australia. This therapy can be~~is~~ administered within several days following lethal infection in animal challenge models. This molecule establishes the feasibility of the concept, but it is not a naturally occurring human mAb (it was derived from a library) and has ~~some~~ limitations in terms of breadth and potency. This work points the way, however, to an opportunity for a fully human broad and ultra-potent neutralizing Ab to serve as a next-generation biologic with improved properties. As shown below, we already have ~~in hand~~identified 10 new fully

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human neutralizing human mAbs, including a putative lead molecule that is ultra-potent and neutralizes both HeV and NiV. In addition, ~~And~~ we have the capacity to generate many more, potentially ~~some~~ with even better activity. Development of new human mAbs for henipavirus infections is of practical utility and also will be of interest to the infectious disease, viral pathogenesis, and immunology communities. Developing and deploying a new ultrahigh-throughput Ab discovery platform capable of rapidly isolating and characterizing hundreds of members of naturally-occurring human Ab clonal lineages at a time could revolutionize the approach to therapeutic Ab science.

B. INNOVATION. We bring several innovative features of research to this proposal.

First, we will refine and deploy one of the highest yield Ab discovery platform for infectious diseases ever described. We already use what is likely the highest efficiency method for isolation of fully human mAbs to date, based on high-efficiency B cell transformation followed by formation of human hybridomas. The methodology is state-of-the-art, and it is a robust procedure in our hands, with high yields of human mAbs from donor specimens. The current yield of discovery efforts with this technology is on the order of several dozen high-affinity human mAbs from the typical 5-10 mL)?) blood specimen studied. In the current proposal, we will leverage this foundation to achieve efficiencies that are ~ two orders of magnitude higher. We will accomplish this goal by coupling the established biological cell cloning and screening procedure with next-generation immune repertoire sequencing, followed by massively parallel DNA synthesis of entire antigen-specific repertoires, using emerging synthesis technologies from Synthetic Genomics and Twist Bioscience. The goal is to isolate and characterize the largest panels of human Abs to any infectious agent ever attempted.

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Second, we have a high level of expertise in the biology of henipavirus pathogens, especially HeV and NiV, and we have recombinant proteins for novel henipavirus protein targets. Our scientists have identified novel mechanisms pertaining to the pathogenesis of henipavirus infection that involve the surface G proteins (references?), and we have defined the impact of blocking attachment of viruses to cells on henipavirus virulence in large and small animal models of infection and disease (references?). We have elucidated the complex biology of these molecules, and demonstrated that their function is critical to replication and pathogenesis of these viruses (references?). Our work reveals that attachment to receptor represents a vulnerability in the viral lifecycle, ~~and therefore~~ providing~~e~~ a promising class of drug target that has not yet been fully exploited. We have already ~~have~~ established a fundamental portfolio of research in our laboratories that is focused on identifying factors and processes involved in viral replication of these pathogens and disease in their hosts. We will use this fundamental knowledge to further develop best-in-class human mAb therapeutics.

Third, there are several innovations in the present application regarding the henipavirus field. In this application, we will develop the very first cell-based HTS assay using an authentic henipavirus infection and replication reporter system based on the non-pathogenic CedPV for identifying inhibitors of henipavirus entry and replication. The basic screening protocol is based on the following two main principles: (1). Using the recombinant CedPV platform (an authentic henipavirus) all stages of virus replication are targeted in the screening assay; binding via henipavirus receptors (ephrins), membrane fusion and nucleocapsid entry, genome transcription and protein synthesis (reporter gene signal). (2). The CedPV F and G envelope glycoproteins could also be replaced within the rCedPV genome with F and G glycoproteins of HeV or NiV and replication competent chimeric viruses based on CedPV can be generated. This will allow for a HTS assay focused only HeV or NiV entry step dependent on the ephrin-B2 and B3 receptors. The proposed cell-based, HTS assay will make use of an authentic henipavirus infection and replication reporter system, which has never been done before. Thus, this assay will provide a HTS platform for antiviral drug discovery that targets all stages of the virus lifecycle. The recombinant CedPV based platform requires only BSL-2 containment, relieving the physical and economic burden of HTS screening in a high-containment environment. Because of the close phylogenic relatedness of CedPV to HeV and NiV, including the use of the same principle cell-surface receptor for entry (ephrin-B2) and replication strategies, there is good potential that identified inhibitory mAbs~~s~~ may have activity across the henipavirus genus. Indeed, this area of research is both timely and needed because there is a complete lack of any effective and approved antiviral therapeutics against henipavirus infection suitable for human use, certainly in the case of human NiV or HeV symptomatic infections.

C. APPROACH

C1. PRELIMINARY DATA

C1.1. Advanced Ab Technologies. We have developed what is arguably the highest yield system ever described for isolation of naturally-occuring human mAbs that inhibit microorganisms. The effectiveness of our pipeline can be measured by the quality of the mAbs we isolate and the rapidity with which we can develop them. For instance, we have participated as leaders in the international response to the three most recent international outbreaks of emerging infections, contributing rapid and seminal contributions to the response to these emergent events, as shown below:

Agent	Result of discovery campaign	Representative publications	Status of human mAb development for clinical testing
Ebola, Marburg	Best-in-class neutralizing human mAbs	***Cell Host Microbe*** 2018 23(1): 101 -109.e4 ***Science Translational Medicine*** 2017; 9(384). ***Nature Microbiology*** 2016; 1:16128. ***Science*** 2016; 354:350-354. ***Cell*** 2016; 164:392-405. ***Cell*** 2015; 160:904-12. ***Cell*** 2015; 160:893-903.	Abs for Ebola and Marburg have been licensed from Vanderbilt (the prime site in this application), to Mapp Bio, the commercial partner, and prepared as cGMP material. One Ab (MR191) was administered to a human in 2017 under emergency IND. BARDA invested over $30M in 2017 in the Mapp Bio program through the Emergent ADM to develop filovirus Abs, including MR191.
Zika	Best-in-class neutralizing human mAbs	***Nature*** 2016; 540:443-447. ***Nature Communications*** 2017; 8:14722.	Abs have been licensed to one biotech, and a second biotech is negotiating for exclusive rights. GMP CHO lines are being prepared by Batavia, in preparation for IND submission by Vanderbilt.
Chikungunya	Best-in-class neutralizing human mAbs	***Cell Host Microbe*** 2015; 18:86-95. ***PNAS USA*** 2015; 112:13898-903. ***Cell Reports*** 2016; 16:1326 -38. ***Science Translational Medicine*** 2017; 9:375. ***PLoS Neglected Tropical Disease*** 2017; 19;11:e0005637.	1. One set of human mAbs licensed to a big pharma, NHP studies are completed, and GMP material has been prepared for human trials. IND in preparation. 2. A second set is in manufacture as mRNA delivery for clinical trials by a large biotech.

We have already shown that we can isolate potently neutralizing Abs to HeV and NiV~~,~~ in preliminary experiments, and will continue this Ab discovery campaign in the first 2 years of this proposal. Targeting G protein of henipaviruses for mAb isolation using our hybridoma technology should be straightforward, but the yield will be uncertain until the discovery campaigns are launched in full. Typically, we have used a method based on EBV transformation followed by human hybridoma formation to isolate antimicrobial human mAbs (**Figure 2**).

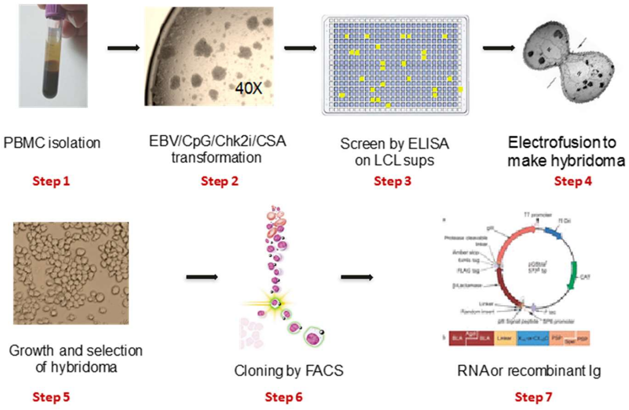

Figure 2. General workflow for generation of human mAbs by hybridoma.

The hybridoma process uses electrofusion of B cell lines with myeloma cells to create hybrids at high-efficiency[32]. This process has enabled us to isolate Abs to a vast array of human viruses. There are many advantages of this system. First, since the protocol uses human memory B cells, the timing after infection is not critical, and unlike mAbs derived from immunization related technologies, isolated mAbs are from a successful human immune response to the pathogen. In fact, we have isolated high potency neutralizing Abs to viruses ≥ 60 years after exposure to 1918 influenza[33,34] after several decades following infection with dengue virus acquired during foreign travel[35-39]. This protocol has been optimized to isolate high potency naturally-occurring Abs, but we also have a number of other techniques that can be used.

Alternative approaches to mAb development. We also have developed or deployed about a dozen additional methods for isolation of human mAbs. We will use each of our memory B cell techniques here. We also

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pioneered and used single-cell sorting of antigen-specific B cells to isolate virus-specific mAbs as early as the late 1990s[40]. This approach can be performed by sorting antigen-specific cells, followed by isolation of Ab variable genes and expression as recombinant IgG. Alternatively, cells can be expanded on feeder layers expressing CD40 ligand, IL-21, and BAFF. The advantage of this approach is that it can be quite quick, and also uses memory cells, so the timing of blood collection after infection is not critical. However, the challenge of this approach is to obtain a conformationally correct protective antigen for sorting. Sorting with an antigen that does not recapitulate the presentation of the antigen on the surface of the virion leads to sorting of B cells secreting Abs that are not of the right specificity or potency. The Crowe laboratory (Vanderbilt) and the Broder laboratory (USUHS) have prepared and validated the recombinant G protein antigens for this approach. One variation of the technique that we will explore is generation of small Ab variable gene libraries from oligoclonal antigen-specific EBV transformed B cell lines. We can express these gene libraries on the surface of yeast, and then select individual yeast clones for binding to the G protein of interest, using flow cytometric sorting. We have experience with this procedure for successful isolation of human Abs, which is particularly successful when the libraries are small due to enrichment for antigen-specific variable genes, accomplished by prescreening for B cell lines as we described above. This multi-pronged approach will give us "more shots on goal" from a technical standpoint, a risk mitigation strategy.

Figure 3. Large-scale collection of human PBMCs from henipavirus immune donor. A) Leukopack. Approximately 40 billion human PBMCs were collected from an immune Australian immune donor by leukoapheresis (ungloved hand is that of the donor, who observed laboratory processing). **B.** Further purification of PBMCs by dozens of density gradients. **C.** Large team required to rapidly process billions of cells. **D.** Hundreds of cryovials of immune donor cells were cryopreserved, enabling repetitive antibody discovery campaigns.

C1.2. Generation of human mAbs that neutralize henipaviruses.

We already have demonstrated the feasibility of the Ab discovery work for henipaviruses, and identified a potential lead Ab, HENV-26. We identified an Australian equine veterinary professional who had ~~had~~ an inadvertent exposure to the HeV equine vaccine (Equivac HeV), a product produced by Zoetis. The vaccine contains a non-infectious G protein component of the virus. We later determined that this individual's serum contains high levels of HeV and NiV neutralizing Abs. The subject flew to Vanderbilt where we obtained ~40 billion PBMCs by leukoapheresis, yielding hundreds of cryovials with millions of cells each (**Figure 3**).

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Human mAbs with high potency. In preliminary experiments with one 10 million cell aliquot of these cells, we made a panel of 10 human mAbs that bound to recombinant HeV G protein. All of the Abs neutralized HeV (**Figure 4A**), with one that was designated HENV-26 being especially potent. This mAb exhibited nearly 100% neutralization activity even at 390 ng/mL Its IC_{50} value is therefore very low. We need to do additional BSL4 experiments with further dilution to determine the final IC_{50}. This finding suggests that HeV is remarkably susceptible to neutralization, when the right naturally occurring human IgG clone is identified.

Human mAbs with breadth for both HeV *and* NiV. We also found that 5 of the 10 Abs also neutralized NiV, with HENV-26 again being the most potent, with near complete neutralization measured still at 195 ng/mL (**Figure 4B**). It is interesting that the activity is apparently even higher for the HENV-26 mAb isolated from a HeV G exposed person. This finding does suggest that NiV also is remarkably susceptible

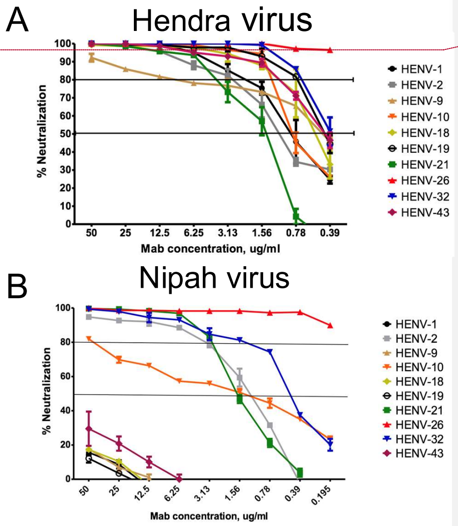

Figure 4. Percent neutralization of henipaviruses mediated by each of 10 new human mAbs, tested in a dilution series (high to low concentration of mAb left to right). **A.** Hendra virus. **B.** Nipah virus Bangladesh

to neutralization, when the right B cell clone is selected.

We performed competition-binding assays on an Octet biolayer interferometry instrument and demonstrated that the panel of 10 mAbs has members that recognize at least 4 different major antigenic sites (**Figure 5; next page**).

Finally, we obtained a crystal structure of the ultra-potent HENV-26 Fab in complex with HeV G protein (**Figure 6).** The structure reveals that this mAb binds to the receptor binding site on HeV G, thus its mechanism of action is to block receptor binding. However, close inspection of the structure in comparison with that of the receptor bound to G revealed that the Ab and the receptor use different critical contact residues to engage the receptor **(Fig 6B**).

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Figure 5. Competition binding experiments reveal the ten new human mAbs, each of which is neutralizing, fall into 4 competition-binding groups, suggesting they recognize diverse major antigenic sites. Hendra G protein was used to coat an Octet biosensor tip, then a mAb was interacted (to determine 100% binding level). Then we measured binding of a second mAb. Reduction of binding (from 100%) of the 2nd in the presence of the 1st was considered full (black), intermediate (grey) or no (white) competition.

These experiments, while preliminary in nature and performed using a single vial of donor cells (with hundreds more available), clearly demonstrates the high ~~feasibility~~ probability of obtaining a large number of broad and potent henipavirus neutralizing Abs. In addition,~~And~~, the HENV-26 already identified is a very promising lead for further development, even if no additional Abs are obtained in future screens. We will use our nextgen sequencing techniques to identify somatic variants of HENV-26 for recombinant expression and determine if any exhib~~v~~it~~s~~ an increase in inhibitory activity. Also, our industrial partner, Mapp, will begin manufacturability assessments at the start of the work using HENV-26. At the beginning of the proposal (prior to new Ab discovery campaigns) we can combine mAbs from each of the other competition-binding groups (*i.e.*, mAbs to other antigenic sites) to see if any cocktail achieves a cooperative or synergistic effect. Monotherapy (with a broad and ultra-potent mAb like HENV-26) is simpler from a manufacturing and regulatory perspective. But cocktails sometimes exhibit cooperative effects and they are more likely to resist viral escape.

Figure 6. Crystal structure of the complex of Hendra G protein and human mAb HENV-26 reveals this potent antibody blocks receptor engagement. Left) The Hendra virus G protein is shown in green, the Fab light chain in yellow, the Fab heavy chain variable domain in magenta and the Fab heavy chain CH1 domain in cyan. For comparison, the receptor structure is shown in grey color in the position was found in the complex of receptor and Hendra G protein. **Center**) Close-up view of the antibody and ephrin-B2 receptor reveals the antibody blocks receptor binding, but the residues on Hendra G protein with which the antibody and receptor interact differ greatly. **Right**) Hendra G protein surface showing binding sites of ephrin B2 and Fab HENV-26. Residues interacting with both HENV-26 and ephrin B2 are shown in green, with only HENV-26 shown in blue, with only ephrin B2 in yellow.

The goal of the proposed discovery efforts here is to identify the most potent neutralizing mAbs possible, with the greatest breadth against henipaviruses possible. Given the excellent potency of HENV-26, the cross-neutralization of NiV and HeV, and the known mechanism of action, HENV-26 appears optimal as a lead candidate. However, we may be able to find a clone with an even better profile. We will conduct discovery campaigns for about 18 months, then choose a lead by the end of Year 2 ~~years~~. Even finding additional clones with the identical specificity, mode of action, potency and breadth would help us when we transition the project to product development and manufacturing. Mapp will perform manufacturing assessments on lead compounds, as discussed in detail below. Having more clones to work with at that stage enhances the likelihood of finding a clone with desirable biologic activity *and* manufacturability.

C2. SPECIFIC AIMS

Specific Aim 1. Isolation of human mAbs from patients previously infected with henipavirus or exposed to henipavirus vaccine antigens

Rationale: We have several powerful B cell technologies that can be brought to bear on this project. The principal approach is to generate human hybridomas from circulating B cells using an electrofusion technique we developed under a previous NIH Bioengineering grant. This approach is the highest efficiency technique for isolating human clones with naturally-matched heavy and light chain genes ever reported. Using this approach, we have generated thousands of virus-specific B cell lines and mAbs to RSV[41-49], MPV[49-51], rotavirus[40,52-59], norovirus[60,61], influenza NA H1/2/3/5/7 and NA9[33,34,62-70], dengue viruses[35-39,71-76], Zika virus[60,77], hepatitis C virus[78], HIV[79-83], chikungunya virus[76,84-87], Ebola viruses[88-91], Marburg virus[92-94], poxviruses[95], *S. aureus*[96] and many others (unpublished). The method is efficient enough~~, for example,~~ that we were able to generate human hybridomas to the 1918 H1N1 pandemic influenza virus from ~ 100-year-old survivors, in whom the average frequency of B cells specific for that early 20th century HA was only 1 in 4 x 10^6 cells (or 0.000025%)[33].

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PBMC samples from human subjects with henipavirus immunity. Fortunately, we have an ongoing donor identification and sample acquisition program in the Vanderbilt Vaccine Center, which obtains serum, plasma and peripheral blood mononuclear cells (PBMC) for isolation and cryopreservation. These materials are processed and stored in a de-identified manner in the Vanderbilt Vaccine Center Biorepository, which is a de-identified sample archive. The acquisition of samples does not use federal funds, as this is an institutionally supported service. The use of these materials by VVC investigators is *Not Human Subjects Research* category activity. The current proposal does not propose to support acquisition of new human subjects. Nevertheless, we are aware that the program is enrolling a survivor of natural HeV infection in early 2018. If that occurs, we would obtain some of those de-identified cells for use in the current proposal if funded. If any NiV immune subject materials are deposited in the Biorepository, we also would request use of those.

Recombinant HeV and NiV glycoproteins. The Crowe laboratory produced the soluble forms of HeV and NiV G proteins that were used in the preliminary Ab screens shown above. The proteins bind to human Abs in human immune serum, and they facilitated isolation of ultra-potent neutralizing antib~~v~~odies already, validating their utility in the proposed work. The Broder laboratory also has extensive experience in the development and characterization of human cell line expressed soluble, oligomeric forms of viral envelope glycoproteins which have been used in a variety of applications in vaccine development, viral entry mechanisms and receptor discovery, and as antigens in biosurveillance [8,9,97-106]. Specifically, we have prepared the NiV and HeV soluble tetrameric G glycoproteins (sG), as well as other henipavirus sG (Kumasi, Cedar, Mojang) that are available to perform various human mAb selections, including multiple antigen selections to identify cross-reactive, pan-henipavirus G mAbs.

Cell isolation and fusion: The specific technique is as follows. We will use cells isolated from cryopreserved PBMCs with Ficoll-Hypaque following an established density gradient method. Transforming EBV (substrain B95.8), and the TLR agonist CpG ODN 2006 (2.5 μg/mL; Invivogen), 10 μM of Chk2 inhibitor II (Sigma C3742), and 1 μg/mL cyclosporine A (Sigma) will be used to stimulate human lymphocytes for transformation. Cells are plated in 384-well tissue culture plates and monitored visually for transformation.

Screening for virus-specific Ab lines: Supernatants from wells containing EBV-transformed lymphoblastoid cell lines (LCL) will be screened for binding activity in several ELISAs against the recombinant G proteins described above. Up to 10 antigens can be used in each screen, facilitating the search for pan-henipavirus mAbs. Positive wells in any of these assays will be selected for cell fusion. EBV-transformed LCLs do secrete human Abs, but these lines are quite unstable and difficult to clone biologically. LCLs secreting antigen-specific Abs will be fused with HMMA 2.5 myeloma cells to form human hybridomas, which are much more stable. The system used is a programmable electrofusion source (BTX). The fusion volume is 500 μL. HMMA2.5 myeloma cells and EBV-transformed human B cells are washed with RPMI-1640 and cytofusion medium. Large volume fusion chambers are commercially available and interface well with this unit. The key to the fusion is bringing the two cell types into close physical contact using dielectrophoresis in a low-voltage AC field (in which cells developed a dipole, essentially becoming like little magnets stuck together in long linear chains). After fusion, cells will be allowed to recover in the fusion electrode for 30 min, harvested, and then washed with medium prior to plating for selection. **Hybridoma selection and cloning:** After fusion, fused cells are seeded into 384-well plates in complete medium containing HAT and ouabain. After five days of culture, cells are fed by removing 100 µL of the culture medium and adding an equal volume of fresh medium containing hypoxanthine/thymidine or HT. We use a series of STEMCELL Technologies media for these procedures, most importantly the ClonaCell®-HY Medium E for growth and expansion. After initial screening for human Ig production by ELISA, the hybridoma cells from positive wells are expanded into 96- and then 24-well plates and cultured in complete medium, and then hybridoma cells are seeded in 96-well plates at 0.3 cell-per-well density. Finally, we single-cell sort the lines on a FACSAria III flow cytometer or with a ClonePix.

Ab sequences: After clonal hybridomas are obtained that secrete mAbs, we will determine the genetic basis for development of high-potency Abs to henipaviruses by sequence analysis and interpretation of Ab variable gene regions from HeV G protein-reactive human mAbs. **Prioritization scheme:** We are likely to isolate very large numbers of mAbs from these samples. If we isolate hundreds (or more likely thousands), we can retain the mAbs but we will not be able to perform complete characterization of all of these mAbs in the scale of this application. We will use several criteria to determine the highest priority clones to study. First, we will focus on Abs that neutralize with high potency (<0.1 µg/mL IC_{50}). Second, if there is still a large number, we will perform competitive binding assays by ELISA, Wasatch SPR and Octet biosensors to determine groups of mAbs with a similar binding pattern. We will make sure to characterize mAbs from each competition group, and within those groups, focus the most effort on the mAbs with the highest inhibitory potency. Third, we will focus on clones that neutralize both HeV and NiV, or, when identified, on pan-henipavirus clones. **Nextgen library preparation and sequencing:** PBMCs isolated from subjects ~~also~~ will also either be sequenced directly or enriched for specific B cell populations of interest such as antigen-specific memory B cells. We will extract total RNA and construct bulk B cell libraries as previously described. Sequencing will be performed according to standard Illumina 2 x 250 amplicon sequencing protocols that are well-established in the Crowe laboratory. In some cases, we will perform single-cell sequencing according to the 10X Genomics Single Cell VDJ B cell protocol and sequencing on an Illumina HiSeq 2500 to capture heavy-chain and light-chain linked information. Finally, we will perform an alternate single-cell approach in which single B cell are sequestered into water-in-oil emersions using a Dolomite µencapsulator system and full-length V_H or V_L sequences are physically linked via overlap extension PCR in the final form of a single-chain fragment variable region (scFv). These scFv libraries will be sequenced on a PacBio Sequel sequencing platform and used in a yeast display assay to test for additional functionality aspects. Following collection of raw data, we will analyze heavy and light chain variable region sequences using the proprietary PyIR software that we developed for large-scale immune repertoire studies. These differing approaches eliminate common bottlenecks related to B cell repertoire sequencing.

Recombinant Ab cloning and expression: Following sequencing of clones from hybridomas, yeast display, or repertoire studies, we will synthesize heavy and light chain variable region sequences of interest to the project for recombinant cloning and expression and downstream functional assays. Due to the potentially large number of interesting Ab sequences we are likely to discover, we will perform high-throughput synthesis of Ab genes using our 3 BioXP synthesizers (Synthetic Genomics) and Twist Bio custom synthesis, which can provide unrivaled scalability and throughput for synthesizing DNA at a high-quality level and low associated cost. Twist Bio and SGI DNA offer DNA synthesis services with the ability to produce large, small, and complex DNA constructs using the Gibson assembly method and error correction technology. Using in-house SGI DNA BioXP 3200 DNA synthesizers, we are able to synthesize and clone up to 1,400 genes between 300bp and 1.8 kbp in length per week at a cost of $0.11/base. Alternatively, for larger scale gene synthesis efforts Twist Bio has the capacity to synthesize and clone up to 10,000 genes between 300 and 3.2 kb in length per month at a cost of $0.09/base. Following synthesis, Ab genes will be Gibson-cloned in a 96-well format using a gateway-based Ig cloning platform to allow for rapid recombinant subcloning between different Fc variant expression vectors that are well established with the Crowe laboratory (70 Fc variant expression vectors are available). This high-throughput and modular recombinant Ab system allows for not only rapid expression of Ab genes of interest, but also rapid functional testing of different Fc-mediated Ab effects. This entire recombinant Ab cloning platform can be scaled from a low-throughput and manual-based preparation to a high-throughput and automated preparation on Tecan liquid handlers in the Crowe laboratory.

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Parallelized micro-scale Ab expression: We achieved a recent expansion in capabilities for micro-scale Ab expression and purification. Methods are now in place to express Abs either as hybridomas or recombinantly in a CHO cell transient transfection system across a broad range of scales from 96-well plates to multi-liter bioreactors. The lower volume end of this range allows parallelized expression of 100s to 1,000s of lead Abs, which will feed into the automated Ab purification on our Tecan automation platforms. This approach will allow early-stage assays to identify leads definitively that can then be iteratively produced, purified, and screened across our wide range of volume options (6-well plates, small volume shake flasks, large-volume shake flasks, and bioreactors). This approach will allow us to broadly screen entire panels of purified Abs at micro-scales, and then specifically tailor our time and reagent investment to prioritization of the clone. **Ab preparations:** Hybridoma cell lines will be adapted to serum-free medium. Supernatants of high-density cultures will be produced in CELLine Devices (BD Biosciences). Purified and concentrated preparations of each of the Abs will be prepared by FPLC using protein G resin on an AKTA followed by buffer exchange using ultracentrifugal filter devices, followed by quantitative ELISAs to define concentration.

Potential Pitfalls and Alternative Approaches. There are a number of technical challenges in the work that we propose. First, the frequency of human B cells in human subjects with immunity to viruses is uncertain. The occurrence of such circulating cells could be rare in some individuals. Therefore, our method must be capable of isolating rare B cell clones in a comprehensive manner. This risk is mitigated however because we have identified a donor with high frequency of HeV and NiV cross reactive B cells, and we have billions of PBMCs stored in the VVC Repository. We also will address this challenge in several experimental ways. First, our Ab discovery platform will incorporate not only state-of-the-art antigen-specific B cell isolations, but also, we will couple this with comprehensive sequencing of all Ab genes in the donor specimen. This repertoire sequencing approach will define billions of Ab variable gene sequences (likely reduced to about 20 million circulating clonotypes), allowing us to examine the entire lineage of somatic variants for any Ab isolated. This information is fascinating in terms of shining a light on the extreme diversity of the human immune response. However, in this proposal, we will turn that data into actionable information for the first time by large-scale synthesis of entire antigen-specific immune repertoires. To our knowledge, no group has ever attempted to synthesize Ab gene repertoires of the scale, much less expressed and characterized every member of a clonal lineage in this way for hundreds of lineages. This massive and comprehensive survey of Ab specificity and function in individuals with recent infection should allow us to identify the optimal functional members amongst large clonal lineages. This approach should allow us to find the rarest of clones with high therapeutic potential. In the past, contemplating such a large-scale Ab discovery campaign was unthinkable. However, the high-efficiency technique that we propose, using biological isolation coupled with next-generation sequencing and high-throughput gene synthesis and expression, makes such a survey possible.

The purification of membrane proteins can pose challenges that can affect the ability to prepare relevant microbial antigens. We have significant experience in purification of these surface proteins and have confidence in our ability to purify these proteins using our established protocols.

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***In vitro* functional screens to test human Ab efficacy and specificity:** Once candidate Abs are identified, they will be screened using *in vitro* assays to identify the most functional molecules. We will target henipavirus G proteins, as described above. First, Abs generated against HeV and other viruses will be evaluated for the ability to neutralize HeV and NiV. Initially, we will use *wt* viruses, in the BSL4 laboratories at UTMB, but in the first year of the proposed studies we also will test using BSL2 surrogate virus systems developed by the Broder laboratory (described below). Abs will be used alone or in combination to determine the most effective cocktails that inhibit virus replication. With Abs that block the receptor binding domain (such as the HENV-26 mAb described in preliminary data above) we expect to achieve near complete blockade of henipavirus attachment to receptor. Considering that attachment is required for viral replication, these mAbs will represent powerful therapeutics to protect against infection by henipaviruses. The most active Ab and Ab combinations will be advanced to *in vivo* protection studies to define their therapeutic efficacy.

Cedar virus-based neutralization assay development: The ability to carry-out serum neutralization tests (SNTs) against infectious NiV or HeV is challenging because of the requirements for BSL-4 containment. The development of a robust surrogate assay for NiV and HeV SNTs that could be conducted at BSL-2 lab conditions would be a significant advancement for this proposed project but also to the henipavirus research field as a whole. Cedar virus (CedPV) was isolated from pooled bat urine collected in Queensland, Australia and is the only other authentic henipavirus isolate to date[5]. CedPV is closely related to HeV and NiV, genetically and antigenically. The Broder lab has been studying the functional characteristics and cellular receptor use profile of CedPV and its interactions with host cells of varied species. A reverse genetics system has recently been established for CedPV and this platform represents a new system which can be exploited in the present application that can be used safely under BSL-2 containment. ***Rationale:*** An authentic henipavirus CedPV-based neutralization assay will provide a long-term henipavirus neutralization testing platform that will alleviate the requirement for costly high-containment BSL-4 testing against NiV and HeV. This assay will facilitate the extensive combination (multiple mAbs) neutralization testing *in vitro* to identify the most potent antiviral combinations of mAbs, prior to conducting initial testing *in vivo* in hamsters. ***Innovation:*** The difficulties faced in the quantitation and assessment of vaccine-induced neutralizing Ab responses to NiV/HeV are significant owing to the requirements of BSL-4 containment for use of infectious virus. Typically, all SNT assays using mAbs or polyclonal sera derived from animals, humans, or from experimental vaccine protocols are carried using infectious wild-type NiV and/HeV isolates. A classical plaque reduction neutralization test (PRNT) to evaluate neutralizing polyclonal sera or mAbs (Abs reactive to the NiV/HeV G and F envelope glycoproteins) is wildly used, and PRNT titers will give an indication of protective immunity. PRNT using live NiV has been developed [107]. However, for NiV and HeV, PRNT assays must be performed in BSL-4 containment and takes 5–7 days to complete. Alternatives to assessing neutralization of NiV/HeV by Abs have been explored such as recombinant VSV-based pseudotyped reporter-gene virions bearing NiV or HeV F and G [108], however this assay is not as sensitive as the PRNT using authentic NiV. Other reporter virus platforms using VSV and HIV-1 have described as well but again, consistent production of quality pseudotyped virus stocks is difficult and neither have offered real improvements over the classical PRNT with infectious NiV and/or HeV[109,110]. Finally, because of the known details of NiV and HeV host cell infection process and receptor use, cell-free systems have also been explored. Bossart *et al*.,[111] developed a Luminex-based NiV/HeV sG and ephrin receptor Ab blocking assay, and although in general it can assess relative potency of neutralizing Abs as a surrogate assay, it cannot assess all types of neutralizing Abs reactive to G. Indeed, the ephrin-B2/B3 receptor binding site on G is only one of 7 known virus neutralizing determinants (Broder *et al*, unpublished). The innovation here is two-fold: First, we propose here a cell-based SNT that will make use of an authentic henipavirus infection and replication reporter system (a replication competent, reporter gene-encoding CedPV) which has never been done before. Second, the recombinant CedPV-based platform requires only BSL-2 containment, relieving the cost and technical challenges of the high-containment environment when high numbers of samples are to be tested. ***Approach:*** *Develop, characterize and adapt a rCedPV luciferase reporter virus for use in SNT*. Here, we will develop the very first cell-based, authentic henipavirus infection, replication and reporter system based on the non-pathogenic CedPV. We will rescue a second pair of rCedPV chimeras, bearing the HeV and NiV F and G glycoproteins, as reporter viruses encoding luciferase. These novel viruses will serve as authentic replication competent henipaviruses with the same tropism and biology of HeV and NiV but that can be grown and employed in virus infection assays outside of BSL-4 containment. We will provide these reagents to the Geisbert lab to characterize and compare them to wild-type NiV and HeV in cell-based infection tropism and growth kinetics assays and validate their use as surrogates in neutralization assays. The novelty of our approach is that the virus reporter system is an authentic henipavirus and one that may be used in the BSL-2 setting. ***Experimental Methods and Design:*** Generation of plasmids and rescue of recombinant viruses: We have already constructed a recombinant CedPV antigenome clone by synthesis of several large segments of the genome into pOLTV5[112] between a T7 RNA promoter and hepatitis delta virus (HDV) ribozyme. The pOLTV5 vector was similarly used for cloning and expression of recombinant HeV[113] and methods similar to those used in the rescue of recombinant NiV[114]. The first reporter virus chosen was using Turbo-GFP, and the GFP gene was modified to be flanked by the CedPV intragenic P stop and M start sequences conforming to the “rule of six”. As with recombinant HeV[113] the GFP gene was inserted between the P and M genes to maintain the levels of N and P transcripts. Helper plasmids for the N, P, and L genes from CedPV were also prepared (pCMV). To generate recombinant CedPV, pCMV CedPV N, pCMV CedPV P, pCMV CedPV L, and pOLTV5 full-length rCedPV-GFP anti-genome plasmid were mixed with Lipofectamine LTX (Invitrogen) in OptiMEM, and used to transfect 5 x 10^5 BSR-T7 cells. At day 4, GFP was observed in BSR-T7 cells transfected with the rCedPV-GFP antigenome and N, P, and L helper plasmids. Non-adherent BSR-T7 cells and culture supernatant were collected and freeze-thawed 3-times. Vero cells at a density of 1 x 10^6 cells/well in 6-well plates were inoculated with the freeze-thaw supernatant. Maximal GFP signal and syncytia were observed after 6 days, supernatant was clarified by centrifugation and 300µL of supernatant was passaged to fresh 1 x 10^6 Vero cells in 6-well plates. Within 24h, GFP signal and syncytia were observed. At 3 days, maximal GFP signal was observed and supernatant was passaged on fresh Vero cells (75 cm^2 flasks) for amplification of rCedPV-GFP. We also plan to increase the efficiency of CedPV rescue, and the pOLTV5-CedPV vector has been modified to express a 5′ HDV ribozyme site anterior to the CedPV 3′ leader sequence[115]. With this modified anti-genome two additional recombinant viruses have already been made; a CedPV void of any reporter gene but retaining the introduced restriction site (rCedPV+6) which is our wild-type CedPV, and luciferase encoding virus. We chose nanoluciferase (NanoLuc) a newly developed small luciferase reporter (brightest bioluminescence reported to date), which is a small luciferase subunit (19 kDa) from the deep-sea shrimp *Oplophorus gracilirostris*[116]. The rCedPV-GFP virus and wild-type rCedPV are amplified in Vero cells, and typically 8 T-75 cm^2 flasks, after 4 days in culture yield viral stocks of 1 x 10^8

pfu/mL in ~3 mL after purification and resuspension and can be stored aliquoted at -80ºC. We have also determined that CedPV exhibits a remarkably broader ephrin receptor usage profile, and cells bearing CedPV G and F can also mediate fusion with cells expressing ephrin-B1 and the glycosylphosphatidylinositol (GPI) anchored ephrin-A proteins; ephrin-A1, A2 and A5. Shown in **Figure 7** is the CedPV F and G cell fusion obtained with all B and A class ephrins.

Figure 7. <u>CedPV (CeV) glycoprotein-mediated fusion with a panel of A-type and B-type ephrin-expressing cells.</u> Target cells were prepared by transfecting a panel of ephrin expression plasmids into HeLa USU cells infected with vCB21R(Laz-reporter vaccinia virus) and allowed to fuse with CedPV F+G expressing HeLa USU effector cells infected with vTF7.3 (T7-polymerase reporter vaccine virus. Fusion is quantitated as the rate of β-galactosidase activity[117]. CedPV mediated fusion with cells expressing ephrins-A1, -A2, -A5, -B1, and -B2.

CedPV cell fusion also correlates with CedPV G glycoprotein binding to ephrins and this data has been confirmed by surface plasmon resonance experiments with soluble ephrins and CedPV soluble G. (C. Navaratnarajah, R. Cattaneo, and C. Broder, data not shown).

<u>*Generation of rCedPV Chimeras*</u>: The Broder lab was the first to show the unique functional similarities between NiV and HeV G and F glycoproteins by demonstrating that they are the only paramyxoviruses that can bi-directionally functionally complement heterotypically[118] perhaps owing to their aa identity (~83% and 89% for G and F). Our new data with CedPV has provided a breakthrough in finally distinguishing between a functional vs. non-functional F-G interaction. The CedPV-F is only 42% and 43% identical with HeV-F and NiV-F respectively; and CedPV-G is 29% and 30% identical with HeV-G and NiV-G. Performing F and G co-expression and fusion assays using combinations of CedPV, HeV and NiV (**Figure 8**) has provided some very important observations. Ephrin-B2 and -B3 negative HeLa-USU cells are negative for CedPV fusion, as are ephrin-B3 bearing Hela-B3 cells. Fusion does occur with 293T and ephrin-B2 bearing HeLa-B2 cells. Thus, CedPV-F has heterotypic function with both HeV-G and NiV-G, but CedPV-G has heterotypic function with only HeV-F and not NiV-F and correlates with ephrin use. Of significance, these data support the hypothesis that we will be able to construct and generate novel rCedPV isolates using our reverse genetics system, whereby the F and G genes within the rCedPV genome maybe removed and replaced by with NiV F and G or HeV F and G. These new reporter gene encoding authentic henipaviruses that may be used under BSL-2 containment and they will be the ideal tools in establishing a robust SNT assay to assess virus neutralizing Ab responses in HeV-sG vaccinated subjects. Experiments on the generation and manipulation of the non-pathogenic rCedPVs, including the proposed development of novel chimeric viruses using the NiV and HeV F and G glycoproteins, have been approved by the Institutional Biosafety Committee (IBC). The chimeric viruses will in fact be loss-of-function recombinant viruses because of the reduced ephrin receptor use profiles between CedPV and either NiV or HeV.

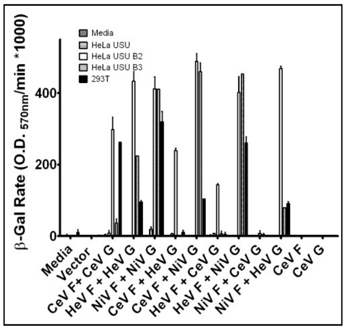
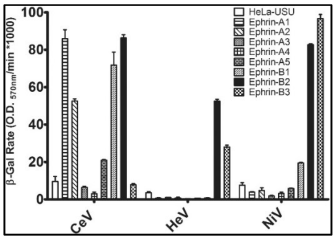


Figure 8. <u>CedPV mediated cell fusion and heterotypic mixing with HeV and NiV.</u> Various target cell populations are shown in the legend.

<u>Characterization of the recombinant CedPV viruses:</u> We expect the reporter gene encoding virus will not be hindered in any way based on the amplification results and titer of rCedPV-GFP, but we will analyze the replication kinetics of rCedPV-Luc and also compare those results to wild-type rCedPV (no reporter gene). Each virus will be used in experiments to determine receptor tropism and compared to the CedPV cell fusion assays using CedPV F and G expression as done with HeV and NiV[105,117-119]. <u>*Expected Results and Data Analysis:*</u> Based on the preliminary data in hand, we do not expect any unforeseen difficulties in generating the novel rCedPV/NiV-HeV chimeric viruses. We expect that all recombinant viruses will have an infection profile identical to the cell-cell fusion assays using recombinant expressed F and G from CedPV, NiV and HeV. Because we have rescued the GFP-encoding CedPV, we do not anticipate any unexpected limitations in recusing the rCedPV chimeras encoding NiV or HeV F and G. Based on the replication results with rCedPV-GFP, we expect that large amounts of reporter virus stocks can be prepared, providing a consistent source of material for conducting the number of SNT assays expected to be carried out.



<u>**Specific Aim 2: Develop immuno-therapeutics for the treatment of henipavirus infections.**</u>

Rationale: In Specific Aim 1 we will isolate human mAbs targeting conserved G protein domains. Experiments in Aim 2 will evaluate the therapeutic value of these Abs in animal models of viral infection. The goal is to create broadly reactive Abs that both inhibit viral replication, while simultaneously promoting immune-mediated clearance. Considering the conservation of the selected target across henipavirus strains, mAbs developed through this campaign have the potential to be valuable for treatment of a variety of diseases.

***In vivo* protective effect of Abs against invasive infection caused by henipaviruses:** This project will employ golden Syrian hamsters as the small animal model of NiV and HeV disease since they develop late stage lesions similar to those in humans and because they are more suitable than other small animals for screening large numbers of MAbs. Using hamsters will allow us to select optimal MAb formulations and regimens prior to efficacy testing in NHPs. African Green Monkeys (AGMs) are the gold standard animal models for henipaviruses and can provide a model of ~~immunogenicity and~~ efficacy that has ~~some~~ predictive value for therapeutic interventions. The AGM model was developed by Dr. Geisbert for HeV and NiV infection [2, 3]. The AGM model results in neurological disease and severe respiratory pathology along with generalized vasculitis that provides an accurate reflection of what is observed in henipavirus-infected humans [1-3]. The model yields a consistent lethal infection and disease with low dose challenge (less than 5000 pfu for Nipah virus, Bangladesh strain) given by the intratracheal (i.t) and/or intranasal (i.n.) route (TW Geisbert, unpublished data). The virus spreads rapidly within 3-4 days and the infection is established in multiple organ systems. Animals develop a severe ARDS-like disease, associated with copious amounts of sanginous fluid and froth. Death usually occurs within 7-9 days after infection. Evidence of endothelial syncytia are prominent in most of the tissues and vasculitis is systemic. Virus can be found in virtually every organ system at the time of death in the AGM, and most infected AGMs show evidence of henipavirus-induced neurological disease with congestion of the brain and evidence of meningeal hemorrhage and edema. The cynomolgus macaque model is not useful for demonstrating the effectiveness of therapeutic interventions or vaccines because the virus is not lethal in cynomolgus macaques when given by the combined i.n. and i.t. routes at a dose that is lethal in AGMs (TW Geisbert, unpublished data) and animals universally recover. Therefore, AGMs will be used as the NHP species of choice for the proposed research.

The Geisbert laboratory will test the *in vivo* efficacy of human mAbs using the ~~(~~Syrian golden hamster (*Mesocricetus auratus*) and AGM primate model of infection. This laboratory is one of the most experienced groups in the world in the use of animal models of henipavirus infection. Once Abs are demonstrated to recognize and block virus attachment to receptor, or other functions in the life cycle, they will be assessed for protection in the appropriate infection model. Varying concentrations of specific Abs, used alone or in combination, as well as control Abs will be tested in Syrian golden hamsters aged 5 to 8 weeks of age, weighing 50-150 grams with equal numbers of males and females. The total number of animals that will be used over the course of these studies is 240. Once the number of candidate mAbs/mAb cocktails is reduced to x from the hamster testing, ~~The lead mAbs also~~they will be tested in African green monkeys (*Chlorocebus aethiops*) using adult animals 3 to 12 years of age, weighing 2.5-9 kg, and using equal numbers of males and females. The total number of monkeys that will be used over the course of these studies is 36. Animals will be obtained from a commercial vendor. In years 1 and 2 of the project, hamsters will be exposed to HeV or NiV by the intranasal (i.n.) administration of up to 1 x 10^6 pfu of virus and treated at various times or with varying doses after henipavirus exposure by i.p. administration of mAbs to establish the therapeutic window and dose response,, respectively. In years 3 to 5, work will focus on assessing the protective efficacy of the most promising mAbs identified from the hamster studies. African green monkeys (AGMs) will be challenged by the intratracheal (i.t.) and/or i.n. routes using up to 1 x 10^6 PFU of HeV or NiV and treated at various times after exposure by intravenous (i.v.) administration of mAbs. Blood collection will be done as described in Section 3 of the Vertebrate Animals document.

Manufacturability. To augment the measures of biological activity, we will design and execute a manufacturability assessment (MA) study with desired product quality attributes and ensure the lead molecule(s) demonstrate adequate expression, process yield, processing and storage stability. We will continue to refine the Target Product Profile (TPP; see Product Development Strategy) since some components of the MA study design will be dependent on the TPP. This will allow us to focus on the most critical biophysical properties for the indication, route of administration and target concentration resulting in an optimized MA study design to rank the candidate molecules. We will take a tiered approach to maximize the use of resources and material. The first step, *in-silico* assessment of potential liabilities will include identifying unpaired Cys and exposed Trp/Met in variable regions and either re-engineer or de-prioritize, accordingly. Expression levels will be evaluated using Octet RED96 (Pall) with Protein A/anti-Fc biosensors. The top 10-20 candidates will then be subjected to protein A elution and low pH hold steps to evaluate the propensity to aggregate by SEC, form fragments by CE-SDS, intact mass by LC-MS and total yield by UV spectroscopy. Candidates will be ranked based on purity assessed by the methods mentioned above and the total yield. The

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top 3-5 candidates that result will then be subjected to biophysical analysis determined by the TPP. For example, thermal stability will be evaluated by pH ranging studies (pH 5 – 8) in 2 platform buffers and if target concentration is ≥ 50 mg/mL, viscosity and solubility as a function of pH will be investigated to determine the optimal pH range. The top 2-3 candidates will be advanced with the chosen formulation buffers into stress assessments such as accelerated temperature at 40ºC for 4 weeks, 3X Freeze-Thaw and Agitation studies with and without surfactants. Readouts will be aggregation, fragmentation, particulate formation and protein concentration. Evaluation of binding to GP will be included. At this stage, charge heterogeneity and glycosylation analysis of the top candidates will be performed in chosen formulation buffers. Detailed biophysical profiling with material from the final host cell platform and process conditions will be performed later during development to understand the behavior of representative material for scale up and manufacturing.

Generation of the CHO line. CHO cells are the standard of choice for manufacturing complex biomolecules like IgG fusion proteins for therapeutic purposes. Due to its robust nature and track record as a proven host for biologics manufacturing, there is substantial infrastructure within the US to assure GMP production of CHO material at virtually any scale. **Figure 9** shows Mapp's approach to producing the material to be used in the later stages of the proposal as well as the CHO cell line to support advanced development of the product. The sequence of the lead mAb(s) will be optimized *in silico* for expression in CHO cells and synthesized by DNA2.0. The synthesized sequences will be inserted into DNA2.0's IP-free plasmid DNA expression vector for stable integration in CHO (CHOZN®; Sigma; fucosylated N-glycans) or Mapp's CHOK1-AF (afucosylated N-glycans) host cell line depending upon the preliminary results. Following transfection of the CHO host with the mAb vectors, stable pools will be generated. Following selection, the pools will be fractionated into mini-pools for analysis using a Molecular Devices Clonepix™ 2 automated high-throughput colony picker in use at Mapp. Approximately 100 high expressing single-cell clones will be selected using the ClonePix 2 system, expanded, and screened for production by ELISA. Productive subclones will be screened and a Research Cell Bank (RCB) for up to 5 of the best subclones will be created. The RCBs will be tested for Mycoplasma, sterility, and relevant viruses. These banks will contain several vials of each subclone and safely stored under appropriate conditions.

Figure 9. Strategy for production of mAbs and generation of stable CHO cell lines.

Production of mAbs. Mapp will perform early manufacturing using the polyclonal mini-pools in advance of a clonal cell line in order to develop the manufacturing process and prepare for production of material to support IND-enabling NHP testing and GLP tissue cross reactivity (TCR) testing. MAbs for the NHP and TCR study will be generated using the clonal cell lines identified. Manufacturability is critical to the success of any potential biotherapeutic. Therefore, to ensure the cell lines and product meet the requirements necessary for a successful clinical campaign, Mapp will employ commonly accepted industry practices for evaluating and selecting a CHO cell line suitable for this task. Polyclonal mini-pools and clonal cell lines will be subjected to a standard platform fed-batch process for determination of the cell line most suitable for manufacturing purposes. Cells will be grown in shake flasks or larger systems (*e.g.*, Wave bioreactor; 50L) as appropriate. Supernatant from the bioreactors will be harvested via filtration and subsequently processed using a 2-step purification process. The first chromatography step will be Protein-A affinity. The subsequent chromatography step will likely be an ion exchange step similar to the current platform process in use at Mapp (Q-sepharose). Bulk drug substance will be formulated and stored at an appropriate temperature for future use. The resulting product will be tested using a set of assays typical for mAbs as well as those specific to the NiV/HeV mAbs. Research-grade material for use in biochemical characterization and *in vivo* efficacy testing will be generated from polyclonal pools of transfectants; material to support future toxicology studies will be generated from the clinical cell line using bioreactor and/or shake-flask vessels.

Evaluation of cross-reactivity to human tissues under Good Laboratory Practices (GLP). These studies are conducted by Charles River Laboratories in accordance with FDA guidelines in compliance with GLP regulations. Biotinylated mAb will be incubated with frozen normal human-tissue specimens (all major organs, including adrenal, brain, eye, kidney, bone marrow, liver, lung) for measurement of cross-reactivity. Tissues from three adults and one neonate donor are used and Synagis® (MedImmune) serves as a control mAb. All slides are examined and scored by a board-certified veterinary pathologist.

Commented [LZ8]: Jim, not in our budget…you can say that Mapp will be responsible for funding this activity here or in the budget justification

Submission of pre-IND to FDA (Final Milestone). Upon conclusion of the work effort, a pre-IND will be submitted to the FDA. This regulatory filing will outline the data accumulated to date including the *in vitro* and *in vivo* efficacy data. In addition, our proposed pharmacology/toxicology studies and Phase 1 clinical safety trial design will be presented. The purpose of this submission is to solicit feedback on the current set of data as well as the future plans to best prepare a future IND submission to support the Phase 1 trial.

Potential Pitfalls and Alternative Approaches. We do not anticipate any challenges with the viral G protein binding assays, virus neutralizing assays or hamster infection models as we have considerable experience in each of these techniques and these assays are established in our laboratory. The studies described above aim to generate human Abs that recognize important viral protein epitopes expressed on the surface of the virus during infection. Production of high concentrations of human Abs can be challenging for some clones. We have described the approach above to enhance manufacturability of the Abs. Also, we anticipate having hundreds of mAbs from which to choose, with similar potency and mechanism of action, but diverse expression profiles. We will focus on development of ultra-potent neutralizing Abs that produce well in CHO cells early in the process.

Commented [LZ9]: Perhaps something about PK not being a problem? i.e. mAbs have a long half-life and the course of disease is short,,,

References

1 Desselberger, U. Emerging and re-emerging infectious diseases. *J Infect* **40**, 3-15 (2000).
2 Taylor, L. H., Latham, S. M. & Woolhouse, M. E. Risk factors for human disease emergence. *Philos Trans R Soc Lond B Biol Sci* **356**, 983-989 (2001).
3 Luby, S. P. & Broder, C. C. in *Viral Infections of Humans, Epidemiology and Control* (eds R. A. Kaslow, L. R. Stanberry, & J. W. Le Duc) Ch. 22, 519-536 (Springer Science+Business Media, 2014).
4 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects African green monkeys from Hendra virus challenge. *Sci Transl Med* **3**, 105ra103, doi:10.1126/scitranslmed.3002901 (2011).
5 Marsh, G. A. *et al.* Cedar virus: a novel Henipavirus isolated from Australian bats. *PLoS Pathog* **8**, e1002836, doi:10.1371/journal.ppat.1002836 (2012).
6 Clayton, B. A., Wang, L. F. & Marsh, G. A. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. *Zoonoses and public health* **60**, 69-83, doi:10.1111/j.1863-2378.2012.01501.x (2013).
7 Halpin, K. *et al.* Pteropid Bats are Confirmed as the Reservoir Hosts of Henipaviruses: A Comprehensive Experimental Study of Virus Transmission. *Am J Trop Med Hyg* **85**, 946-951, doi:85/5/946 [pii]

10.4269/ajtmh.2011.10-0567 [doi] (2011).
8 Bossart, K. N., Fusco, D. L. & Broder, C. C. Paramyxovirus entry. *Advances in experimental medicine and biology* **790**, 95-127, doi:10.1007/978-1-4614-7651-1_6 (2013).
9 Bossart, K. N. *et al.* A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. *Sci Transl Med* **4**, 146ra107, doi:10.1126/scitranslmed.3004241 (2012).
10 Broder, C. C. *et al.* Immunization Strategies Against Henipaviruses. *Curr Top Microbiol Immunol* **359**, 197-223, doi:10.1007/82_2012_213 (2012).
11 Wacharapluesadee, S. *et al.* Bat Nipah virus, Thailand. *Emerg Infect Dis* **11**, 1949-1951 (2005).
12 Iehle, C. *et al.* Henipavirus and Tioman virus antibodies in pteropodid bats, Madagascar. *Emerg Infect Dis* **13**, 159-161 (2007).
13 Epstein, J. H. *et al.* Henipavirus infection in fruit bats (Pteropus giganteus), India. *Emerg Infect Dis* **14**, 1309-1311 (2008).
14 Hayman, D. T. *et al.* Evidence of henipavirus infection in West African fruit bats. *PLoS ONE* **3**, e2739, doi:10.1371/journal.pone.0002739 [doi] (2008).
15 Yob, J. M. *et al.* Nipah virus infection in bats (order Chiroptera) in peninsular Malaysia. *Emerg Infect Dis* **7**, 439-441. (2001).
16 Drexler, J. F. *et al.* Henipavirus RNA in African bats. *PLoS One* **4**, e6367, doi:10.1371/journal.pone.0006367 [doi] (2009).
17 Hasebe, F. *et al.* Serologic Evidence of Nipah Virus Infection in Bats, Vietnam. *Emerging Infectious Diseases* **18**, 536-537, doi:http://dx.doi.org/10.3201/eid1803.111121 (2012).
18 Li, Y. *et al.* Antibodies to Nipah or Nipah-like viruses in bats, China. *Emerg Infect Dis* **14**, 1974-1976 (2008).
19 Hayman, D. T. *et al.* Antibodies to henipavirus or henipa-like viruses in domestic pigs in ghana, west Africa. *PLoS One* **6**, e25256, doi:10.1371/journal.pone.0025256 [doi]

PONE-D-11-12752 [pii] (2011).
20 Drexler, J. F. *et al.* Bats host major mammalian paramyxoviruses. *Nat Commun* **3**, 796, doi:10.1038/ncomms1796 (2012).

21 Field, H., de Jong, C. E., Halpin, K. & Smith, C. S. Henipaviruses and fruit bats, Papua New Guinea. *Emerg Infect Dis* **19**, 670-671, doi:10.3201/eid1904.111912 (2013).

22 Breed, A. C. *et al.* Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua New Guinea. *Emerg Infect Dis* **16**, 1997-1999, doi:10.3201/eid1612.100879 [doi] (2010).

23 Lieu, K. G., Marsh, G. A., Wang, L. F. & Netter, H. J. The non-pathogenic Henipavirus Cedar paramyxovirus phosphoprotein has a compromised ability to target STAT1 and STAT2. *Antiviral research* **124**, 69-76, doi:10.1016/j.antiviral.2015.09.017 (2015).

24 Wu, Z. *et al.* Novel Henipa-like virus, Mojiang Paramyxovirus, in rats, China, 2012. *Emerg Infect Dis* **20**, 1064-1066, doi:10.3201/eid2006.131022 (2014).

25 Mire, C. E. *et al.* Pathogenic Differences between Nipah Virus Bangladesh and Malaysia Strains in Primates: Implications for Antibody Therapy. *Scientific reports* **6**, 30916, doi:10.1038/srep30916 (2016).

26 Geisbert, T. W. *et al.* Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. *Sci Transl Med* **6**, 242ra282, doi:10.1126/scitranslmed.3008929 (2014).

27 Xu, K. *et al.* Crystal structure of the hendra virus attachment g glycoprotein bound to a potent cross-reactive neutralizing human monoclonal antibody. *PLoS Pathog* **9**, e1003684, doi:10.1371/journal.ppat.1003684 (2013).

28 Bossart, K. N. *et al.* A neutralizing human monoclonal antibody protects against lethal disease in a new ferret model of acute Nipah virus infection. *PLoS Pathog* **5**, e1000642, doi:10.1371/journal.ppat.1000642 [doi] (2009).

29 Chan, Y. P. *et al.* Biochemical, conformational, and immunogenic analysis of soluble trimeric forms of henipavirus fusion glycoproteins. *J Virol* **86**, 11457-11471, doi:10.1128/jvi.01318-12 (2012).

30 Zhu, Z. *et al.* Exceptionally potent cross-reactive neutralization of Nipah and Hendra viruses by a human monoclonal antibody. *J Infect Dis* **197**, 846-853, doi:10.1086/528801 [doi] (2008).

31 Broder, C. C., Weir, D. L. & Reid, P. A. Hendra virus and Nipah virus animal vaccines. *Vaccine* **34**, 3525-3534, doi:10.1016/j.vaccine.2016.03.075 (2016).

32 Yu, X., McGraw, P. A., House, F. S. & Crowe, J. E., Jr. An optimized electrofusion-based protocol for generating virus-specific human monoclonal antibodies. *J Immunol Methods* **336**, 142-151, doi:10.1016/j.jim.2008.04.008 (2008).

33 Yu, X. *et al.* Neutralizing antibodies derived from the B cells of 1918 influenza pandemic survivors. *Nature* **455**, 532-536, doi:10.1038/nature07231 (2008).

34 Xu, R. *et al.* Structural basis of preexisting immunity to the 2009 H1N1 pandemic influenza virus. *Science* **328**, 357-360, doi:10.1126/science.1186430 (2010).

35 de Alwis, R. *et al.* Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. *Proc Natl Acad Sci U S A* **109**, 7439-7444, doi:10.1073/pnas.1200566109 (2012).

36 Smith, S. A. *et al.* Human monoclonal antibodies derived from memory B cells following live attenuated dengue virus vaccination or natural infection exhibit similar characteristics. *J Infect Dis* **207**, 1898-1908, doi:10.1093/infdis/jit119 (2013).

37 Smith, S. A. *et al.* Isolation of dengue virus-specific memory B cells with live virus antigen from human subjects following natural infection reveals the presence of diverse novel functional groups of antibody clones. *J Virol* **88**, 12233-12241, doi:10.1128/JVI.00247-14 (2014).

38 Smith, S. A. *et al.* The potent and broadly neutralizing human dengue virus-specific monoclonal antibody 1C19 reveals a unique cross-reactive epitope on the bc loop of domain II of the envelope protein. *MBio* **4**, e00873-00813, doi:10.1128/mBio.00873-13 (2013).

39 Smith, S. A. *et al.* Persistence of circulating memory B cell clones with potential for dengue virus disease enhancement for decades following infection. *J Virol* **86**, 2665-2675, doi:10.1128/JVI.06335-11 (2012).

40 Weitkamp, J. H. *et al.* Generation of recombinant human monoclonal antibodies to rotavirus from single antigen-specific B cells selected with fluorescent virus-like particles. *J Immunol Methods* **275**, 223-237 (2003).

41 Alvarado, G. & Crowe, J. E., Jr. Development of Human Monoclonal Antibodies Against Respiratory Syncytial Virus Using a High Efficiency Human Hybridoma Technique. *Methods Mol Biol* **1442**, 63-76, doi:10.1007/978-1-4939-3687-8_6 (2016).

42 Barbas, C. F., 3rd *et al.* Human monoclonal Fab fragments derived from a combinatorial library bind to respiratory syncytial virus F glycoprotein and neutralize infectivity. *Proc Natl Acad Sci U S A* **89**, 10164-10168 (1992).

43 Bates, J. T. *et al.* Escape from neutralization by the respiratory syncytial virus-specific neutralizing monoclonal antibody palivizumab is driven by changes in on-rate of binding to the fusion protein. *Virology* **454-455**, 139-144, doi:10.1016/j.virol.2014.02.010 (2014).

44 Bates, J. T. *et al.* Reversion of somatic mutations of the respiratory syncytial virus-specific human monoclonal antibody Fab19 reveal a direct relationship between association rate and neutralizing potency. *J Immunol* **190**, 3732-3739, doi:10.4049/jimmunol.1202964 (2013).

45 Crowe, J. E., Jr. *et al.* Recombinant human respiratory syncytial virus (RSV) monoclonal antibody Fab is effective therapeutically when introduced directly into the lungs of RSV-infected mice. *Proc Natl Acad Sci U S A* **91**, 1386-1390 (1994).

46 Crowe, J. E., Jr. *et al.* Isolation of a second recombinant human respiratory syncytial virus monoclonal antibody fragment (Fab RSVF2-5) that exhibits therapeutic efficacy in vivo. *J Infect Dis* **177**, 1073-1076 (1998).

47 Mousa, J. J., Kose, N., Matta, P., Gilchuk, P. & Crowe, J. E., Jr. A novel pre-fusion conformation-specific neutralizing epitope on the respiratory syncytial virus fusion protein. *Nat Microbiol* **2**, 16271, doi:10.1038/nmicrobiol.2016.271 (2017).

48 Mousa, J. J. *et al.* Structural basis for nonneutralizing antibody competition at antigenic site II of the respiratory syncytial virus fusion protein. *Proc Natl Acad Sci U S A* **113**, E6849-E6858, doi:10.1073/pnas.1609449113 (2016).

49 Wen, X. *et al.* Structural basis for antibody cross-neutralization of respiratory syncytial virus and human metapneumovirus. *Nat Microbiol* **2**, 16272, doi:10.1038/nmicrobiol.2016.272 (2017).

50 Wen, X. *et al.* Structure of the human metapneumovirus fusion protein with neutralizing antibody identifies a pneumovirus antigenic site. *Nat Struct Mol Biol* **19**, 461-463, doi:10.1038/nsmb.2250 (2012).

51 Williams, J. V. *et al.* A recombinant human monoclonal antibody to human metapneumovirus fusion protein that neutralizes virus in vitro and is effective therapeutically in vivo. *J Virol* **81**, 8315-8324, doi:10.1128/JVI.00106-07 (2007).

52 Aiyegbo, M. S. *et al.* Differential accessibility of a rotavirus VP6 epitope in trimers comprising type I, II, or III channels as revealed by binding of a human rotavirus VP6-specific antibody. *J Virol* **88**, 469-476, doi:10.1128/JVI.01665-13 (2014).

53 Aiyegbo, M. S. *et al.* Human rotavirus VP6-specific antibodies mediate intracellular neutralization by binding to a quaternary structure in the transcriptional pore. *PLoS One* **8**, e61101, doi:10.1371/journal.pone.0061101 (2013).

54 Cho, M. J. *et al.* Determinants of VH1-46 Cross-Reactivity to Pemphigus Vulgaris Autoantigen Desmoglein 3 and Rotavirus Antigen VP6. *J Immunol* **197**, 1065-1073, doi:10.4049/jimmunol.1600567 (2016).

55 Kallewaard, N. L. *et al.* Functional maturation of the human antibody response to rotavirus. *J Immunol* **180**, 3980-3989 (2008).

56 Tian, C. *et al.* Immunodominance of the VH1-46 antibody gene segment in the primary repertoire of human rotavirus-specific B cells is reduced in the memory compartment through somatic mutation of nondominant clones. *J Immunol* **180**, 3279-3288 (2008).

57 Weitkamp, J. H. *et al.* Infant and adult human B cell responses to rotavirus share common immunodominant variable gene repertoires. *J Immunol* **171**, 4680-4688 (2003).

58 Weitkamp, J. H. *et al.* VH1-46 is the dominant immunoglobulin heavy chain gene segment in rotavirus-specific memory B cells expressing the intestinal homing receptor alpha4beta7. *J Immunol* **174**, 3454-3460 (2005).

59 Weitkamp, J. H., Lafleur, B. J. & Crowe, J. E., Jr. Rotavirus-specific CD5+ B cells in young children exhibit a distinct antibody repertoire compared with CD5- B cells. *Hum Immunol* **67**, 33-42, doi:10.1016/j.humimm.2006.02.024 (2006).

60 Sapparapu, G. *et al.* Neutralizing human antibodies prevent Zika virus replication and fetal disease in mice. *Nature* **540**, 443-447, doi:10.1038/nature20564 (2016).

61 Shanker, S. *et al.* Structural basis for norovirus neutralization by an HBGA blocking human IgA antibody. *Proc Natl Acad Sci U S A* **113**, E5830-E5837, doi:10.1073/pnas.1609990113 (2016).

62 Bangaru, S. *et al.* Recognition of influenza H3N2 variant virus by human neutralizing antibodies. *JCI Insight* **1**, doi:10.1172/jci.insight.86673 (2016).

63 Krause, J. C. *et al.* A broadly neutralizing human monoclonal antibody that recognizes a conserved, novel epitope on the globular head of the influenza H1N1 virus hemagglutinin. *J Virol* **85**, 10905-10908, doi:10.1128/JVI.00700-11 (2011).

64 Krause, J. C. *et al.* Epitope-specific human influenza antibody repertoires diversify by B cell intraclonal sequence divergence and interclonal convergence. *J Immunol* **187**, 3704-3711, doi:10.4049/jimmunol.1101823 (2011).
65 Krause, J. C. *et al.* Naturally occurring human monoclonal antibodies neutralize both 1918 and 2009 pandemic influenza A (H1N1) viruses. *J Virol* **84**, 3127-3130, doi:10.1128/JVI.02184-09 (2010).
66 Thornburg, N. J. *et al.* Human antibodies that neutralize respiratory droplet transmissible H5N1 influenza viruses. *J Clin Invest* **123**, 4405-4409, doi:10.1172/JCI69377 (2013).
67 Thornburg, N. J. *et al.* H7N9 influenza virus neutralizing antibodies that possess few somatic mutations. *J Clin Invest* **126**, 1482-1494, doi:10.1172/JCI85317 (2016).
68 Tsibane, T. *et al.* Influenza human monoclonal antibody 1F1 interacts with three major antigenic sites and residues mediating human receptor specificity in H1N1 viruses. *PLoS Pathog* **8**, e1003067, doi:10.1371/journal.ppat.1003067 (2012).
69 Winarski, K. L. *et al.* Vaccine-elicited antibody that neutralizes H5N1 influenza and variants binds the receptor site and polymorphic sites. *Proc Natl Acad Sci U S A* **112**, 9346-9351, doi:10.1073/pnas.1502762112 (2015).
70 Xu, R. *et al.* A recurring motif for antibody recognition of the receptor-binding site of influenza hemagglutinin. *Nat Struct Mol Biol* **20**, 363-370, doi:10.1038/nsmb.2500 (2013).
71 de Alwis, R. *et al.* Dengue viruses are enhanced by distinct populations of serotype cross-reactive antibodies in human immune sera. *PLoS Pathog* **10**, e1004386, doi:10.1371/journal.ppat.1004386 (2014).
72 Fibriansah, G. *et al.* DENGUE VIRUS. Cryo-EM structure of an antibody that neutralizes dengue virus type 2 by locking E protein dimers. *Science* **349**, 88-91, doi:10.1126/science.aaa8651 (2015).
73 Fibriansah, G. *et al.* A potent anti-dengue human antibody preferentially recognizes the conformation of E protein monomers assembled on the virus surface. *EMBO Mol Med* **6**, 358-371, doi:10.1002/emmm.201303404 (2014).
74 Fibriansah, G. *et al.* A highly potent human antibody neutralizes dengue virus serotype 3 by binding across three surface proteins. *Nat Commun* **6**, 6341, doi:10.1038/ncomms7341 (2015).
75 Nivarthi, U. K. *et al.* Mapping the Human Memory B Cell and Serum Neutralizing Antibody Responses to Dengue Virus Serotype 4 Infection and Vaccination. *J Virol* **91**, doi:10.1128/JVI.02041-16 (2017).
76 Smith, S. A. *et al.* Dengue Virus prM-Specific Human Monoclonal Antibodies with Virus Replication-Enhancing Properties Recognize a Single Immunodominant Antigenic Site. *J Virol* **90**, 780-789, doi:10.1128/JVI.01805-15 (2015).
77 Hasan, S. S. *et al.* A human antibody against Zika virus crosslinks the E protein to prevent infection. *Nat Commun* **8**, 14722, doi:10.1038/ncomms14722 (2017).
78 Bailey, J. R. *et al.* Broadly neutralizing antibodies with few somatic mutations and hepatitis C virus clearance. *JCI Insight* **2**, doi:10.1172/jci.insight.92872 (2017).
79 Hicar, M. D. *et al.* Pseudovirion particles bearing native HIV envelope trimers facilitate a novel method for generating human neutralizing monoclonal antibodies against HIV. *J Acquir Immune Defic Syndr* **54**, 223-235, doi:10.1097/QAI.0b013e3181dc98a3 (2010).
80 Hicar, M. D. *et al.* Human Antibodies that Recognize Novel Immunodominant Quaternary Epitopes on the HIV-1 Env Protein. *PLoS One* **11**, e0158861, doi:10.1371/journal.pone.0158861 (2016).
81 Hicar, M. D. *et al.* Low frequency of broadly neutralizing HIV antibodies during chronic infection even in quaternary epitope targeting antibodies containing large numbers of somatic mutations. *Mol Immunol* **70**, 94-103, doi:10.1016/j.molimm.2015.12.002 (2016).
82 Willis, J. R. *et al.* Long antibody HCDR3s from HIV-naive donors presented on a PG9 neutralizing antibody background mediate HIV neutralization. *Proc Natl Acad Sci U S A* **113**, 4446-4451, doi:10.1073/pnas.1518405113 (2016).
83 Willis, J. R. *et al.* Redesigned HIV antibodies exhibit enhanced neutralizing potency and breadth. *J Clin Invest* **125**, 2523-2531, doi:10.1172/JCI80693 (2015).
84 Broeckel, R. *et al.* Therapeutic administration of a recombinant human monoclonal antibody reduces the severity of chikungunya virus disease in rhesus macaques. *PLoS Negl Trop Dis* **11**, e0005637, doi:10.1371/journal.pntd.0005637 (2017).
85 Hawman, D. W. *et al.* Pathogenic Chikungunya Virus Evades B Cell Responses to Establish Persistence. *Cell Rep* **16**, 1326-1338, doi:10.1016/j.celrep.2016.06.076 (2016).

86 Long, F. *et al.* Cryo-EM structures elucidate neutralizing mechanisms of anti-chikungunya human monoclonal antibodies with therapeutic activity. *Proc Natl Acad Sci U S A* **112**, 13898-13903, doi:10.1073/pnas.1515558112 (2015).

87 Miner, J. J. *et al.* Therapy with CTLA4-Ig and an antiviral monoclonal antibody controls chikungunya virus arthritis. *Sci Transl Med* **9**, doi:10.1126/scitranslmed.aah3438 (2017).

88 Bornholdt, Z. A. *et al.* Host-Primed Ebola Virus GP Exposes a Hydrophobic NPC1 Receptor-Binding Pocket, Revealing a Target for Broadly Neutralizing Antibodies. *MBio* **7**, e02154-02115, doi:10.1128/mBio.02154-15 (2016).

89 Flyak, A. I. *et al.* Cross-Reactive and Potent Neutralizing Antibody Responses in Human Survivors of Natural Ebolavirus Infection. *Cell* **164**, 392-405, doi:10.1016/j.cell.2015.12.022 (2016).

90 Pallesen, J. *et al.* Structures of Ebola virus GP and sGP in complex with therapeutic antibodies. *Nat Microbiol* **1**, 16128, doi:10.1038/nmicrobiol.2016.128 (2016).

91 Wec, A. Z. *et al.* A "Trojan horse" bispecific-antibody strategy for broad protection against ebolaviruses. *Science* **354**, 350-354, doi:10.1126/science.aag3267 (2016).

92 Flyak, A. I. *et al.* Mechanism of human antibody-mediated neutralization of Marburg virus. *Cell* **160**, 893-903, doi:10.1016/j.cell.2015.01.031 (2015).

93 Hashiguchi, T. *et al.* Structural basis for Marburg virus neutralization by a cross-reactive human antibody. *Cell* **160**, 904-912, doi:10.1016/j.cell.2015.01.041 (2015).

94 Mire, C. E. *et al.* Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci Transl Med* **9**, doi:10.1126/scitranslmed.aai8711 (2017).

95 Gilchuk, I. *et al.* Cross-Neutralizing and Protective Human Antibody Specificities to Poxvirus Infections. *Cell* **167**, 684-694 e689, doi:10.1016/j.cell.2016.09.049 (2016).

96 Thomsen, I. P. *et al.* Monoclonal Antibodies Against the Staphylococcus aureus Bicomponent Leukotoxin AB Isolated Following Invasive Human Infection Reveal Diverse Binding and Modes of Action. *J Infect Dis* **215**, 1124-1131, doi:10.1093/infdis/jix071 (2017).

97 Laing, E. D. *et al.* Serologic Evidence of Fruit Bat Exposure to Filoviruses, Singapore, 2011-2016. *Emerg Infect Dis* **24**, 114-117, doi:10.3201/eid2401.170401 (2018).

98 Mire, C. E. *et al.* A recombinant Hendra virus G glycoprotein subunit vaccine protects nonhuman primates against Hendra virus challenge. *J Virol* **88**, 4624-4631, doi:10.1128/jvi.00005-14 (2014).

99 Middleton, D. *et al.* Hendra virus vaccine, a one health approach to protecting horse, human, and environmental health. *Emerg Infect Dis* **20**, 372-379, doi:10.3201/eid2003.131159 (2014).

100 Peel, A. J. *et al.* Continent-wide panmixia of an African fruit bat facilitates transmission of potentially zoonotic viruses. *Nat Commun* **4**, 2770, doi:10.1038/ncomms3770 (2013).

101 Pallister, J. A. *et al.* Vaccination of ferrets with a recombinant G glycoprotein subunit vaccine provides protection against Nipah virus disease for over 12 months. *Virol J* **10**, 237, doi:10.1186/1743-422x-10-237 (2013).

102 Pallister, J. *et al.* A recombinant Hendra virus G glycoprotein-based subunit vaccine protects ferrets from lethal Hendra virus challenge. *Vaccine* **29**, 5623-5630, doi:10.1016/j.vaccine.2011.06.015 (2011).

103 Chan, Y. P., Yan, L., Feng, Y. R. & Broder, C. C. Preparation of Recombinant Viral Glycoproteins for Novel and Therapeutic Antibody Discovery. *Methods Mol Biol* **In Press** (2008).

104 Mungall, B. A. *et al.* Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. *J Virol* **80**, 12293-12302 (2006).

105 Bossart, K. N. *et al.* Receptor binding, fusion inhibition, and induction of cross-reactive neutralizing antibodies by a soluble G glycoprotein of Hendra virus. *J Virol* **79**, 6690-6702 (2005).

106 Bonaparte, M. I. *et al.* Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. *Proc Natl Acad Sci U S A* **102**, 10652-10657 (2005).

107 Weingartl, H. M. *et al.* Recombinant nipah virus vaccines protect pigs against challenge. *J Virol* **80**, 7929-7938 (2006).

108 Tamin, A. *et al.* Development of a neutralization assay for Nipah virus using pseudotype particles. *J Virol Methods* **160**, 1-6, doi:10.1016/j.jviromet.2009.02.025 (2009).

109 Kaku, Y. *et al.* Second generation of pseudotype-based serum neutralization assay for Nipah virus antibodies: sensitive and high-throughput analysis utilizing secreted alkaline phosphatase. *J Virol Methods* **179**, 226-232, doi:10.1016/j.jviromet.2011.11.003 (2012).

110 Khetawat, D. & Broder, C. C. A functional henipavirus envelope glycoprotein pseudotyped lentivirus assay system. *Virol J* **7**, 312, doi:1743-422X-7-312 [pii]

10.1186/1743-422X-7-312 [doi] (2010).
111 Bossart, K. N. *et al.* Neutralization assays for differential henipavirus serology using Bio-Plex Protein Array Systems. *J Virol Methods* **142**, 29-40 (2007).
112 Peeters, B. P., de Leeuw, O. S., Koch, G. & Gielkens, A. L. Rescue of Newcastle disease virus from cloned cDNA: evidence that cleavability of the fusion protein is a major determinant for virulence. *J Virol* **73**, 5001-5009 (1999).
113 Marsh, G. A. *et al.* Recombinant Hendra viruses expressing a reporter gene retain pathogenicity in ferrets. *Virol J* **10**, 95, doi:10.1186/1743-422x-10-95 (2013).
114 Yoneda, M. *et al.* Establishment of a Nipah virus rescue system. *Proc Natl Acad Sci U S A* **103**, 16508-16513, doi:0606972103 [pii]

10.1073/pnas.0606972103 [doi] (2006).
115 Yun, T. *et al.* Efficient Reverse Genetics Reveals Genetic Determinants of Budding and Fusogenic Differences between Nipah and Hendra Viruses and Enables Real-Time Monitoring of Viral Spread in Small Animal Models of Henipavirus Infection. *J Virol* **89**, 1242-1253, doi:10.1128/jvi.02583-14 (2015).
116 Hall, M. P. *et al.* Engineered luciferase reporter from a deep sea shrimp utilizing a novel imidazopyrazinone substrate. *ACS chemical biology* **7**, 1848-1857, doi:10.1021/cb3002478 (2012).
117 Bossart, K. N. & Broder, C. C. Viral glycoprotein-mediated cell fusion assays using vaccinia virus vectors. *Methods Mol Biol* **269**, 309-332 (2004).
118 Bossart, K. N. *et al.* Membrane fusion tropism and heterotypic functional activities of the nipah virus and hendra virus envelope glycoproteins. *J Virol* **76**, 11186-11198. (2002).
119 Bossart, K. N., Wang, L. F., Eaton, B. T. & Broder, C. C. Functional expression and membrane fusion tropism of the envelope glycoproteins of Hendra virus. *Virology* **290**, 121-135. (2001).

From: Larry Zeitlin on behalf of Larry Zeitlin <Larry.Zeitlin@mappbio.com>
To: Crowe, James; Broder, Christopher; Geisbert, Thomas W.
Subject: Re: Version 5 henipavirus partnership grant
Date: Thursday, January 11, 2018 9:15:47 PM
Attachments: henipavirus science 5 LZ pictures deleted.docx

Jim,

Looks like its coming together…some edits/comments for your consideration.

Good luck
LZ

From: James Crowe <james.crowe@vanderbilt.edu>
Date: Thursday, January 11, 2018 at 4:33 PM
To: Larry Zeitlin <Larry.Zeitlin@mappbio.com>, Christopher Broder <christopher.broder@usuhs.edu>, Thomas Geisbert <twgeisbe@utmb.edu>
Subject: Version 5 henipavirus partnership grant

This is a little cleaned up compared the version i sent earlier.
If you haven't started, use this to edit
Its getting big so i zipped it
It you need to , you can just send edited pieces back, or share a dropbox or something

---

From: "Larry Zeitlin, Ph.D." <Larry.Zeitlin@mappbio.com>
Date: Thursday, January 11, 2018 at 2:39 PM
To: "James Crowe, Jr." <james.crowe@Vanderbilt.Edu>, Chris Broder <christopher.broder@usuhs.edu>, Thomas Geisbert <twgeisbe@utmb.edu>
Subject: Re: nipah

Jim,
What is the drop dead deadline to get edits back to you?
Best
LZ

From: James Crowe <james.crowe@vanderbilt.edu>
Date: Thursday, January 11, 2018 at 12:26 PM
To: Christopher Broder <christopher.broder@usuhs.edu>, Thomas Geisbert <twgeisbe@utmb.edu>, Larry Zeitlin <Larry.Zeitlin@mappbio.com>
Subject: Re: nipah

Here is what I have. It's a little bit of a shambles, but it has most of the pieces and parts.
Each of you could make your parts much better i am sure
Also, i haven't really integrated it top to bottom to make it smooth, and formatting is bad and inconsistent

Sorry!!!! Embarrassed

From: Chris Broder <christopher.broder@usuhs.edu>
Date: Thursday, January 11, 2018 at 1:44 PM
To: Thomas Geisbert <twgeisbe@utmb.edu>
Cc: "Larry Zeitlin, Ph.D." <Larry.Zeitlin@mappbio.com>, "James Crowe, Jr." <james.crowe@Vanderbilt.Edu>
Subject: Re: nipah

yup, on board as well

On Thu, Jan 11, 2018 at 2:18 PM, Geisbert, Thomas W. <twgeisbe@utmb.edu> wrote:

> Me too
>
> ---
>
> **From:** Larry Zeitlin [Larry.Zeitlin@mappbio.com]
> **Sent:** Thursday, January 11, 2018 1:02 PM
> **To:** Crowe, James; Geisbert, Thomas W.
> **Cc:** Broder, Christopher
> **Subject:** Re: nipah
>
> I'll be available this afternoon and in the morning for any edits you need from me.
>
> Safe travels
> LZ
>
> ---
>
> **From:** James Crowe <james.crowe@vanderbilt.edu>
> **Date:** Thursday, January 11, 2018 at 10:57 AM
> **To:** Thomas Geisbert <twgeisbe@utmb.edu>
> **Cc:** Christopher Broder <christopher.broder@usuhs.edu>, Larry Zeitlin <Larry.Zeitlin@mappbio.com>
> **Subject:** Re: nipah
>
> Not really. I'm struggling. I am boarding a plane for 4 hours so hope to make some progress if my battery lasts
>
> If I can get WiFi on plane I will send you all he current draft
>
> The places that each of you would be responsible for need help. But hey are not long and you'll be able to fix them quickly
>
> Sent from my iPhone
>
> On Jan 11, 2018, at 12:12 PM, Geisbert, Thomas W. <twgeisbe@UTMB.EDU> wrote:

> Jim,
>
> Are you OK on everything?
>
> Tom
>
> ---
>
> **From:** Crowe, James [james.crowe@Vanderbilt.Edu]
> **Sent:** Tuesday, January 09, 2018 11:55 AM
> **To:** Broder, Christopher; Geisbert, Thomas W.
> **Cc:** Larry Zeitlin
> **Subject:** Re: nipah
>
> Thanks Chris!
>
> ---
>
> **From:** Chris Broder <christopher.broder@usuhs.edu>
> **Date:** Tuesday, January 9, 2018 at 10:56 AM
> **To:** Thomas Geisbert <twgeisbe@utmb.edu>
> **Cc:** "James Crowe, Jr." <james.crowe@Vanderbilt.Edu>, "Larry Zeitlin, Ph.D." <Larry.Zeitlin@mappbio.com>
> **Subject:** Re: nipah
>
> Jim.
>
> for pt #4 to Tom,   here is an additional section of grant text. Also., you can capture the issue of new SNT idea from the document i sent before xmas.
> I highlighted in blue. and added some red text.
>
> Chris
>
> On Tue, Jan 9, 2018 at 10:02 AM, Geisbert, Thomas W. <twgeisbe@utmb.edu> wrote:
>
>> Chris,
>>
>> See below. Larry and I are providing the requested info. Do you have any time to help Jim with this? On number 4 to me below I sent him a few of our pubs. Not sure if you have anything from grants that could be co-opted here.
>>
>> Many thanks!
>>
>> Tom
>>
>> ---
>>
>> **From:** Crowe, James [james.crowe@Vanderbilt.Edu]
>> **Sent:** Monday, January 08, 2018 9:42 PM
>> **To:** Geisbert, Thomas W.; Larry Zeitlin

Subject: Re: nipah

I'm kinda hurting bad on this. I made the mistake/choice/blessing of taking ~ 8 days off at the end of the year, and have been completely underwater ever since. I still want to submit this, but it's going to be a scramble. A lot (but not all) of the admin stuff is done. But the science is still in shambles. I need to put it together over the next day.

What I need, or could benefit from, from you guys:
TOM

1. Select agent section, since there is Hendra and Nipah
2. Biosafety section
3. Vertebrate Animals section

4. Any basic (even extended) text or old grant sections on "HeV and NiV are xxx viruses", "bad for you", and "neut test is done by x, with these strains: yy", "animal models are . . . "

Larry

1. Anything you would do for characterizing manufacturability or development of the lead mabs, for the science section
2. A "product development plan section" on human mAbs for these viruses. Kinda boilerplate, which i am guessing you have already

---

From: Thomas Geisbert <twgeisbe@UTMB.EDU>
Date: Sunday, January 7, 2018 at 3:57 PM
To: "Larry Zeitlin, Ph.D." <Larry.Zeitlin@mappbio.com>, "James Crowe, Jr." <james.crowe@Vanderbilt.Edu>
Subject: RE: nipah

Jim,

Likewise. Please let me know if you need anything on the proposal.

Best,

Tom

From: Larry Zeitlin [Larry.Zeitlin@mappbio.com]

> > **Sent:** Thursday, January 04, 2018 1:40 PM
> > **To:** James Crowe
> > **Cc:** Geisbert, Thomas W.
> > **Subject:** nipah
> >
> > Hi Jim,
> >
> > Just checking on what you'll need from me for the proposal.
> >
> > Best wishes for 2018
> >
> > LZ
>
> --
> Christopher C. Broder, Ph.D.
> Professor of Microbiology and Immunology
> and Emerging Infectious Diseases
> Director, Emerging Infectious Diseases Graduate Program
>
> Department of Microbiology and Immunology, B4106
> Uniformed Services University
> 4301 Jones Bridge Rd, Bethesda, MD 20814-4799
>
> **USU is "America's Medical School"**
> Email: christopher.broder@usuhs.edu
> http://www.usuhs.mil/eid/
> TEL: 301-295-3401
> FAX: 301-295-1545/3773
>
> (b) (6) [redacted]
>
>

--
Christopher C. Broder, Ph.D.
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and Emerging Infectious Diseases
Director, Emerging Infectious Diseases Graduate Program

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FAX: 301-295-1545/3773

(b) (6)

DRAFT V5 —NOT FOR DISTRIBUTION— January 1, 2018

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, outlining the underpinning strategic goals, and prioritizing research areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus (NiV) infection.

*(**Note:** The content below is organized into the three main categories for R&D of MCMs [diagnostics, therapeutics, and vaccines] to allow reviewers an opportunity to follow the lines of thinking for each category. Some issues are cross cutting and apply to more than one category. As this draft is refined, the content may be reorganized to create one over all roadmap and to group cross-cutting issues together. In addition, this document is relatively detailed to ensure that the most important issues are identified and discussed. As the document evolves, certain items may be "rolled up" into a smaller number of broader issues, strategic goals, and primary activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak occurred in pigs and humans in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the natural reservoir for NiV (Pteropus bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, and Ghana. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and human-to-human NiV transmission. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a role, with human consumption of bat-contaminated raw date palm sap serving as the primary NiV source. In humans, NiV infection has neurological and respiratory syndromes, with fever, headache, dizziness, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and/or relapsing encephalitis, and survivors may experience long-term neurological deficits. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B). NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). To date, HeV disease has been confined to Australia. An outbreak of an unidentified Henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2015.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and

timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's initial list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no medical countermeasures [MCMs] exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of medical countermeasures to prevent and control LF outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment, effective community engagement, and workforce development and training in at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV (and other closely related henipaviruses) that are available and readily deployable for use when needed. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

Clinical aspects:

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. Additionally, latent disease can occur long after initial infection. Both of these factors can hinder accurate diagnosis.
- Quality, quantity, types and timing of collection of clinical samples can greatly affect the accuracy of laboratory test results.

Laboratory aspects:

- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- High-level biocontainment requirements may pose an impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- Various types of test methods/platforms are required to cover testing at different phases of Nipah virus infections. As diagnostic capabilities and resources are often inadequate in endemic

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and at risk areas, test platforms/types to be developed for these areas need to be cheap, simple to perform and require minimum instrumentation.

Virus aspects:

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- As Nipah virus is PC4 virus, ~~D~~diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- NiV appears to have the potential for strain heterogeneity and antigenic variability, which could potentially influence accuracy of diagnostic testing over time.
- The natural reservoir for NiV is fruit bats of the Pteropus genus, which have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.
- Pteropus bat species also appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.

Financial constraints:

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- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- ~~High-level biocontainment requirements may pose an impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*~~

Key needs

- A WHO clinical guideline on early diagnosis and management of Nipah virus infection need to be developed to guide clinicians/epidemiologists on early detection of cases (based on clinical and epidemiological data), samples collection/transport and management of cases.
- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.

- Diagnostic preparedness to detect HeV and other emergent henipaviruses in humans and animals as they arise.
- Proficiency testing, to monitor reproducibility and performance of NiV diagnostic assays in the field.
- Enhanced laboratory infrastructure in at-risk areas, with a particular focus on improving regional and national laboratory capacity for case and outbreak confirmation.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the DIVA test.
- Long-term need: multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or additional high-consequence pathogens.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, other body fluids such as saliva, urine and respiratory fluid, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, and limits of detection) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs).
- Additional validation data are needed to assess performance characteristics of NiV diagnostic assays against the various strains of NiV.
- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor antigenic changes over time that may impact diagnostic testing and the epidemiologic and clinical features of disease.
- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats. *(*cross cutting)*

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Promote the development and assessment of affordable, highly sensitive and specific, point-of-care diagnostic tests for use in humans and animals that have minimal requirements for biosafety precautions and staff training and that can detect NiV and other related henipaviruses.
3. Strengthen laboratory infrastructure and capacity in the endemic and at-risk areas to improve early diagnostic capabilities.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, other body fluids such as saliva, urine and respiratory fluid, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and develop appropriate standards for their use.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Expand research** to further understand the ecologic and epidemiology of NiV and other henipaviruses in human and animal populations over time, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to research** development of serologic tests to distinguish vaccinated from infected animals, if henipavirus vaccines are to be widely used in livestock populations.

Product development

- **Generate** a TPP for NiV diagnostics.
- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other high-consequence pathogens in humans and animals.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure for detection of NiV in endemic and at-risk areas, particularly in support of enhanced national and regional diagnostic capabilities.
- **Improve surveillance capacity** to: (1) better define the incidence of disease in NiV-endemic areas and (2) promote surveillance in non-endemic areas to enhance detection of emergent NiV disease or disease caused by other related henipaviruses.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV point-of-care diagnostics.
- **Ensure** access to regulatory review and authorization of NiV diagnostic assays.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostic products to endemic and at risk areas.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV disease occurs in relatively small outbreak settings, the low incidence of disease poses a major challenge for conducting phase 3 clinical trials; therefore, alternative regulatory pathways will need to be considered for licensure of vaccines or therapeutics. *(*cross cutting)*
- Differences between the NiV_M and NiV_B strains may impact the effectiveness of developed MCMs, yet NiV_M has been used significantly more often than NiV_B in research. *(*cross cutting)*
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier and can access the brain and spinal cord to inhibit viral replication. This is particularly important for preventing late-onset CNS disease.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons. In addition, the healthcare systems in endemic areas are not able to rapidly identify contacts most likely to benefit from PEP. *(*cross cutting)*
- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.

- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat both acute and chronic (relapsing) NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock. *(*cross cutting)*

Knowledge gaps

- Additional information is needed on the immunology and pathogenesis of NiV in order to develop appropriate treatment options. This includes evaluating the pathophysiologic differences between NiV_B and NiV_M infection, determining the mechanisms of late-onset and relapsing NiV encephalitis, identifying factors influencing the development of permanent neurological sequela, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ribavirin was found to reduce mortality by 36% in the 1998-1999 NiV outbreak in Malaysia and is considered an option for treatment of NiV infection. However, animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to HeV-infected horses. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.
- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV_B and NiV_M, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV_B to be shorter than for NiV_M.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the type of exposures that warrant such intervention and the most appropriate agents to administer.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of individual components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform supportive care and best-practice guidelines.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), such as determining the most appropriate lethal NiV dose for MCM development and

identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection and ensure that therapies are prepositioned in endemic areas for use as needed.
2. Stimulate basic research for better understanding of NiV pathogenesis and the immune response to infection.
3. Strengthen clinical and laboratory infrastructure in endemic and at-risk areas to promote early diagnosis of NiV cases so that effective treatment and PEP can be rapidly administered.
4. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Conduct** basic science research on the immunology and pathogenesis of NiV infections to inform development of new therapeutic agents.
- **Determine** the key differences between NiV_B and NiV_M that may have implications for the development of safe and effective NiV therapeutics.
- **Continue to research** the safety, tolerability, and efficacy of investigational therapies for NiV infection, including animal studies and clinical trials as appropriate and feasible.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against both NiV_B and NiV_M and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Refine, standardize, and validate** animal challenge models for future assessment of promising therapeutic candidates.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV therapeutics.
- **Support** plans for licensure/registration, adequate manufacturing, and subsequent distribution of safe and effective therapies to endemic areas and to other at-risk areas on an as-needed basis for outbreak control.
- **Secure** financing for procurement and deployment of NiV therapeutics for outbreak control once treatment agents are available.
- **Develop** treatment and PEP guidance as new therapies demonstrate safety and efficacy.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV occurs as relatively small, focal outbreaks, the low incidence of infection poses a major challenge for conducting phase 3 clinical trials for efficacy demonstration; therefore, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics. *(*cross cutting)*
- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- The absence of improved diagnostic assays for timely diagnosis of infection creates a challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control, once vaccines are available for use. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, African green monkeys (AGMs) are regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*

Key needs

- Safe and effective PEP (either vaccines or therapeutic agents) to prevent infection following exposure to NiV and guidance on their use. Such countermeasures are important tools to protect healthcare workers, family caregivers, and livestock workers. *(*cross cutting)*
- Nipah vaccines that can protect against both NiV_M and NiV_B in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks. Research tools, such as standardized neutralization assays, to compare immunogenicity of different vaccines.
- Enhanced surveillance capacity to provide more accurate information on the current incidence and geographic distribution of NiV in endemic and at-risk areas in order to refine potential vaccination strategies, particularly over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- The mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis are unknown. Further research to better understand viral clearance has important implications for development of vaccines and therapeutics.
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B; only a few studies demonstrating cross-protection have been performed to date.
- Further research is needed to determine durability and safety profiles of candidate vaccines, such as VSV recombinants, where concerns regarding potential neurotropism have been raised.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoestis).
- The identification of specific correlates of protection and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines and in simulating various epidemiologic scenarios that may impact strategies for vaccine use.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), including determining the most appropriate NiV dose for MCM development (Johnston 2015) and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses. Ongoing research into developing NiV/HeV vaccines for livestock is needed to further assess the merit of this potential control strategy.

- If evidence at some point supports the need for a broader, population-based vaccination strategy, additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.

Strategic Goals

1. Develop, evaluate, and license NiV vaccines for use in humans and potentially livestock, primarily for reactive/outbreak use in endemic and at-risk areas.
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection.
- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research efforts and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Generate** research tools (e.g., standardized neutralization assays) to compare immunogenicity of different vaccines.
- **Complete preclinical evaluation** of promising candidate NiV vaccines for safety, immunogenicity, efficacy, correlates of protection, and durability.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and livestock, primarily for reactive use during outbreaks.

- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.

Key capacities

- **Refine and validate** relevant animal challenge models for NiV infection.
- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the true burden and incidence of NiV disease, which may in turn, inform vaccination strategies.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV vaccines.
- **Ensure** access to regulatory review and authorization of NiV vaccines, particularly if non-traditional pathways are needed for licensure.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.
- **Provide** ongoing assessment to inform development and maintenance of NiV vaccine stockpiles over time; mathematical modelling may be used to contribute to this assessment.
- **Continue** to provide guidance on vaccination strategies for various target populations and epidemiologic scenarios, once vaccines are available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis, Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, outlining the underpinning strategic goals, and prioritizing research areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus (NiV) infection.

*(**Note:** The content below is organized into the three main categories for R&D of MCMs [diagnostics, therapeutics, and vaccines] to allow reviewers an opportunity to follow the lines of thinking for each category. Some issues are cross cutting and apply to more than one category. As this draft is refined, the content may be reorganized to create one over all roadmap and to group cross-cutting issues together. In addition, this document is relatively detailed to ensure that the most important issues are identified and discussed. As the document evolves, certain items may be "rolled up" into a smaller number of broader issues, strategic goals, and primary activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak occurred in pigs and humans in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the natural reservoir for NiV (Pteropus bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, and Ghana. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and human-to-human NiV transmission. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a role, with human consumption of bat-contaminated raw date palm sap serving as the primary NiV source. In humans, NiV infection has neurological and respiratory syndromes, with fever, headache, dizziness, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and/or relapsing encephalitis, and survivors may experience long-term neurological deficits. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B). NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). To date, HeV disease has been confined to Australia. An outbreak of an unidentified Henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2015.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and

timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's initial list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no medical countermeasures [MCMs] exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of medical countermeasures to prevent and control LF outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment, effective community engagement, and workforce development and training in at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV (and other closely related henipaviruses) that are available and readily deployable for use when needed. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. Additionally, latent disease can occur long after initial infection. Both of these factors can hinder accurate diagnosis.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- NiV appears to have the potential for strain heterogeneity and antigenic variability, which could potentially influence accuracy of diagnostic testing over time.
- The natural reservoir for NiV is fruit bats of the Pteropus genus, which have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis

more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.

- Pteropus bat species also appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- High-level biocontainment requirements may pose an impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Diagnostic preparedness to detect HeV and other emergent henipaviruses in humans and animals as they arise.
- Proficiency testing, to monitor reproducibility and performance of NiV diagnostic assays in the field.
- Enhanced laboratory infrastructure in at-risk areas, with a particular focus on improving regional and national laboratory capacity for case and outbreak confirmation.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the DIVA test.
- Long-term need: multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or additional high-consequence pathogens.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, and limits of detection) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs).
- Additional validation data are needed to assess performance characteristics of NiV diagnostic assays against the various strains of NiV.

- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor antigenic changes over time that may impact diagnostic testing and the epidemiologic and clinical features of disease.
- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats. *(*cross cutting)*

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Promote the development and assessment of affordable, highly sensitive and specific, point-of-care diagnostic tests for use in humans and animals that have minimal requirements for biosafety precautions and staff training and that can detect NiV and other related henipaviruses.
3. Strengthen laboratory infrastructure and capacity in the endemic and at-risk areas to improve early diagnostic capabilities.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and develop appropriate standards for their use.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Expand research** to further understand the ecologic and epidemiology of NiV and other henipaviruses in human and animal populations over time, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to research** development of serologic tests to distinguish vaccinated from infected animals, if henipavirus vaccines are to be widely used in livestock populations.

Product development

- **Generate** a TPP for NiV diagnostics.

- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other high-consequence pathogens in humans and animals.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure for detection of NiV in endemic and at-risk areas, particularly in support of enhanced national and regional diagnostic capabilities.
- **Improve surveillance capacity** to: (1) better define the incidence of disease in NiV-endemic areas and (2) promote surveillance in non-endemic areas to enhance detection of emergent NiV disease or disease caused by other related henipaviruses.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV point-of-care diagnostics.
- **Ensure** access to regulatory review and authorization of NiV diagnostic assays.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostic products to endemic and at risk areas.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV disease occurs in relatively small outbreak settings, the low incidence of disease poses a major challenge for conducting phase 3 clinical trials; therefore, alternative regulatory pathways will need to be considered for licensure of vaccines or therapeutics. *(*cross cutting)*
- Differences between the NiV_M and NiV_B strains may impact the effectiveness of developed MCMs, yet NiV_M has been used significantly more often than NiV_B in research. *(*cross cutting)*
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier and can access the brain and spinal cord to inhibit viral replication. This is particularly important for preventing late-onset CNS disease.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons. In addition, the healthcare systems in endemic areas are not able to rapidly identify contacts most likely to benefit from PEP. *(*cross cutting)*

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat both acute and chronic (relapsing) NiV infection to improve survival and decrease associated morbidity and long-term disability.
- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock. *(*cross cutting)*

Knowledge gaps

- Additional information is needed on the immunology and pathogenesis of NiV in order to develop appropriate treatment options. This includes evaluating the pathophysiologic differences between NiV_B and NiV_M infection, determining the mechanisms of late-onset and relapsing NiV encephalitis, identifying factors influencing the development of permanent neurological sequela, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Ribavirin was found to reduce mortality by 36% in the 1998-1999 NiV outbreak in Malaysia and is considered an option for treatment of NiV infection. However, animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number of individuals exposed to HeV-infected horses. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional

animal studies and clinical trials are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV_B and NiV_M, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV_B to be shorter than for NiV_M.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the type of exposures that warrant such intervention and the most appropriate agents to administer.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of individual components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform supportive care and best-practice guidelines.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), such as determining the most appropriate lethal NiV dose for MCM development and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection and ensure that therapies are prepositioned in endemic areas for use as needed.
2. Stimulate basic research for better understanding of NiV pathogenesis and the immune response to infection.
3. Strengthen clinical and laboratory infrastructure in endemic and at-risk areas to promote early diagnosis of NiV cases so that effective treatment and PEP can be rapidly administered.
4. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Conduct** basic science research on the immunology and pathogenesis of NiV infections to inform development of new therapeutic agents.
- **Determine** the key differences between NiV_B and NiV_M that may have implications for the development of safe and effective NiV therapeutics.

- **Continue to research** the safety, tolerability, and efficacy of investigational therapies for NiV infection, including animal studies and clinical trials as appropriate and feasible.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against both NiV_B and NiV_M and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Refine, standardize, and validate** animal challenge models for future assessment of promising therapeutic candidates.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV therapeutics.
- **Support** plans for licensure/registration, adequate manufacturing, and subsequent distribution of safe and effective therapies to endemic areas and to other at-risk areas on an as-needed basis for outbreak control.
- **Secure** financing for procurement and deployment of NiV therapeutics for outbreak control once treatment agents are available.
- **Develop** treatment and PEP guidance as new therapies demonstrate safety and efficacy.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV occurs as relatively small, focal outbreaks, the low incidence of infection poses a major challenge for conducting phase 3 clinical trials for efficacy demonstration; therefore, alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics. *(*cross cutting)*

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- The absence of improved diagnostic assays for timely diagnosis of infection creates a challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control, once vaccines are available for use. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, African green monkeys (AGMs) are regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*

Key needs

- Safe and effective PEP (either vaccines or therapeutic agents) to prevent infection following exposure to NiV and guidance on their use. Such countermeasures are important tools to protect healthcare workers, family caregivers, and livestock workers. *(*cross cutting)*
- Nipah vaccines that can protect against both NiV_M and NiV_B in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks. Research tools, such as standardized neutralization assays, to compare immunogenicity of different vaccines.
- Enhanced surveillance capacity to provide more accurate information on the current incidence and geographic distribution of NiV in endemic and at-risk areas in order to refine potential vaccination strategies, particularly over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- The mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis are unknown. Further research to better understand viral clearance has important implications for development of vaccines and therapeutics.
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B; only a few studies demonstrating cross-protection have been performed to date.
- Further research is needed to determine durability and safety profiles of candidate vaccines, such as VSV recombinants, where concerns regarding potential neurotropism have been raised.

- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoestis).
- The identification of specific correlates of protection and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines and in simulating various epidemiologic scenarios that may impact strategies for vaccine use.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), including determining the most appropriate NiV dose for MCM development (Johnston 2015) and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses. Ongoing research into developing NiV/HeV vaccines for livestock is needed to further assess the merit of this potential control strategy.
- If evidence at some point supports the need for a broader, population-based vaccination strategy, additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.

Strategic Goals

1. Develop, evaluate, and license NiV vaccines for use in humans and potentially livestock, primarily for reactive/outbreak use in endemic and at-risk areas.
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection.
- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.

- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research efforts and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Generate** research tools (e.g., standardized neutralization assays) to compare immunogenicity of different vaccines.
- **Complete preclinical evaluation** of promising candidate NiV vaccines for safety, immunogenicity, efficacy, correlates of protection, and durability.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and livestock, primarily for reactive use during outbreaks.

- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.

Key capacities

- **Refine and validate** relevant animal challenge models for NiV infection.
- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the true burden and incidence of NiV disease, which may in turn, inform vaccination strategies.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV vaccines.
- **Ensure** access to regulatory review and authorization of NiV vaccines, particularly if non-traditional pathways are needed for licensure.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.
- **Provide** ongoing assessment to inform development and maintenance of NiV vaccine stockpiles over time; mathematical modelling may be used to contribute to this assessment.
- **Continue** to provide guidance on vaccination strategies for various target populations and epidemiologic scenarios, once vaccines are available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis, Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

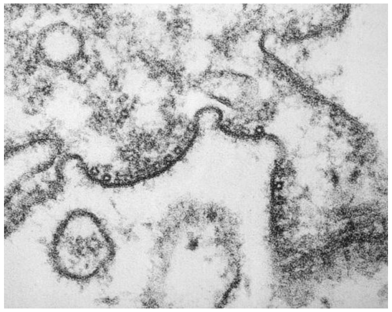
Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

Nipah Virus R&D Roadmap Development Taskforce Conference Call

January 9/10, 2018



Agenda

1. Welcome and roll call
2. R&D roadmap architecture
3. Role of roadmap taskforce members
4. Roadmap development and timeline
5. Discussion of draft R&D roadmap for Nipah virus
6. Next steps

World Health Organization

Developing and Implementing R&D Roadmaps *for priority pathogens with epidemic potential*

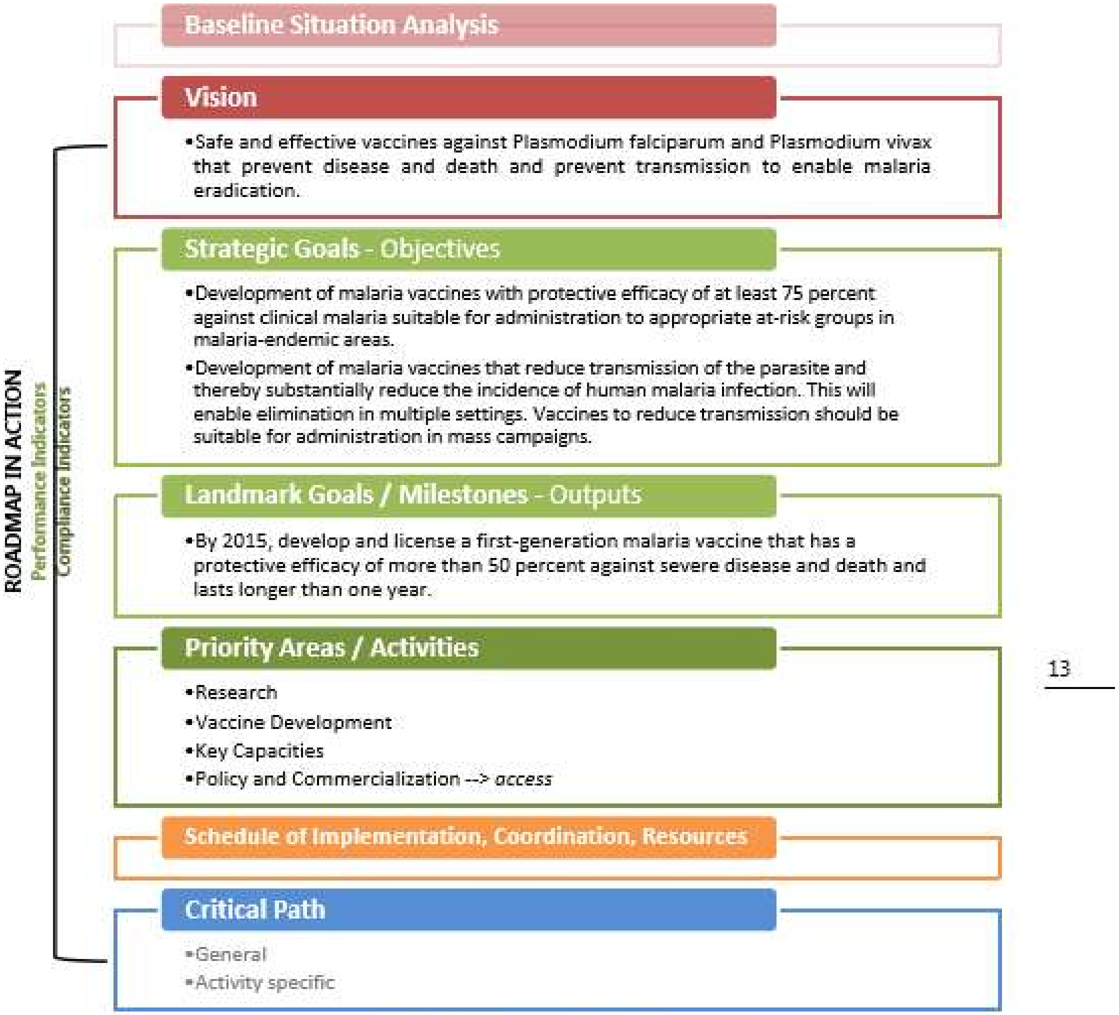
A generic methodology

R&D BLUEPRINT

Working Draft, version 30 August 2017

WHO | R&D Blueprint | Geneva, 30 August 2017

Example of Roadmap Architecture: Malaria Vaccine



Baseline Situation Analysis

Vision

- Safe and effective vaccines against Plasmodium falciparum and Plasmodium vivax that prevent disease and death and prevent transmission to enable malaria eradication.

Strategic Goals - Objectives

- Development of malaria vaccines with protective efficacy of at least 75 percent against clinical malaria suitable for administration to appropriate at-risk groups in malaria-endemic areas.
- Development of malaria vaccines that reduce transmission of the parasite and thereby substantially reduce the incidence of human malaria infection. This will enable elimination in multiple settings. Vaccines to reduce transmission should be suitable for administration in mass campaigns.

Landmark Goals / Milestones - Outputs

- By 2015, develop and license a first-generation malaria vaccine that has a protective efficacy of more than 50 percent against severe disease and death and lasts longer than one year.

Priority Areas / Activities

- Research
- Vaccine Development
- Key Capacities
- Policy and Commercialization --> *access*

Schedule of Implementation, Coordination, Resources

Critical Path

- General
- Activity specific

Figure 3 Key chapters of a roadmap,
examples from the *2013 Malaria Vaccine Technology Roadmap*

COMMENTARY

A roadmap for MERS-CoV research and product development: report from a World Health Organization consultation

Kayvon Modjarrad, Vasee S Moorthy, Peter Ben Embarek, Maria Van Kerkhove, Jerome Kim & Marie-Paule Kieny

As part of the World Health Organization (WHO) R&D Blueprint initiative, leading stakeholders on Middle East respiratory syndrome coronavirus (MERS-CoV) convened to agree on strategic public-health goals and global priority research activities that are needed to combat MERS-CoV.

The emergence and persistence of MERS-CoV as a cause of severe respiratory disease 10 years after the outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV) highlights the need for the rapid development of effective interventions against highly pathogenic human coronaviruses. As MERS-CoV grows in global importance—causing disease and death in more than 1,700 and 600 people, respectively, across 27 countries[1]—research and print for emergency R&D to prevent, or at least mitigate, the impact of infectious-disease outbreaks. MERS-CoV is one of eight pathogens prioritized in the WHO blueprint (http://www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/), and it was selected as a case study to demonstrate how accelerated basic and applied research, as well as product development, could be better supported and coordinated. The WHO therefore scientific community that dromedary camels are the main animal reservoir and source of zoonotic transmission to human populations[3]. However, the dynamics of transmission events from dromedaries to humans and between humans are poorly understood. Although studies have demonstrated that individuals with close and frequent contact (i.e., occupationally exposed) with dromedaries are at much higher risk for MERS-CoV infection than the general population[4], it remains

Supplemental note to commentary:

A Roadmap for Research and Product Development against Middle East Respiratory Syndrome-Coronavirus (MERS-CoV)

Background on Technology Roadmaps

Technology roadmaps have been successfully used in several sectors where large-scale, collaborative efforts are required to deliver outcomes related to innovation including product development. In all such processes, it is critical to understand and articulate the goals and markets for innovative products. High-level priority objectives are identified, followed by agreement on specific activities to reach the objectives. It is critical to map baseline knowledge gaps and then develop a strategic plan to address those deficiencies. This also requires an assessment of capacity needs that can support these activities. Project management and implementation structures are subsequently established to pursue agreed activities to reach the goals. An example of this process can be found in the WHO Malaria Vaccine Technology Roadmap.

Introduction

The Scientific Advisory Group convened by WHO to advise the Blueprint included MERS CoV as one of the disease priorities, and this document was prepared for MERS as a prototype. It represents the results of a consultation process facilitated by WHO, working with international stakeholders, to develop a MERS-CoV Research and Product Development Technology Roadmap.

Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) is an emerging pathogen of growing importance. As of May 2016, MERS-CoV has been confirmed as cause of severe acute respiratory disease in 1728 people, resulting in 624 deaths. The high case fatality rate, growing geographic

Nipah Virus Taskforce Members

- Christopher Broder
- Kaw Bing Chua
- Emily Gurley
- Stephen Luby
- Gregg Milligan
- Mahmudur Rahman
- Pierre Rollin
- Lin-Fa Wang

Role of Taskforce Members

- Contribute to the initial development of the draft roadmap outline (via early document review and a conference call prior to the in-person consultation).
- Participate in the in-person consultation (if available): March 1-2, 2018.
- Review subsequent roadmap drafts and provide ongoing input.
- Provide assistance with dissemination of the roadmaps.

Timeline for Nipah Virus Roadmap Development

Pre-consultation conference call with TF members to review draft outline	January 9/10
Obtain written feedback from TF members on outline (if feasible)	By January 19
Revise outline and send out to SMEs who will be participating in the in-person consultation	Mid-February
Hold in-person SME consultation on Nipah roadmap	March 1-2
Incorporate feedback from SMEs and create new draft (text format)	March/April
Seek additional input from TF members on the new version	April

Timeline for Nipah Virus Roadmap Development (cont.)

Post draft roadmap online for public comment	April/May*
Incorporate feedback, adjudicate comments, and create final draft	May*
Send final draft to TF members for review (if necessary)	June*
Continue to review and revise final draft with input from WHO and others	July/August*
Publish final roadmap document	Late August
Begin dissemination process	September

*Tentative

Considerations for Roadmap Review

- Organizational approach
- Level of detail
- Questions
  - Are the right issues covered?
  - What is missing?
  - Is anything inaccurate?
  - What should be removed?
  - Other comments, questions, or concerns

Discussion of Draft Roadmap Outline

- **Diagnostics**
  - Background: primary challenges, key needs, knowledge gaps
  - Strategic goals
  - Priority activities
    - Research
    - Product development
    - Key capacities
    - Policy and commercialization

Discussion of Draft Roadmap Outline (cont.)

- **Therapeutics**
  - Background: primary challenges, key needs, knowledge gaps
  - Strategic goals
  - Priority activities
    - Research
    - Product development
    - Key capacities
    - Policy and commercialization

Discussion of Draft Roadmap Outline (cont.)

- **Vaccines**
  - Background: primary challenges, key needs, knowledge gaps
  - Strategic goals
  - Priority activities
    - Research
    - Product development
    - Key capacities
    - Policy and commercialization

Next steps . . .

Thank you!!!!

A roadmap for MERS-CoV research and product development: report from a World Health Organization consultation

Kayvon Modjarrad, Vasee S Moorthy, Peter Ben Embarek, Maria Van Kerkhove, Jerome Kim & Marie-Paule Kieny

As part of the World Health Organization (WHO) R&D Blueprint initiative, leading stakeholders on Middle East respiratory syndrome coronavirus (MERS-CoV) convened to agree on strategic public-health goals and global priority research activities that are needed to combat MERS-CoV.

The emergence and persistence of MERS-CoV as a cause of severe respiratory disease 10 years after the outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV) highlights the need for the rapid development of effective interventions against highly pathogenic human coronaviruses. As MERS-CoV grows in global importance—causing disease and death in more than 1,700 and 600 people, respectively, across 27 countries[1]—research and development (R&D) efforts to design diagnostic, prophylactic and therapeutic products are gaining momentum. In the aftermath of the 2014–16 Ebola epidemic in West Africa and the current Zika virus outbreak, it has become clear that more strategic investments are needed in the early development of diagnostics, therapeutics and preventives against pathogens of pandemic potential[2]. The ultimate goal is to reduce delays between the identification of a public-health emergency and the deployment of effective medical interventions that will save lives and minimize socioeconomic disruption. Toward this objective, the WHO is developing a blueprint for emergency R&D to prevent, or at least mitigate, the impact of infectious-disease outbreaks. MERS-CoV is one of eight pathogens prioritized in the WHO blueprint (http://www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/), and it was selected as a case study to demonstrate how accelerated basic and applied research, as well as product development, could be better supported and coordinated. The WHO therefore convened a consultation of leading experts (**Supplementary Note 1**) on 10–11 December 2015 to develop a roadmap for MERS-CoV activities as part of the blueprint agenda.

Baseline assessment and epidemiology

The WHO's assistant director-general for health systems and innovation, Marie-Paule Kieny, opened the consultation by framing the meeting in the context of the broader blueprint for R&D preparedness and emergency response for known priority pathogens. The first author of this report then presented a landscape analysis of MERS-CoV diagnostics, preventives and therapeutics, highlighting the major gaps and advances in ongoing research and product-development activities. This baseline analysis summarized a report that was written for the consultation and that can be accessed on the WHO website (http://www.who.int/csr/research-and-development/mers-landscape.pdf?ua=1). After these overviews, a series of presentations described current knowledge about MERS-CoV epidemiology.

There is already broad consensus within the scientific community that dromedary camels are the main animal reservoir and source of zoonotic transmission to human populations[3]. However, the dynamics of transmission events from dromedaries to humans and between humans are poorly understood. Although studies have demonstrated that individuals with close and frequent contact (i.e., occupationally exposed) with dromedaries are at much higher risk for MERS-CoV infection than the general population[4], it remains unclear what routes of exposure mediate viral transmission most efficiently. Since the recognized introduction and entrenchment of MERS-CoV in human populations, the majority of MERS-CoV outbreaks have occurred in the nosocomial setting[5]. Although surveillance programs, observational studies and enhanced infection-control systems are now being prioritized and implemented throughout the Middle East, there is still wide variability across the region in the reach of surveillance efforts, the depth of case investigations and adherence to infection-control protocols. Improvements in these efforts will require political will, coordination across multiple sectors within countries at highest risk for future outbreaks, more streamlined communication, engagement with affected communities and cross-validation of diagnostics already in use and in development.

Diagnostic-assay development and standardization

During the early stages of infection, MERS-CoV cannot be clinically distinguished from other types of respiratory infections. Thus,

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Table 1 MERS-CoV monoclonal antibodies in development. Several groups have identified monoclonal antibodies that have at least shown potent neutralization against MERS-CoV, and in some cases, that have protected transgenic mice and NHPs from MERS-CoV disease after viral challenge.

Institution	Name	Source	Target	R&D
Chinese Academy of Sciences, China	4C2, 2E6	RBD-immunized mouse	RBD	Mouse efficacy
Dana-Farber Cancer Institute, USA; Abviro, USA	3B11 (AV-3)	Human-antibody library	RBD	Mouse and NHP efficacy
HUMABS Biomed, Switzerland	LCA60	Human survivor	RBD	Mouse and NHP efficacy
New York Blood Center, USA; Fudan University, China	Mersmab1	S1 immunized mouse	RBD	*In vitro*
Organic Vaccines, USA	m336, m337, m338	Human-antibody library	RBD	Mouse, rabbit, and NHP efficacy
National Institutes of Health, USA	D12, F11, G2, G4	S/S1 immunized mouse	RBD, S1, S2	NHP efficacy
Regeneron, USA	REGN3048/REGN3051	Humanized mouse	RBD	Mouse and NHP efficacy
Tsinghua University, China	MERS-4, MERS-27	Human-antibody library	RBD	*In vitro*

S1, spike-domain-containing RBD; S2, spike-domain-containing fusion machinery.

as the current case definition of MERS-CoV infection is based on laboratory confirmation[6], the development and harmonization of sensitive, specific and easily administered diagnostic assays are crucial to the success of surveillance systems, epidemiologic studies and efficacy assessments in clinical trials. One of the principal challenges of developing useful diagnostic assays is that they are dependent on high levels of virus replication and thus cannot detect infection until several days after viral exposure. During this time, the individual is infectious and poses great risk to others. Speaking to these issues, several participants surveyed the diagnostics in use and commented on their current utility and future viability for both clinical and research purposes.

Nucleic acid–amplification tests (NAATs) are currently the gold standard of MERS-CoV diagnostic platforms[7]. Although these tests have become substantially easier to implement, their performance is still dependent on specimen quality and technician training, because environmental contamination can easily confound accurate interpretation of results. Serologic assays—such as those based on immunofluorescence, immunochromatography, enzyme-linked immunosorbence and live-virus or pseudovirus neutralization—vary in their performance characteristics, but provide benefits over NAATs in the form of easier implementation and more functionally relevant readouts[8]. The performance of any assay, particularly with respect to MERS-CoV, depends on when it is administered during the natural history of disease. A more detailed understanding of the key features of the clinical course of MERS-CoV infection is, therefore, needed to inform the optimization of existing assays and the development of next-generation diagnostics. It was generally agreed that, even in the absence of these data, there is an urgent need for rapid, point-of-care diagnostics for both human and animal populations. The suggestion was made to develop commercially available—or at least adequately validated—simple, dipstick immunochromatographic assays that are suitable for use on livestock and humans (before confirmatory testing of positive results in humans). It was also recommended that more-advanced diagnostics, such as real-time reverse-transcriptase polymerase chain reaction (rRT–PCR) assays, be developed as part of a multivalent respiratory panel. There is also a need for more coordinated efforts to sequence circulating viruses and to correlate those data with phenotypic outcomes, such as viral fitness, virulence and structure–function relationships of the surface Spike (S) and other MERS-CoV proteins. There was a general call for the development of commercial tests and quality assurance of existing assays, although some of this work has already been started[7]. The validation of diagnostic tests will be essential for the execution and interpretation of epidemiologic studies that can better define viral reservoirs, transmission dynamics and correlates of protection.

Therapeutics

There are currently no licensed treatments for MERS-CoV. The discovery of antivirals for MERS-CoV has been limited to the repurposing of compounds already licensed or in development for other diseases. Some of the experimental treatments used sporadically during this outbreak are the same as those used for SARS-CoV. As in the SARS-CoV epidemic, however, the use of treatments such as ribavirin, interferons and corticosteroids have yielded little to no clinical benefit, despite showing efficacy in nonhuman primates (NHPs)[9]. High-throughput screens of large libraries have uncovered pharmaceutical agents across several classes that inhibit MERS-CoV replication *in vitro* and a few that improve survival in marmoset models[10]. Investigational agents are also being repurposed from other infectious diseases, such as Ebola virus disease, for potential use against MERS-CoV. Data were presented on the antiviral furthest along in development: GS-5734, an adenine analog that incorporates into viral RNA to disrupt replication. It has been shown to protect NHPs from Ebola virus disease and is now advancing through a phase 1 dose-escalation trial[11]. So far, its activity against MERS-CoV has been tested only in cell lines.

Antibodies, both monoclonal and polyclonal, have eclipsed antivirals as the focus of MERS-CoV therapeutic R&D. Initially, convalescent plasma administration, which had been used in other emerging infection outbreaks, was seen as a potentially expeditious and effective means of post-exposure prophylaxis in the setting of cluster outbreaks[12]. A regional protocol was developed, but ultimately could not be implemented, owing to a lack of sufficient convalescent donors[13]. Meanwhile, a different formulation of polyclonal antibodies, derived from a transchromosomal humanized bovine model, has been moving forward in development. Both prophylactic and therapeutic use of this polyclonal preparation significantly reduces viral lung titers in mice that were intranasally transduced with adenoviral vectors that expressed the human MERS-CoV cognate protein receptor, DPP4 (ref. 14). However, it was noted by several experts that therapeutic studies conducted thus far in animals may not be relevant to human outbreaks, given that products are administered only hours after challenge, probably before symptoms in humans would appear.

Among products being researched for pre- or post-exposure prophylaxis, monoclonal antibodies (mAbs) targeting the receptor-binding domain (RBD) of S are furthest

Table 2 MERS-CoV vaccine candidates in development. There are five general vaccine platforms in development for MERS-CoV. At the time of this report, all candidates are still in different preclinical stages of development.

Vaccine platform	Institution	Product	Stage of preclinical development			Stage of clinical development		
			In vitro	Immunogenicity	Efficacy	Phase 1	Phase 2	Phase 3
Live attenuated	Universidad Autonoma de Madrid, Spain	Recombinant MERS-CoV	▨					
Subunit	Novavax, USA	Full-length S trimers	▨	▨				
	Central South University, China	RBD fused with human Fc	▨	▨				
	New York Blood Center, USA Fudan University, China	RBD fused with human Fc	▨	▨				
	Chinese CDC, China	Truncated RBD	▨	▨	▨			
DNA	GeneOne Life Sciences, South Korea	Full-length S	▨	▨	▨	▨		
Prime-boost	National Institutes of Health, USA	Full length S DNA prime, S1 subunit protein boost	▨	▨	▨			
Recombinant vector	Greffex, USA	Ad5 S	▨	▨				
	Chinese CDC, China	Ad5 S or S1	▨	▨				
	University of Pittsburgh, USA Erasmus Medical Center, the Netherlands	Ad5 or Ad41	▨	▨				
	University of Oxford, UK	ChAd5 S	▨	▨				
	Paul Ehrlich Institute, Germany	Measles S	▨	▨	▨			
	Ludwig Maximilian University of Munich, Germany	MVA S	▨	▨	▨			

Fc, crystallizeable fraction of a human antibody; RBD, receptor-binding domain of the spike glycoprotein; Ad, adenovirus; Ad41, adenovirus serotype 41; MVA, modified vaccinia Ankara virus.

along in the product-development pipeline[15] (**Table 1**), some of which were presented at the meeting. Although representatives from each of the groups developing MERS-CoV mAbs presented data on the origin, potency, breadth and animal efficacy of their respective mAbs, some common themes emerged from the session as a whole and from the discussion that followed. Because most of the antibodies that have been developed target the RBD, there is a potential for viral escape from any one mAb. Thus, there should be greater efforts to (i) monitor circulating strains to assess viral evolution; (ii) define and measure phenotypic correlates of viral sequences; (iii) investigate the use of combination mAbs or polyclonal sera to overcome the potential emergence of therapeutic resistance; and (iv) study the serum of human survivors to better understand the response to natural infection and to develop reference reagents.

Vaccines

The global will to develop a coronavirus vaccine faded in the aftermath of the SARS-CoV pandemic, but has since gained renewed momentum in the face of the current MERS-CoV outbreak. This session started with a broad overview of vaccine-development pipelines and stressed that most vaccines fail to advance beyond phase 1 testing because of a lack of interest from funders and limited industrial support. In the realm of emerging infectious diseases with suspected or known pandemic potential, governmental agencies and nongovernmental organizations might have a key role in the development of interventions against diseases that do not provide a strong incentive for private-sector investments, but that are still relevant to public health and global security.

There are currently a dozen vaccine candidates in preclinical development (**Table 2**). Seven of the groups presented their products at the meeting. All developers are basing their immunogen designs on the S surface glycoprotein, the primary target for neutralizing antibodies during natural MERS-CoV infection[16]. Multiple platforms can be used to produce S, including but not limited to those presented at the meeting, such as live-attenuated viruses, DNA vectors, protein subunits and viral vectors (i.e., adenovirus, modified vaccinia virus Ankara and measles virus)[17]. Several of the products presented have demonstrated protection in at least one animal model. Although most of the vaccine candidates in the pipeline are being developed for human use, two of the vaccines have been tested in camels, which, if effective, would interrupt transmission of the virus to humans. A successful example of this "OneHealth" strategy—in which human, animal and environmental concerns are all considered—was described for the vaccine against Hendra virus in horses[18]. One of the lessons learned from the Hendra experience is that preclinical development and animal-model testing in relevant smaller animal models should be extensive before efficacy trials are commenced in larger-animal target populations, such as horses, or in the case of MERS-CoV, dromedary camels. Downselection of vaccine candidates in smaller animals increases the likelihood of success in large animals, which is crucial because the costs are much higher and logistical challenges much greater in the latter models. An additional lesson to take from previous experiences with animal vaccines for human health is to engage affected populations and educate them on the potential benefits and risks of a vaccine for their animals (i.e., camels) and communities.

One of the difficulties facing the development of effective vaccines for MERS-CoV is the absence of an animal model that recapitulates the pathogenesis and natural history of severe human disease. Two presentations and a robust discussion centered on this limitation in the field. Several mouse models that are transgenic for the human DPP4 protein receptor have now been developed[19]. Despite their manifestation of clinical disease, data from these transgenic mice might need to be supplemented with that of other, larger animal models for clinical advancement and ultimate licensing. Semi-permissive NHPs have been used as an animal surrogate in vaccine-efficacy testing thus far[20], but it is not clear whether either the rhesus or marmoset NHP models will serve as an accurate proxy for human disease, given that knowledge of the human pathology of MERS-CoV infection is limited to a single autopsy[21]. The development of more relevant animal models requires parallel investigation and elucidation of the virus's pathogenesis in humans. Additionally, there is some concern that a vaccine developed against a new coronavirus may induce antibody-dependent enhancement of infectivity and eosinophilic pulmonary infiltrates, as was observed among mice vaccinated with a virus-like particle (VLP) or killed–inactivated SARS-CoV vaccine[22]. However, none of the vaccines currently being developed for MERS-CoV includes the VLP or killed-inactivated platforms.

The roadmap for MERS-CoV R&D (**Supplementary Note 2**) will focus on vaccines that are indicated for populations and purposes of priority to the WHO perspective. Three broad indications for vaccination were discussed. The first two indications are for human use. They include a single-dose vaccine to be deployed for individuals at acute risk during outbreaks and a two-dose vaccine to induce durable protection for those at continual risk, such as camel handlers and health-care workers. The third vaccine indication proposed is for dromedaries, particularly juvenile camels, which pose a greater risk than older camels of transmitting virus to humans. The endpoint for a veterinary vaccine is likely to be the reduction or prevention of viral shedding, rather than sterilizing immunity. Despite the great potential for a camel vaccine to interrupt the epidemic, there has been a gap in funding for the development of a camel vaccine from conventional sources that support veterinary vaccines. One reason may be that a MERS-CoV vaccine for camels would be used differently than conventional veterinary vaccines. Usually, animals are vaccinated to prevent illness and death within veterinary populations. In the case of a MERS-CoV camel vaccine campaign, however, the primary intent is to prevent infection and disease in human populations. It will be important to shore up funds for a camel vaccination option, as this may be the fastest developmental and regulatory route toward licensing a product that can prevent human MERS-CoV infections and deaths.

Drafting a research and product development roadmap for MERS-CoV

R&D roadmaps have been used successfully in many sectors in which large-scale, collaborative efforts are required to deliver outcomes related to the innovation and development of new products. In all such processes, it is essential to start by clearly articulating and understanding the goals and markets for these products. High-level priority areas are first identified, after which an agreement on specific activities is decided. It is also crucial to first map out the baseline knowledge gaps and then to develop a strategic plan to address those deficiencies. This also requires an assessment of capacity needs that can support these activities. Project management and implementation structures are subsequently established to pursue agreed-upon activities to reach these goals. An example of this process can be found in the WHO Malaria Vaccine Technology Roadmap, which has culminated in a first-generation malaria vaccine and catalyzed the development of second-generation products (http://www.who.int/immunization/topics/malaria/vaccine_roadmap/en/).

At the meeting, four strategic goals were agreed upon in principle. The first is to establish a surveillance network of coronavirus laboratories as an early warning system to identify circulating species and strains in animal populations, new outbreaks in human populations and emerging strains in all populations. The second is to acquire a better understanding of MERS-CoV pathogenesis, natural history and veterinary and human epidemiology. The third is to develop, manufacture, test, license and use improved diagnostics, preventives and therapeutics that enable the interruption of transmission between humans and from dromedary camels to humans. The fourth, and perhaps most important, is for the global donor community to establish a mechanism that provides a line-of-sight for manufacturers from preclinical proof-of-concept studies to post-licensing procurement of MERS-CoV products, by initiating a public-health financial model for emerging pathogens prioritized by the WHO blueprint process.

Priority activities to be pursued through the MERS-CoV roadmap

A series of activities was prioritized and divided into the following categories: (i) basic, translational, epidemiological and social research; (ii) cross-cutting product development that includes refining more relevant animal models, developing reference reagents and designing tools and methods for rational prioritization between products; (iii) improved diagnostics; (iv) therapeutics, mAbs and polyclonal-antibody preparations; (v) vaccines for humans and camels; (vi) capacity development; and (vii) policy and commercialization. These will be further developed in consultation with potential funding stakeholders such as the Biomedical Advanced Research and Development Authority (BARDA), the US National Institutes of Health (NIH), the Wellcome Trust, the European Commission and the International Vaccine Initiative, among others, and with the broader MERS-CoV research community, to include public-health officials, manufacturers, regulators and product-development partnerships. The draft roadmap was posted for public consultation on WHO's website throughout the month of February 2016 and was finalized in May 2016 (http://www.who.int/csr/research-and-development/roadmap-consultation/en/).

Summary

The 2014 Ebola epidemic in West Africa revealed both great potential and pernicious deficiencies within existing mechanisms for rapid medical-product development and deployment. In the aftermath of the epidemic, global health communities coalesced around the realization that a multifaceted plan was required to respond quickly and efficiently to the next outbreak. The WHO is currently developing an R&D blueprint by which such preparation and response can follow, highlighting MERS-CoV as a case study. Although global coordination has resulted in the maturation of the preclinical pipeline for novel interventions for MERS-CoV, products will have to be developed along faster than normal timelines, with greater investments by multiple agencies for development, manufacturing and preclinical and clinical testing, as well as preparations for timely efficacy testing in affected populations if the incidence of disease rises sharply. As the global community takes lessons from the recent Ebola crisis, applies them to the current Zika virus outbreak and prepares for the potential of another regional epidemic or broader pandemic, stakeholders in research and product development on emerging pathogens must set out a sound strategy now for where to best target their investments in anticipation of future outbreaks. The current consultation is a first step toward that end, having opened a forum for global dialogue between public-health agencies, scientists, product developers and funders to engage in joint planning of MERS-CoV R&D activities.

Note: Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We gratefully acknowledge input to the roadmap-development process from all those who attended the WHO consultation in December 2015. A supplementary file lists all those who attended. The views, findings and conclusions within are those of the authors and should not be construed to represent the positions of the US Department of Defense or the World Health Organization.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

1. WHO. http://www.who.int/csr/disease/coronavirus_infections/MERS-CoV_summary_update_20140611.pdf (World Health Organization, Geneva, 2014).
2. Cohen, J. *Science* **351**, 16–19 (2016).
3. Ben Embarek, P.K. & Van Kerkhove, M.D. *Wkly. Epidemiol. Rec.* **90**, 245–250 (2015).
4. Reusken, C.B. *et al. Emerg. Infect. Dis.* **21**, 1422–1425 (2015).
5. Drosten, C. *et al. Clin. Infect. Dis.* **60**, 369–377 (2015).
6. World Health Organization. (WHO/MERS/LAB/15.1) (World Health Organization, Geneva, 2015).
7. Pas, S.D. *et al. J. Clin. Virol.* **69**, 81–85 (2015).
8. Park, S.W. *et al. Euro Surveill.* **20**, 30042 (2015).
9. Falzarano, D. *et al. Nat. Med.* **19**, 1313–1317 (2013).
10. Dyall, J. *et al. Antimicrob. Agents Chemother.* **58**, 4885–4893 (2014).
11. Warren, T. *et al.* (IDWeek, San Diego, abstract no. LB-2) (IDWeek, Arlington, Virginia, USA, 2015).
12. Mair-Jenkins, J. *et al. Convalescent Plasma Study Group. J. Infect. Dis.* **211**, 80–90 (2015).
13. Arabi, Y. *et al. Springerplus* **4**, 709 (2015).
14. Zhao, J. *et al. Proc. Natl. Acad. Sci. USA* **111**, 4970–4975 (2014).
15. Modjarrad, K. *J. Virus Erad.* **2**, 1–4 (2016).
16. Wang, L. *et al. Nat. Commun.* **6**, 7712 (2015).
17. Modjarrad, K. *Virus Res.* (in the press).
18. Middleton, D. *et al. Emerg. Infect. Dis.* **20**, 372–379 (2014).
19. Agrawal, A.S. *et al. J. Virol.* **89**, 3659–3670 (2015).
20. Falzarano, D. *et al. PLoS Pathog.* **10**, e1004250 (2014).
21. Ng, D.L. *et al. Am. J. Pathol.* **186**, 652–658 (2016).
22. Tseng, C.T. *et al. PLoS One* **7**, e35421 (2012).

A Roadmap for Research and Product Development against Middle East Respiratory Syndrome-Coronavirus (MERS-CoV)

Background on Technology Roadmaps

Technology roadmaps have been successfully used in several sectors where large-scale, collaborative efforts are required to deliver outcomes related to innovation including product development. In all such processes, it is critical to understand and articulate the goals and markets for innovative products. High-level priority objectives are identified, followed by agreement on specific activities to reach the objectives. It is critical to map baseline knowledge gaps and then develop a strategic plan to address those deficiencies. This also requires an assessment of capacity needs that can support these activities. Project management and implementation structures are subsequently established to pursue agreed activities to reach the goals. An example of this process can be found in the WHO Malaria Vaccine Technology Roadmap.

Introduction

The Scientific Advisory Group convened by WHO to advise the Blueprint included MERS CoV as one of the disease priorities, and this document was prepared for MERS as a prototype. It represents the results of a consultation process facilitated by WHO, working with international stakeholders, to develop a MERS-CoV Research and Product Development Technology Roadmap.

Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) is an emerging pathogen of growing importance. As of May 2016, MERS-CoV has been confirmed as cause of severe acute respiratory disease in 1728 people, resulting in 624 deaths. The high case fatality rate, growing geographic distribution, and poorly defined epidemiology have created an urgent need for applied research and product development in order to better characterise epidemiology, diagnose, treat and prevent transmission and disease related to MERS-CoV.

Since the identification of MERS-CoV in the Kingdom of Saudi Arabia in 2012, human cases have been confirmed in 27 countries, including a large outbreak in the Republic of Korea. It is generally believed that dromedary camels are the intermediate animal reservoir; a high prevalence of MERS binding or inhibitory antibodies has been reported in camels across the Middle East, North Africa, and in sub-Saharan Africa. Outbreak investigation reports are strongly suggestive of a link between contact with camels and human cases of MERS-CoV. However many gaps remain in understanding of epidemiology and transmission of MERS-CoV.

Strategic Goals

- A strengthened network of laboratories able to act as an early warning system for emerging highly pathogenic viruses with the potential to cause public health emergencies
- Stimulate basic research for better understanding of the MERS-CoV pathogen, pathogenesis, immunity, epidemiology, transmission including the intermediate animal reservoir as well as person to person transmission
- Development, testing, manufacturing, licensure and use for improved diagnostics, therapeutics and preventives, including preventives for dromedary camels.

- ***Enabling environment strategic goal***: Establish line of sight for manufacturers from pre-clinical proof-of-concept to procurement of MERS products once licensed, by establishing public health-oriented financing model for MERS products, and other products for emerging pathogens prioritized by WHO blueprint process

Priority Areas

Research

- **Acquire** understanding of naturally acquired immunity, and immunity acquired through vaccination
- **Explore** social and cultural questions related to camel vaccination in most affected countries
- **Develop** an immune correlate of protection during pre-clinical and clinical efficacy trials
- **Urgently define** and address epidemiological, biological, behavioural and environmental knowledge gaps related to the definitive host, the intermediate animal reservoir, transmission to humans, person to person transmission, and characterisation of target groups for deployment of preventives
- **Characterise** pathogen diversity of MERS-CoV isolates in different outbreaks as basis for diagnostics, therapeutics and preventives development
- **Model** possible progression of outbreaks of MERS-CoV in various countries, and model impact of alternate approaches to vaccination in camels and humans
- **Research** improved infection control strategies, including appropriate models for triage in healthcare facilities

Cross-Cutting Product Development Related Priority Areas

- **Improved** animal models more representative of human disease for evaluation of different classes of products, including an animal model for enhanced disease. It is expected that animal model data evaluating enhanced disease will be available before licensure of MERS products
- **Develop and endorse** an international standard panel of calibration reagents to allow for comparability between different operators, labs, and assays, for nucleic acid, binding antibody and inhibitory assays
- **Prioritization** for potential products through use of **Target Product Profiles**, head-to-head testing and use of comparable assays with reference reagents

Diagnostic

- **Develop** quality assured, point of care diagnostics for MERS-CoV.
- ***Preferred diagnostic goal***: Multivalent MERS-CoV point of care diagnostic as part of a panel, including RSV, influenza, and other respiratory infections.

Treatment

- **Develop** quality assured therapeutics with acceptable safety and proven efficacy against MERS-CoV in those at high risk of mortality without treatment
- **Pursue evaluations of GMP grade therapeutics in parallel** on an urgent basis with at least 2 products to be tested during 2016

  - **Phase 1 selection based on pre-clinical proof of concept data, time of GMP availability and regulatory/ethics approvals**
  - **Timings for Phase 1 start, and go/no go to Phase 2-3 is critical factor in decision-making**
  - **Proceed** into phase 2-3 evaluations in MERS affected countries and patients, once supportive phase 1 data is available.
- **Assess** the most effective approaches for supportive care, including for low technology settings.

Preventive

- Human vaccine 1: **Develop and license** single dose MERS-CoV vaccine suitable for reactive use in outbreak settings with rapid onset of immunity
- Human vaccine 2: **Develop and license** two dose vaccine with durable protection for administration to those at high ongoing risk of MERS-CoV such as healthcare workers, and those working with potentially infected animals
  - At least 2 different MERS vaccine approaches to be tested in Phase 1 during 2016, with contingency planning to proceed to Phase 2-3 if Phase 1 data supportive
- Dromedary camel vaccine: **Develop and license** a vaccine suitable for administration to camels to prevent transmission of MERS-CoV from animal reservoir to humans
  - Note that recombinant viral vectors and DNA vaccines are already licensed and in use as veterinary vaccines
  - Evaluation in camels of promising approaches to be conducted during 2016

Key capacities

- **Support** regulatory and ethics capacity strengthening in MERS-CoV most affected countries for oversight of clinical trials
- **Build** GCP Clinical Trial Capacity for product evaluation
- **Establish and coordinate** GCLP Laboratory Testing Capacity for product evaluation

Policy and commercialization

- **Define** scale-up needs and **develop** GMP Manufacturing capacity to meet these needs
- **Develop and encourage** responsible stewardship and support for MERS-CoV research and product development and implementation through appropriate project management and investment strategies (supported by Target Product Profiles vetted by key stakeholders).
- **Establish regulatory pathways** for MERS-CoV products
- **Secure** financing for procurement and deployment of MERS-CoV products once available
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring

The Stakeholders Group for the WHO Blueprint

Starting in 2015, WHO convened a group of key stakeholders in R&D preparedness for emergencies. These include the following funding agencies: BARDA, NIAID, IVI, Wellcome Trust, European Commission Directorate-General Research & Innovation, the GLOPID-R network of funders coordinated via the European Commission and the Bill and Melinda Gates Foundation. It is envisaged that this group will meet by teleconference on a regular basis with WHO to review gaps in key activities at the global level, minimize unhelpful overlaps, and stimulate priority activities to maintain momentum. The roadmap can also be updated at the annual meetings if necessary. Other stakeholders include public health agencies particularly in affected countries, academia, the biotech sector, industry, regulators and ethics committees amongst others. A mechanism will be developed to implement the MERS R&D Roadmap by agreeing which activities should be carried out by the different stakeholders.

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Subject: RE: Agenda and materials for the Nipah WHO R&D Roadmap Taskforce conference call to be held on Tuesday, January 9 or Wednesday January 10, depending on time zone
Date: Wednesday, January 10, 2018 4:39:58 AM
Attachments: Nipah Draft Roadmap.V5 ckb.docm

Dear Dr Micheal Osterholm,

Please find the attached file having some of my suggestions.

Thanks.

Bing

From: Michael Osterholm [mailto:mto@umn.edu]
Sent: Thursday, 4 January, 2018 9:51 AM
To: christopher.broder@usuhs.edu; Chua Kaw Bing; egurley1@jhu.edu; sluby@stanford.edu; gnmillig@utmb.edu; mahmudur57@gmail.com; pyr3@cdc.gov; linfa.wang@duke-nus.edu.sg
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Subject: Agenda and materials for the Nipah WHO R&D Roadmap Taskforce conference call to be held on Tuesday, January 9 or Wednesday January 10, depending on time zone
Importance: High

Dear Colleagues:

I am writing to remind you of our Nipah Roadmap Taskforce conference call **on Tuesday, January 9 at 5:00 pm PST, 7:00 pm CST, 8:00 pm EST, and Wednesday, January 10 at 1:00 am GMT, 2:00 am CET, 7:00 am BST, and 9:00 am SGT.**

Each of you should have received a calendar invite with the call-in information and I have repeated that information below:

- Conference code: 998-3378#
    - USA Toll Free: 866-767-9978
    - UK Toll Free: 0808 1017 535
    - Switzerland Toll Free: 0800 5537 12
    - Singapore Toll Free: 800 101 2395
- For Bangladesh, we do not have a toll free line; we will contact you separately to make logistical arrangements.
- As a BACKUP: International callers may use the following conference call information: Conference Line 205-254-8650 Conference Code: 998-3378#. However, please note that this is NOT a toll-free number.

This email includes the following attachments:

- The Nipah draft roadmap outline. **Please review this document before the call** because most of our call will be devoted to discussing the document. Also, we would very much appreciate any written edits or comments that you have on the document, particularly if you are not able to participate in the call. In order to keep to our development timeline, we need to receive any written comments from you no later than Friday, **January 19.** Please send comments directly to kamoore@umn.edu.
- A short slide deck for our call on Tuesday.
- Supplemental materials on the MERS-CoV Roadmap as examples, including a Nature Medicine article on that Roadmap and an outline of the roadmap, similar to the outline we have developed for the Nipah Roadmap.

The agenda for the call is as follows (most of the time will be devoted to discussing the draft Nipah Roadmap outline):

1. Welcome and roll call
2. R&D roadmap architecture
3. Role of roadmap taskforce members
4. Roadmap development and timeline
5. Discussion of draft R&D roadmap for Nipah
6. Next steps

Thank you again for agreeing to be part of this important work. We look forward to speaking with you next week and to receiving your comments on the draft Roadmap outline by January 19.

Warm regards,

Mike

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Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, outlining the underpinning strategic goals, and prioritizing research areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus (NiV) infection.

*(**Note:** The content below is organized into the three main categories for R&D of MCMs [diagnostics, therapeutics, and vaccines] to allow reviewers an opportunity to follow the lines of thinking for each category. Some issues are cross cutting and apply to more than one category. As this draft is refined, the content may be reorganized to create one over all roadmap and to group cross-cutting issues together. In addition, this document is relatively detailed to ensure that the most important issues are identified and discussed. As the document evolves, certain items may be "rolled up" into a smaller number of broader issues, strategic goals, and primary activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak occurred in pigs and humans in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the natural reservoir for NiV (Pteropus bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, and Ghana. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and human-to-human NiV transmission. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a role, with human consumption of bat-contaminated raw date palm sap serving as the primary NiV source. In humans, NiV infection has neurological and respiratory syndromes, with fever, headache, dizziness, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and/or relapsing encephalitis, and survivors may experience long-term neurological deficits. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B). NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). To date, HeV disease has been confined to Australia. An outbreak of an unidentified Henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2015.

Commented [EG1]: There were a couple of suspect cases of this in Malaysia, but this has been a prominent feature of the outbreaks in India and Bangladesh.

Commented [EG2]: Cough has been more common than vomiting in the Bangladesh case series.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and

timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's initial list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no medical countermeasures [MCMs] exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of medical countermeasures to prevent and control LF outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment, effective community engagement, and workforce development and training in at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

Commented [SPL3]: I consider this paragraph as essential framing and urge that it be retained, even as we work towards a more focused document. It is difficult, because medical countermeasures do not stand alone. They need to interface with a system that constructively deploys them. At a minimum we should strive for countermeasures that are fit for purpose for the contexts where they are needed.

Commented [EG4R3]: Complete agree with Steve on this.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV (and other closely related henipaviruses) that are available and readily deployable for use when needed. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. Additionally, latent disease can occur long after initial infection. Both of these factors can hinder accurate diagnosis.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- NiV appears to have the potential for strain heterogeneity and antigenic variability, which could potentially influence accuracy of diagnostic testing over time.
- The natural reservoir for NiV is fruit bats of the Pteropus genus, which have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis

Commented [EG5]: While this is true, even when testing is nearby, it can still take days to get back a PCR or ELISA result as these are not rapid tests as current used. Within the context of very quick progression to death, we need a bedside rapid diagnostic.

more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.

- Pteropus bat species also appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- High-level biocontainment requirements may pose an impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Diagnostic preparedness to detect HeV and other emergent henipaviruses in humans and animals as they arise.
- Proficiency testing, to monitor reproducibility and performance of NiV diagnostic assays in the field.
- Enhanced laboratory infrastructure in at-risk areas, with a particular focus on improving regional and national laboratory capacity for case and outbreak confirmation.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the DIVA test.
- Long-term need: multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or additional high-consequence pathogens.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, and limits of detection) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs).
- Additional validation data are needed to assess performance characteristics of NiV diagnostic assays against the various strains of NiV.

Commented [SPL6]: Given the wide range of Pteropus bats this needs to be defined. Clearly, we need low cost accurate diagnostics, but we also need to consider the system that needs to be in place in order to make use of the diagnostics. We risk mission creep if we include development of global surveillance as part of medical countermeasures, but there is a difference between how we would optimally deploy point of care diagnostics in Bangladesh and West Bengal, India, compared with the Philipines or Cameroon. We should consider the different needs in these different contexts, to assure that we have an approach that is fit for purpose.

Commented [EG7R6]: Agree

Commented [EG8]: Or, other pathogens on a differential diagnosis for humans?

Commented [SPL9]: Salivary pcr is often positive in Bangladesh before antibodies are detectable.

- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor antigenic changes over time that may impact diagnostic testing and the epidemiologic and clinical features of disease.
- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats. *(*cross cutting)*

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Promote the development and assessment of affordable, highly sensitive and specific, point-of-care diagnostic tests for use in humans and animals that have minimal requirements for biosafety precautions and staff training and that can detect NiV and other related henipaviruses.
3. Strengthen laboratory infrastructure and capacity in the endemic and at-risk areas to improve early diagnostic capabilities.

Commented [SPL10]: Because this is a wildlife shed virus and these creatures live near food sources, spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh. The health care facilities that serve these communities not only have very rudimentary laboratory infrastructure, they also do not have the routine practice of using diagnostic tests to manage patients. Thus, achieving the stated strategic goals requires a much broader, deeper effort, than providing reagents and testing for proficiency at national labs.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and develop appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Expand research** to further understand the ecology~~ic~~ and epidemiology of NiV and other henipaviruses in human and animal populations over time, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to research** development of serologic tests to distinguish vaccinated from infected animals, if henipavirus vaccines are to be widely used in livestock populations.

Commented [SPL11]: Consider whether efforts to promote sharing of sequence and strains would benefit from attention.

Product development

- **Generate** a TPP for NiV diagnostics.

- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other high-consequence pathogens in humans and animals.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure for detection of NiV in endemic and at-risk areas, particularly in support of enhanced national and regional diagnostic capabilities.
- **Improve surveillance capacity** to: (1) better define the incidence of disease in NiV-endemic areas and (2) promote surveillance in non-endemic areas to enhance detection of emergent NiV disease or disease caused by other related henipaviruses.

Commented [EG12]: Perhaps targeted research to identify evidence of spillover and human risk might come before investments in broader surveillance efforts.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV point-of-care diagnostics.
- **Ensure** access to regulatory review and authorization of NiV diagnostic assays.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostic products to endemic and at risk areas.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV disease occurs in relatively small outbreak settings, the low incidence of disease poses a major challenge for conducting phase 3 clinical trials; therefore, alternative regulatory pathways will need to be considered for licensure of vaccines or therapeutics. *(*cross cutting)*
- Differences between the NiV_M and NiV_B strains may impact the effectiveness of developed MCMs, yet NiV_M has been used significantly more often than NiV_B in research. *(*cross cutting)*
- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier and can access the brain and spinal cord to inhibit viral replication. This is particularly important for preventing late-onset CNS disease.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons. In addition, the healthcare systems in endemic areas are not able to rapidly identify contacts most likely to benefit from PEP. *(*cross cutting)*

Commented [SPL13]: Moreover, if we actually had more strains we may identify more heterogeneity than is currently apparent.

Commented [EG14]: And can pose a dilemma if therapeutics prolong life and improve survival but are unable to prevent severe neurologic damage.

Commented [EG15]: Or, infection control measures which are not standard practice in many at-risk countries.

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*

Commented [EG16]: Studies in animals often test usefulness of therapeutics when delivered prior to disease onset or very early during the disease course. Since humans with Nipah virus are often only detected once they are ill, the findings are less useful for understanding how the drug might work in humans.

- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat both acute and chronic (relapsing) NiV infection to improve survival and decrease associated morbidity and long-term disability.

Commented [SPL17]: Would be ideal, but, given the biology, may be much more difficult than an acute countermeasure. Might separate these into 2, so that the ideal does not become the enemy of the good.

Commented [EG18]: Patients in Malaysia were much more likely to survive than patients in Bangladesh. One hypothesis is that this is because patients in Malaysia had access to ventilator support, which is typically unavailable in Bangladesh. There may be health systems approaches that could improve survival.

- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock. *(*cross cutting)*

Knowledge gaps

- Additional information is needed on the immunology and pathogenesis of NiV in order to develop appropriate treatment options. This includes evaluating the pathophysiologic differences between NiV$_B$ and NiV$_M$ infection, determining the mechanisms of late-onset and relapsing NiV encephalitis, identifying factors influencing the development of permanent neurological sequela, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Patients treated with Ribavirin late in the 1998-1999 NiV outbreak in Malaysia experienced 36% lower mortality than persons from earlier in the outbreak who were not treated with Ribavirin.~~was found to reduce mortality by 36% in the 1998-1999 NiV outbreak in Malaysia and is considered~~Ribaviran may be an option for treatment of NiV infection. However, animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number

of individuals exposed to HeV-infected horses. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV$_B$ and NiV$_M$, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV$_B$ to be shorter than for NiV$_M$.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the type of exposures that warrant such intervention and the most appropriate agents to administer.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of individual components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform supportive care and best-practice guidelines.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), such as determining the most appropriate lethal NiV dose for MCM development and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*

Commented [SPL19]: An important related issue is how we protect the health care workers and fellow patients in these settings. Part of this is covered in the vaccine section, but the capacity to protect health care workers and other patients is extremely limited in the contexts where we see recurrant NiV outbreaks. A clear eyed understanding of the context, and piloting approaches within these contexts is essential to develop and refine medical counter measures to that they can reduce pandemic risk. To be effective these countermeasures need to work, not only in idealized trial conditions, but in the everyday setting where high risk patients present.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection and ensure that therapies are prepositioned in endemic areas for use as needed.
2. Stimulate basic research for better understanding of NiV pathogenesis and the immune response to infection.
3. Strengthen clinical and laboratory infrastructure in endemic and at-risk areas to promote early diagnosis of NiV cases so that effective treatment and PEP can be rapidly administered.
4. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Commented [EG20]: Median days from onset to death for patients in Bangladesh who die from Nipah virus is 6 days. Typically, they present unconscious on around day 3. So, there is little time to act but even less when healthcare seeking is delayed, because progression is so fast. This is a worthy goal, but the limited access to healthcare will reduce the number patients we can find in time to treat and will have an impact on field trials.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Conduct** basic science research on the immunology and pathogenesis of NiV infections to inform development of new therapeutic agents.

- **Determine** the key differences between NiV$_B$ and NiV$_M$ that may have implications for the development of safe and effective NiV therapeutics.
- **Continue to research** the safety, tolerability, and efficacy of investigational therapies for NiV infection, including animal studies and clinical trials as appropriate and feasible.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against both NiV$_B$ and NiV$_M$ and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV$_B$ and NiV$_M$ and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Refine, standardize, and validate** animal challenge models for future assessment of promising therapeutic candidates.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV therapeutics.
- **Support** plans for licensure/registration, adequate manufacturing, and subsequent distribution of safe and effective therapies to endemic areas and to other at-risk areas on an as-needed basis for outbreak control.
- **Secure** financing for procurement and deployment of NiV therapeutics for outbreak control once treatment agents are available.
- **Develop** treatment and PEP guidance as new therapies demonstrate safety and efficacy.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV occurs as relatively small, focal outbreaks, the low incidence of infection poses a major challenge for conducting phase 3 clinical trials for efficacy demonstration; therefore,

alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics. *(*cross cutting)*

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- The absence of improved diagnostic assays for timely diagnosis of infection creates a challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control, once vaccines are available for use. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, African green monkeys (AGMs) are regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*

Key needs

- Safe and effective PEP (either vaccines or therapeutic agents) to prevent infection following exposure to NiV and guidance on their use. Such countermeasures are important tools to protect healthcare workers, family caregivers, and livestock workers. *(*cross cutting)*
- Nipah vaccines that can protect against both NiV_M and NiV_B in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks. Research tools, such as standardized neutralization assays, to compare immunogenicity of different vaccines.
- Enhanced surveillance capacity to provide more accurate information on the current incidence and geographic distribution of NiV in endemic and at-risk areas in order to refine potential vaccination strategies, particularly over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- The mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis are unknown. Further research to better understand viral clearance has important implications for development of vaccines and therapeutics.
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B; only a few studies demonstrating cross-protection have been performed to date.

- Further research is needed to determine durability and safety profiles of candidate vaccines, such as VSV recombinants, where concerns regarding potential neurotropism have been raised.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoestis).
- The identification of specific correlates of protection and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines and in simulating various epidemiologic scenarios that may impact strategies for vaccine use.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), including determining the most appropriate NiV dose for MCM development (Johnston 2015) and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses. Ongoing research into developing NiV/HeV vaccines for livestock is needed to further assess the merit of this potential control strategy.
- If evidence at some point supports the need for a broader, population-based vaccination strategy, additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.

Commented [EG21]: And for the estimation of stockpiles that would be required to effectively respond to outbreaks, under various transmission assumptions.

Commented [SPL22]: I suspect that this is an easier technical problem than it is an economic problem. Only 15% of Australian horses are vaccinated against HeV. Pigs in low income countries are rarely protected against swine cholera or Japanese Encepahlitis. The incentives for livestock raisers are not well aligned for deployment as a human public health tool.

Commented [EG23R22]: Indeed, even when we offered free JE vaccine to pig raisers in northern Bangladesh, many refused due to lack of trust in the formal vet care system.

Commented [SPL24]: Should consider surge capacity to rapidly ramp up vaccine supply in case NiV is used as a bioterror agent, or we face a strain that naturally develops increased capacity for person to person transmission.

Strategic Goals

1. Develop, evaluate, and license NiV vaccines for use in humans and potentially livestock, primarily for reactive/outbreak use in endemic and at-risk areas.
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection.

- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research efforts and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Generate** research tools (e.g., standardized neutralization assays) to compare immunogenicity of different vaccines.
- **Complete preclinical evaluation** of promising candidate NiV vaccines for safety, immunogenicity, efficacy, correlates of protection, and durability.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and livestock, primarily for reactive use during outbreaks.
- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.

Key capacities

- **Refine and validate** relevant animal challenge models for NiV infection.
- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the true burden and incidence of NiV disease, which may in turn, inform vaccination strategies.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV vaccines.
- **Ensure** access to regulatory review and authorization of NiV vaccines, particularly if non-traditional pathways are needed for licensure.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.
- **Provide** ongoing assessment to inform development and maintenance of NiV vaccine stockpiles over time; mathematical modelling may be used to contribute to this assessment.
- **Continue** to provide guidance on vaccination strategies for various target populations and epidemiologic scenarios, once vaccines are available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis, Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

From:	Emily Gurley on behalf of Emily Gurley <egurley1@jhu.edu>
To:	Steve Luby; Michael Osterholm
Cc:	christopher.broder@usuhs.edu; chuakb@tll.org.sg; gnmillig@utmb.edu; mahmudur57@gmail.com; pyr3@cdc.gov; linfa.wang@duke-nus.edu.sg; K.Cook@wellcome.ac.uk; benassiv@who.int; kamoore@umn.edu; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; crcruz@umn.edu; oneil005@umn.edu; preziosim@who.int
Subject:	RE: Agenda and materials for the Nipah WHO R&D Roadmap Taskforce conference call to be held on Tuesday, January 9 or Wednesday January 10, depending on time zone
Date:	Monday, January 8, 2018 8:40:10 PM
Attachments:	Nipah Draft Roadmap.V5 sl eg.docx

Mike and colleagues,

I very much look forward to working with you all on this endeavor.

I will be on a flight to Dhaka during the call, so unable to participate. I'll look forward to hearing the updates.

I've attached a few comments to the draft that Steve sent.

All the best,
Emily

From: Steve Luby [mailto:sluby@stanford.edu]
Sent: Saturday, January 6, 2018 1:38 AM
To: Michael Osterholm <mto@umn.edu>
Cc: christopher.broder@usuhs.edu; chuakb@tll.org.sg; Emily Gurley <egurley1@jhu.edu>; gnmillig@utmb.edu; mahmudur57@gmail.com; pyr3@cdc.gov; linfa.wang@duke-nus.edu.sg; K.Cook@wellcome.ac.uk; benassiv@who.int; kamoore@umn.edu; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; crcruz@umn.edu; oneil005@umn.edu; preziosim@who.int
Subject: Re: Agenda and materials for the Nipah WHO R&D Roadmap Taskforce conference call to be held on Tuesday, January 9 or Wednesday January 10, depending on time zone

Mike,

Thanks so much for moving this forward. I am looking forward to the call.

I will be in Bangladesh, but I should be able to connect.

Attached are some comments on the roadmap outline.

Steve

Michael Osterholm

Thursday, January 04, 2018 8:50 AM

Dear Colleagues:

I am writing to remind you of our Nipah Roadmap Taskforce conference call **on Tuesday, January 9 at 5:00 pm PST, 7:00 pm CST, 8:00 pm EST, and Wednesday, January 10 at 1:00 am GMT, 2:00 am CET, 7:00 am BST, and 9:00 am SGT.**

Each of you should have received a calendar invite with the call-in information and I have repeated that information below:

- Conference code: 998-3378#
  - USA Toll Free: 866-767-9978
  - UK Toll Free: 0808 1017 535
  - Switzerland Toll Free: 0800 5537 12
  - Singapore Toll Free: 800 101 2395
- For Bangladesh, we do not have a toll free line; we will contact you separately to make logistical arrangements.
- As a BACKUP: International callers may use the following conference call information: Conference Line 205-254-8650 Conference Code: 998-3378#. However, please note that this is NOT a toll-free number.

This email includes the following attachments:

- The Nipah draft roadmap outline. **Please review this document before the call** because most of our call will be devoted to discussing the document. Also, we would very much appreciate any written edits or comments that you have on the document, particularly if you are not able to participate in the call. In order to keep to our development timeline, we need to receive any written comments from you no later than Friday, **January 19.** Please send comments directly to kamoore@umn.edu.
- A short slide deck for our call on Tuesday.
- Supplemental materials on the MERS-CoV Roadmap as examples, including a Nature Medicine article on that Roadmap and an outline of the roadmap, similar to the outline we have developed for the Nipah Roadmap.

The agenda for the call is as follows (most of the time will be devoted to discussing the draft Nipah Roadmap outline):

1. Welcome and roll call
2. R&D roadmap architecture

3. Role of roadmap taskforce members
4. Roadmap development and timeline
5. Discussion of draft R&D roadmap for Nipah
6. Next steps

Thank you again for agreeing to be part of this important work. We look forward to speaking with you next week and to receiving your comments on the draft Roadmap outline by January 19.

Warm regards,

Mike

Michael T. Osterholm, PhD, MPH
Regents Professor
McKnight Endowed Presidential Chair in Public Health
Director, Center for Infectious Disease Research and Policy
Distinguished University Teaching Professor
Environmental Health Sciences, School of Public Health
Professor, Technological Leadership Institute College of Science and Engineering
Adjunct Professor, Medical School

DRAFT V5 —NOT FOR DISTRIBUTION— January 1, 2018

Nipah Research and Development (R&D) Roadmap

Roadmap purpose: To provide a framework for identifying the vision, outlining the underpinning strategic goals, and prioritizing research areas and activities (from basic research to advanced development, licensure, manufacture, and deployment) for accelerating the collaborative development of medical countermeasures (MCMs) against Nipah virus (NiV) infection.

*(**Note:** The content below is organized into the three main categories for R&D of MCMs [diagnostics, therapeutics, and vaccines] to allow reviewers an opportunity to follow the lines of thinking for each category. Some issues are cross cutting and apply to more than one category. As this draft is refined, the content may be reorganized to create one over all roadmap and to group cross-cutting issues together. In addition, this document is relatively detailed to ensure that the most important issues are identified and discussed. As the document evolves, certain items may be "rolled up" into a smaller number of broader issues, strategic goals, and primary activities.)*

INTRODUCTION

Nipah virus (NiV) is a paramyxovirus that was first identified as a zoonotic pathogen after an outbreak occurred in pigs and humans in Malaysia and Singapore in 1998 and 1999. As part of that outbreak, 265 human cases were identified in Malaysia, and 11 abattoir workers in Singapore became ill following contact with imported pigs. No new outbreaks have been reported in these countries since May 1999. NiV infection was subsequently recognized in Bangladesh in 2001 and nearly annual outbreaks have occurred in that country since, with disease also occurring periodically in eastern India. Other regions may be at risk for NiV infection, as serologic evidence for NiV has been found in the natural reservoir for NiV (Pteropus bat species) in a number of other countries, including Cambodia, Thailand, Indonesia, Madagascar, and Ghana. In the 1998-99 Malaysia outbreak, NiV spillover occurred from bats to pigs, which led to pig-to-pig, pig-to-human, and human-to-human NiV transmission. In the outbreaks in Bangladesh, intermediary hosts between bat and human have not played a role, with human consumption of bat-contaminated raw date palm sap serving as the primary NiV source. In humans, NiV infection has neurological and respiratory syndromes, with fever, headache, dizziness, and vomiting as the primary presenting clinical features. NiV infection may result in late-onset encephalitis and/or relapsing encephalitis, and survivors may experience long-term neurological deficits. Genomic sequencing has demonstrated that two distinct strains of NiV were responsible for the outbreaks in Southeast Asia (NiV_M) and in Bangladesh/India (NiV_B). NiV is part of the Henipavirus genus; this genus also includes another zoonotic pathogen (Hendra virus [HeV]), which predominantly causes infection in horses and also can lead to human disease (usually following contact with infected horses). To date, HeV disease has been confined to Australia. An outbreak of an unidentified Henipavirus (possibly NiV or a closely related virus) occurred among horses and humans in the Philippines in 2015.

The R&D roadmap for NiV infection is a key component of the WHO R&D Blueprint initiative for accelerating research and product development of medical countermeasures to enable effective and

timely emergency response to infectious disease epidemics. NiV is identified in the Blueprint's initial list of "priority pathogens" (defined as pathogens that are likely to cause severe outbreaks in the near future and for which few or no medical countermeasures [MCMs] exist). The Blueprint calls for the development of R&D roadmaps for the priority pathogens to align and stimulate R&D of new or improved countermeasures, such as rapid diagnostic assays, novel therapeutics, and vaccines. The scope of R&D addressed in the roadmap ranges from basic research to late-stage development, licensure, and early use of medical countermeasures to prevent and control LF outbreaks and endemic disease.

Other aspects of public health preparedness and response, in addition to R&D for MCMs, are critical to successful NiV infection prevention and control. Examples include enhanced surveillance systems, minimizing NiV transmission at the human-animal interface, improved personal protective equipment, effective community engagement, and workforce development and training in at-risk regions. Many of these issues are beyond the scope of the R&D roadmap, but need to be addressed as part of a broader public health control strategy.

Commented [SPL1]: I consider this paragraph as essential framing and urge that it be retained, even as we work towards a more focused document. It is difficult, because medical countermeasures do not stand alone. They need to interface with a system that constructively deploys them. At a minimum we should strive for countermeasures that are fit for purpose for the contexts where they are needed.

VISION

Robust MCMs to detect, prevent, and control outbreaks of NiV (and other closely related henipaviruses) that are available and readily deployable for use when needed. These MCMs include: (1) rapid, accurate, point-of-care diagnostics; (2) safe and effective treatment and post-exposure prophylaxis (PEP); and (3) safe and effective vaccines to prevent disease, disability, and death.

DIAGNOSTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Initial signs and symptoms of NiV infection are nonspecific and the diagnosis often is not suspected at the time of presentation. Additionally, latent disease can occur long after initial infection. Both of these factors can hinder accurate diagnosis.
- Laboratory infrastructure and diagnostic capabilities in endemic and at-risk areas are often lacking or inadequate, which can lead to delays in diagnosis.
- Diagnostic testing of clinical specimens for NiV poses safety and logistical challenges in under-resourced areas with regard to collection, handling, transport, and laboratory analysis.
- NiV appears to have the potential for strain heterogeneity and antigenic variability, which could potentially influence accuracy of diagnostic testing over time.
- The natural reservoir for NiV is fruit bats of the Pteropus genus, which have a wide geographic range that stretches across much of the Western Pacific region, Southeast and South Asia, and Madagascar. Evidence also suggests that other fruit bats of the Pteropodidae family may harbor NiV; such bats can be found across Africa and parts of the Middle East. This broad host range increases the likelihood of additional spillover from bats to humans or livestock in new areas where the disease has not yet been detected, which may make accurate and timely diagnosis

more difficult owing to lack of clinical experience with the condition and lack of available laboratory testing.

- Pteropus bat species also appear to carry other henipaviruses (in addition to NiV and HeV), some of which may prove to be pathogenic in humans and livestock. Capacity to identify additional pathogenic henipaviruses is an important challenge for ensuring diagnostic preparedness to respond to future outbreaks.
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- High-level biocontainment requirements may pose an impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*

Key needs

- A target product profile (TPP) for NiV diagnostics, identifying optimal and desirable characteristics to guide the development of promising diagnostic assays.
- Rapid point-of-care diagnostic tests for NiV that involve minimal requirements for laboratory infrastructure, can detect disease early in the clinical course, can be applied in both human and animal populations, and have a high degree of sensitivity and specificity for different NiV strains.
- Diagnostic preparedness to detect HeV and other emergent henipaviruses in humans and animals as they arise.
- Proficiency testing, to monitor reproducibility and performance of NiV diagnostic assays in the field.
- Enhanced laboratory infrastructure in at-risk areas, with a particular focus on improving regional and national laboratory capacity for case and outbreak confirmation.
- Long-term need: if NiV or HeV vaccines become widely used in livestock, serological testing to differentiate vaccinated animals from infected animals, such as the DIVA test.
- Long-term need: multiplex syndrome-based assay panels for use in humans and animals that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or additional high-consequence pathogens.

Commented [SPL2]: Given the wide range of Pteropus bats this needs to be defined. Clearly, we need low cost accurate diagnostics, but we also need to consider the system that needs to be in place in order to make use of the diagnostics. We risk mission creep if we include development of global surveillance as part of medical countermeasures, but there is a difference between how we would optimally deploy point of care diagnostics in Bangladesh and West Bengal, India, compared with the Philipines or Cameroon. We should consider the different needs in these different contexts, to assure that we have an approach that is fit for purpose.

Knowledge gaps

- Further research is needed on the kinetics of NiV detection in cerebrospinal fluid, blood, saliva other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- More information is needed regarding the performance characteristics (including sensitivity, specificity, and limits of detection) for NiV assays, particularly for newer tests (such as pseudotyped neutralization assays and antigen-capture ELISAs).
- Additional validation data are needed to assess performance characteristics of NiV diagnostic assays against the various strains of NiV.

Commented [SPL3]: Salivary pcr is often positive in Bangladesh before antibodies are detectable.

- Ongoing phylogenetic and evolutionary analyses of NiV strains are needed to monitor antigenic changes over time that may impact diagnostic testing and the epidemiologic and clinical features of disease.
- Continued R&D, manufacture, deployment, and assessment of MCMs, as well as other preventive measures, are dependent on accurate and current information on the ecology and epidemiology of NiV infection, using a One Health approach. Improved surveillance is needed to determine the true incidence of disease in endemic areas and to monitor the occurrence of spillover incidents from bats to humans or livestock in new areas. Additionally, continued research is needed to better define and assess the occurrence of NiV and other henipaviruses in the natural reservoir of Pteropus bats and potentially other fruit bats. *(*cross cutting)*

Strategic Goals

1. Obtain a better understanding of the kinetics of NiV detection at various points during the clinical course of illness to allow improved diagnostic capability across the disease spectrum.
2. Promote the development and assessment of affordable, highly sensitive and specific, point-of-care diagnostic tests for use in humans and animals that have minimal requirements for biosafety precautions and staff training and that can detect NiV and other related henipaviruses.
3. Strengthen laboratory infrastructure and capacity in the endemic and at-risk areas to improve early diagnostic capabilities.

Commented [SPL4]: Because this is a wildlife shed virus and these creatures live near food sources, spillovers to human communities have occurred almost exclusively in rural communities in Bangladesh. The health care facilities that serve these communities not only have very rudimentary laboratory infrastructure, they also do not have the routine practice of using diagnostic tests to manage patients. Thus, achieving the stated strategic goals requires a much broader, deeper effort, than providing reagents and testing for proficiency at national labs.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Further evaluate** the kinetics of NiV detection in cerebrospinal fluid, blood, saliva, other body fluids, and tissue samples to enhance the ability to diagnose infection at different stages of disease.
- **Determine** performance characteristics for promising new assays for diagnosis of NiV infection and develop appropriate standards for their use in different contexts.
- **Conduct** field evaluation studies to assess and validate new diagnostic tests for NiV infection.
- **Explore** new diagnostic approaches that may allow for earlier detection of infection.
- **Expand research** to further understand the ecologic and epidemiology of NiV and other henipaviruses in human and animal populations over time, using a One Health approach.
- **Continue to perform** phylogenetic and evolutionary analyses of NiV strains to monitor antigenic changes and characterize genetic diversity over time.
- **Continue to research** development of serologic tests to distinguish vaccinated from infected animals, if henipavirus vaccines are to be widely used in livestock populations.

Commented [SPL5]: Consider whether efforts to promote sharing of sequence and strains would benefit from attention.

Product development

- **Generate** a TPP for NiV diagnostics.

- **Develop, evaluate, and validate** point-of-care rapid diagnostic tests for NiV infection that are affordable, highly sensitive and specific, available for use in humans and animals, and can capture antigenically diverse strains of the virus.
- **Develop** multiplex syndrome-based assay panels that can detect NiV infection while simultaneously screening for the presence of other henipaviruses or other high-consequence pathogens in humans and animals.

Key capacities

- **Create** international partnerships to fund, support, and promote enhanced laboratory capacity, public health surveillance capacity, and infrastructure for detection of NiV in endemic and at-risk areas, particularly in support of enhanced national and regional diagnostic capabilities.
- **Improve surveillance capacity** to: (1) better define the incidence of disease in NiV-endemic areas and (2) promote surveillance in non-endemic areas to enhance detection of emergent NiV disease or disease caused by other related henipaviruses.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV point-of-care diagnostics.
- **Ensure** access to regulatory review and authorization of NiV diagnostic assays.
- **Support** plans for adequate manufacturing and subsequent distribution of NiV diagnostic products to endemic and at risk areas.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

THERAPEUTICS

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV disease occurs in relatively small outbreak settings, the low incidence of disease poses a major challenge for conducting phase 3 clinical trials; therefore, alternative regulatory pathways will need to be considered for licensure of vaccines or therapeutics. *(*cross cutting)*
- Differences between the NiV$_M$ and NiV$_B$ strains may impact the effectiveness of developed MCMs, yet NiV$_M$ has been used significantly more often than NiV$_B$ in research. *(*cross cutting)*

Commented [SPL6]: Moreover, if we actually had more strains we may identify more heterogeneity than is currently apparent.

- Nipah virus can infect the central nervous system (CNS), which creates challenges for generating therapeutic agents that cross the blood-brain barrier and can access the brain and spinal cord to inhibit viral replication. This is particularly important for preventing late-onset CNS disease.
- The absence of improved diagnostic assays for timely diagnosis of infection creates an important challenge in providing early treatment and PEP to exposed persons. In addition, the healthcare systems in endemic areas are not able to rapidly identify contacts most likely to benefit from PEP. *(*cross cutting)*

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, the African green monkey (AGM) is regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*

Key needs

- A TPP for NiV therapeutic agents, identifying optimal and desirable characteristics to guide the development of promising treatment approaches in the context of individual and community priorities.
- Safe, easily administered, well-tolerated, and effective therapeutic agents that treat both acute and chronic (relapsing) NiV infection to improve survival and decrease associated morbidity and long-term disability.

Commented [SPL7]: Would be ideal, but, given the biology, may be much more difficult than an acute countermeasure. Might separate these into 2, so that the ideal does not become the enemy of the good.

- Safe and effective PEP to prevent infection following exposure to NiV and guidance on PEP use. PEP could be used to prevent illness in healthcare workers, family caregivers, and persons exposed to infected livestock. *(*cross cutting)*

Knowledge gaps

- Additional information is needed on the immunology and pathogenesis of NiV in order to develop appropriate treatment options. This includes evaluating the pathophysiologic differences between NiV$_B$ and NiV$_M$ infection, determining the mechanisms of late-onset and relapsing NiV encephalitis, identifying factors influencing the development of permanent neurological sequela, and further characterizing cell-mediated and humoral immune responses to NiV infection. In addition, identifying aspects of the immune response that are absent or counter-effective during human NiV infection may lead to the development of novel targeted intervention strategies.
- Patients treated with Ribavirin late in the 1998-1999 NiV outbreak in Malaysia experienced 36% lower mortality than persons from earlier in the outbreak who were not treated with Ribavirin.~~was found to reduce mortality by 36% in the 1998-1999 NiV outbreak in Malaysia and is considered~~Ribaviran may be an option for treatment of NiV infection. However, animal studies in hamsters and AGMs have not supported efficacy for ribavirin. Further research into the potential effectiveness of ribavirin for NiV infection is needed.
- The human monoclonal antibody (mAb) m102.4 has demonstrated protection against lethal NiV challenge in animal models and has been provided as a compassionate use for a small number

of individuals exposed to HeV-infected horses. Recently, a phase 1 clinical trial for m102.4 with 40 human participants was completed in Australia, but results are not yet available. Additional animal studies and clinical trials are needed to assess the safety, tolerability, and efficacy of m102.4 (and possibly other mAbs) for PEP and potentially early treatment of clinical disease.

- Preclinical and clinical data are needed on the safety, tolerability, and efficacy of the most promising novel treatments (such as fusion inhibitory peptides), used alone or in combination with other therapies. Additionally, the therapeutic windows of each therapy should be determined for both NiV_B and NiV_M, as highlighted by a recent study in AGMs that showed the therapeutic window for m102.4 against NiV_B to be shorter than for NiV_M.
- Additional data are needed to determine the role of PEP and to inform development of guidance on the type of exposures that warrant such intervention and the most appropriate agents to administer.
- Patients may benefit from optimal supportive care independent of treatment with specific NiV therapeutic agents. Key research areas include obtaining data on the safety and efficacy of individual components of supportive care for NiV, such as optimal fluid and respiration management strategies, diagnosis and treatment of organ dysfunction, and the use of empiric antibiotics and/or antimalarials, to inform supportive care and best-practice guidelines.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), such as determining the most appropriate lethal NiV dose for MCM development and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*

Commented [SPL8]: An important related issue is how we protect the health care workers and fellow patients in these settings. Part of this is covered in the vaccine section, but the capacity to protect health care workers and other patients is extremely limited in the contexts where we see recurrant NiV outbreaks. A clear eyed understanding of the context, and piloting approaches within these contexts is essential to develop and refine medical counter measures to that they can reduce pandemic risk. To be effective these countermeasures need to work, not only in idealized trial conditions, but in the everyday setting where high risk patients present.

Strategic Goals

1. Develop, evaluate, and license therapeutic agents for treatment of NiV infection and for PEP to prevent NiV infection and ensure that therapies are prepositioned in endemic areas for use as needed.
2. Stimulate basic research for better understanding of NiV pathogenesis and the immune response to infection.
3. Strengthen clinical and laboratory infrastructure in endemic and at-risk areas to promote early diagnosis of NiV cases so that effective treatment and PEP can be rapidly administered.
4. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Conduct** basic science research on the immunology and pathogenesis of NiV infections to inform development of new therapeutic agents.

- **Determine** the key differences between NiV_B and NiV_M that may have implications for the development of safe and effective NiV therapeutics.
- **Continue to research** the safety, tolerability, and efficacy of investigational therapies for NiV infection, including animal studies and clinical trials as appropriate and feasible.
- **Research** optimal treatment and supportive care strategies for NiV infection and determine best practice guidelines.

Product development

- **Generate** a TPP for NiV infection therapeutics.
- **Develop, evaluate, and license** safe and effective therapeutic agents for treatment of NiV infection that are active against both NiV_B and NiV_M and other henipaviruses, and that can cross the blood-brain barrier to treat or prevent CNS disease.
- **Identify** therapeutic approaches for PEP that are broadly active against both NiV_B and NiV_M and other NiV strains or pathogenic henipaviruses that may emerge.

Key capacities

- **Refine, standardize, and validate** animal challenge models for future assessment of promising therapeutic candidates.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV therapeutics.
- **Support** plans for licensure/registration, adequate manufacturing, and subsequent distribution of safe and effective therapies to endemic areas and to other at-risk areas on an as-needed basis for outbreak control.
- **Secure** financing for procurement and deployment of NiV therapeutics for outbreak control once treatment agents are available.
- **Develop** treatment and PEP guidance as new therapies demonstrate safety and efficacy.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

VACCINES

Current Primary Challenges, Key Needs, and Knowledge Gaps

Primary challenges

- Because NiV occurs as relatively small, focal outbreaks, the low incidence of infection poses a major challenge for conducting phase 3 clinical trials for efficacy demonstration; therefore,

alternative regulatory pathways may need to be considered for licensure of NiV vaccines or therapeutics. *(*cross cutting)*

- High-level biocontainment requirements pose a significant impediment to development of MCMs, as certain materials must be generated under the highest biosafety level (BSL-4) conditions. *(*cross cutting)*
- Economic incentives to invest in Nipah research are not readily apparent, as the disease primarily occurs in the under-resourced areas of South Asia and disease incidence is low; therefore, securing funding for Nipah research represents a substantial challenge. The development of a sustainable value proposition is needed to secure funding to complete development, licensure, manufacture, and deployment of NiV MCMs. *(*cross cutting)*
- The absence of improved diagnostic assays for timely diagnosis of infection creates a challenge in implementing a rapid reactive vaccination strategy for NiV outbreak control, once vaccines are available for use. *(*cross cutting)*
- While ferrets, Syrian hamsters, and INFO KO mice are well-established animal models for NiV research applicable to humans, African green monkeys (AGMs) are regarded as the most relevant animal model for evaluation of candidate therapeutics and vaccines. Additionally, studies involving the AGM model may be required for licensure involving alternative regulatory pathways. Costs, space requirements (particularly in BLS-4 containment facilities), and ethical concerns constrain the use of AGMs. *(*cross cutting)*

Key needs

- Safe and effective PEP (either vaccines or therapeutic agents) to prevent infection following exposure to NiV and guidance on their use. Such countermeasures are important tools to protect healthcare workers, family caregivers, and livestock workers. *(*cross cutting)*
- Nipah vaccines that can protect against both NiV_M and NiV_B in humans and animals, and that provide rapid onset of an immune response to adequately prevent and control outbreaks. Research tools, such as standardized neutralization assays, to compare immunogenicity of different vaccines.
- Enhanced surveillance capacity to provide more accurate information on the current incidence and geographic distribution of NiV in endemic and at-risk areas in order to refine potential vaccination strategies, particularly over time.

Knowledge gaps

- While neutralizing antibodies are likely a primary mediator of protection against NiV infection, cellular immunity appears to also play a role. Additional research is needed regarding the innate, cell-mediated, and humoral immune responses that constitute protective immunity against NiV.
- The mechanisms that allow NiV to escape immunological clearance and cause delayed onset or recurrent encephalitis are unknown. Further research to better understand viral clearance has important implications for development of vaccines and therapeutics.
- Additional research is needed to determine if vaccine candidates are cross-protective between NiV_M and NiV_B; only a few studies demonstrating cross-protection have been performed to date.

- Further research is needed to determine durability and safety profiles of candidate vaccines, such as VSV recombinants, where concerns regarding potential neurotropism have been raised.
- Further research is needed to determine the henipavirus cross-protection efficacy for NiV of the HeV-sG subunit vaccines, such as the recombinant subunit vaccine HeV-sG (Equivac® HeV, Zoestis).
- The identification of specific correlates of protection and standardized mechanisms for measuring immune correlates are needed to facilitate research on promising NiV vaccine candidates, and expedite possible licensing through nontraditional regulatory pathways, such as the US FDA's Animal Rule and accelerated approval mechanisms.
- Mathematical modelling may be useful in estimating the potential impact of NiV vaccines and in simulating various epidemiologic scenarios that may impact strategies for vaccine use.
- Additional research is needed to refine, standardize, and validate relevant animal challenge models for NiV infection in humans (e.g., INFAR KO mouse, ferret, Syrian hamster, and AGM models), including determining the most appropriate NiV dose for MCM development (Johnston 2015) and identifying the best models for studying chronic (relapsing) infection, particularly if investigators use the US FDA's Animal Rule to obtain licensing. *(*cross cutting)*
- Because livestock (e.g., pigs and horses) are intermediary hosts for NiV and HeV, vaccination of livestock populations has been suggested as a possible mitigation strategy for preventing secondary transmission to humans. Currently, one HeV vaccine is available for horses. Ongoing research into developing NiV/HeV vaccines for livestock is needed to further assess the merit of this potential control strategy.
- If evidence at some point supports the need for a broader, population-based vaccination strategy, additional research may be warranted on the development of multivalent vaccines that protect against more than one infection (such as a combined vaccine against NiV and HeV or NiV and measles virus) for use in NiV endemic areas.

Commented [SPL9]: I suspect that this is an easier technical problem than it is an economic problem. Only 15% of Australian horses are vaccinated against HeV. Pigs in low income countries are rarely protected against swine cholera or Japanese Encepahlitis. The incentives for livestock raisers are not well aligned for deployment as a human public health tool.

Commented [SPL10]: Should consider surge capacity to rapidly ramp up vaccine supply in case NiV is used as a bioterror agent, or we face a strain that naturally develops increased capacity for person to person transmission.

Strategic Goals

1. Develop, evaluate, and license NiV vaccines for use in humans and potentially livestock, primarily for reactive/outbreak use in endemic and at-risk areas.
2. Continue to research cross-protection of candidate vaccines against NiV and HeV (and potentially other emergent henipaviruses as needed).
3. Ensure that post-approval pharmacovigilance is in place to support product deployment and monitoring.

Landmark Goals/Milestones

[TBD once the strategic goals have been determined.]

Priority Areas/Activities

Research

- **Determine** the innate, cell-mediated, and humoral immune responses that contribute to protective immunity against NiV infection.

- **Determine** the key differences between NiV_B and NiV_M pathogenesis that may have implications for the development of safe and effective NiV vaccines or therapies.
- **Further study** cross protection of various vaccine candidates against NiV_B and NiV_M strains, and between NiV strains and HeV strains.
- **Identify and standardize** correlates of protection, which are necessary for ongoing research efforts and also may be important for vaccine licensure through non-traditional regulatory pathways.
- **Generate** research tools (e.g., standardized neutralization assays) to compare immunogenicity of different vaccines.
- **Complete preclinical evaluation** of promising candidate NiV vaccines for safety, immunogenicity, efficacy, correlates of protection, and durability.
- **Conduct** mathematical modelling to estimate the potential impact of NiV vaccines and inform strategies for vaccine use.

Product development

- **Develop, clinically evaluate, and license** safe and effective monovalent NiV vaccines for humans and livestock, primarily for reactive use during outbreaks.

- **Develop, clinically evaluate, and license** safe and effective multivalent vaccines that protect against more than one disease (e.g., vaccines that protect against both NiV and MV or HeV), if broader population-based vaccination is warranted at some point in the future.

Key capacities

- **Refine and validate** relevant animal challenge models for NiV infection.
- **Improve** surveillance capabilities, particularly in rural areas of resource-limited settings, to assess the true burden and incidence of NiV disease, which may in turn, inform vaccination strategies.
- **Ensure** post-approval pharmacovigilance and effectiveness evaluations occur to support product deployment and monitoring.

Policy and commercialization

- **Establish** a sustainable value proposition for NiV vaccines.
- **Ensure** access to regulatory review and authorization of NiV vaccines, particularly if non-traditional pathways are needed for licensure.
- **Support** plans for adequate manufacturing and stockpiling of licensed NiV vaccines for use when outbreaks occur.
- **Provide** ongoing assessment to inform development and maintenance of NiV vaccine stockpiles over time; mathematical modelling may be used to contribute to this assessment.
- **Continue** to provide guidance on vaccination strategies for various target populations and epidemiologic scenarios, once vaccines are available.

Schedule of Resources, Coordination, and Implementation

[TBD; will obtain input later in the process.]

Critical Path Analysis

[TBD once the primary activities have been vetted by subject matter experts.]

BACKGROUND INFORMATION

World Health Organization R&D Roadmap documents and guidance:

WHO. Nipah Baseline Situational Analysis, Nov 2017

WHO. WHO target product profile for Nipah virus vaccine. Jun 2017

Other Publications:

Angeletti S, Presti AL, Cella E, et al. Molecular epidemiology and phylogeny of Nipah virus infection: a mini review. Asian Pac J Trop Dis 2016;9(7):630-4. doi: 10.1016/j.apjtm.2016.05.012 [Full text]

ANZCTR (Australian New Zealand Clinical Trials Registry). Trial ACTRN 12615000395538. Last updated 2016 Nov 29; site accessed 2017 Dec 12 [Web page]

Baseler L, Scott, DP, Saturday G, et al. Identifying early target cells of Nipah virus infection in Syrian hamsters. PLoS Negl Trop Dis 2016 Nov; 10(11): e0005120. Published online 2016 Nov 3 [Full text]

Bossart KN, Rockx B, Feldmann F, et al. A Hendra virus G glycoprotein subunit vaccine protects African green monkeys from Nipah virus challenge. Sci Transl Med 2012 Aug 8;4(146): 146ra107. [Full text]

Broder CC. Passive immunization and active vaccination against Hendra and Nipah viruses. Dev Biol (Basel) 2013 May 14;135:125-38 [Abstract]

Broder CC, Weird DL, Reid PA. Hendra virus and Nipah virus animal vaccines. Vaccine 2016 Jun 24;34(30):3525-34 [Full text]

Broder CC, Xu K, Nikolov DB, et al. A treatment for and vaccine against the deadly Hendra and Nipah viruses. Antivir Res 2013 Oct;100(1):8-13. doi: 10.1016/j.antiviral.2013.06.012. [Full text]

CDC (Centers for Disease Control and Prevention). Nipah virus (NiV) signs and symptoms. Last updated 2014 Mar 20 [Web page]

Chen F, Zhao D, Jiao Z. Nipah virus encephalitis. In: Li H, ed. Radiology of Infectious Diseases: Volume 1. Springer, Dordrect; 2015. 541-7

Chiang CF, Lo MK, Rota PA, et al. Use of monoclonal antibodies against Hendra and Nipah viruses in an antigen capture ELISA. Virol J 2010 Jun 3;7(115). doi: 10.1186/1743-422X-7-115. [Full text]

Ching PG, de los Reyes V, Sucaldito M, et al. Outbreak of Henipavirus infection, Philippines, 2014. Emerg Infect Dis 2015;21(2):328-331. doi: 10.3201/eid2102.141433. [Full text]

Chong HT, Kamarulzaman A, Tan CT, et al. Treatment of acute Nipah encephalitis with ribavirin. Ann. Neurol 2001;49:810–813. [Abstract]

Chua KB, Gubler DJ. Perspectives of public health laboratories in emerging infectious diseases. Emerg Microbes Infect 2013 Jun;2(6): doi: 10.1038/emi.2013.34. [Full text]

Clayton BA. Nipah virus: transmission of a zoonotic paramyxovirus Current Opinion in Virology 2017; 22:97–10 [Abstract]

Daniels P, Ksiazek T, Eaton BT. Laboratory diagnosis of Nipah and Hendra virus infections. Microbes Infect 2001Apr;3(4):289-95 [Abstract]

DeBuysscher BL, de Wit E, Munster VJ, et al. Comparison of the pathogenicity of Nipah virus isolates from Bangladesh and Malaysia in the Syrian hamster. PLoS Negl Trop Dis 2013 Jan 17;7(1):e0002024. [Full text]

DeBuysscher BL, Scott D, Marzi A, et al. Single-dose live-attenuated Nipah virus vaccines confer complete protection by eliciting antibodies directed against surface glycoproteins. Vaccine 2014 May 7;32(22):2637-44 [Full text]

Dhondt KP, Horvat B. Henipavirus infections: lessons from animal models. Pathogens 2013 Apr 9;2(2):264-87 [Full text]

de Wit E, Munster VJ. Nipah virus emergence, transmission, and pathogenesis. In: Shapshak P, Sinnott JT, Somboonwit C, Kun JH, eds. Global Virology I – Identifying and Investigating Viral Diseases. New York, NY: Springer US; 2015. 125-46

Foord AJ, White JR, Colling A, et al. Microsphere Suspension Array Assays for Detection and Differentiation of Hendra and Nipah Viruses. Biomed Res Int. 2013; 2013: 289295. Published online 2013 Feb 6. doi: 10.1155/2013/289295. [Full text]

Freiberg AN, Worthy MN, Lee B, et al. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. J Gen Virol 2010 Mar;91(Pt 3):765-72 [Full text]

Geisbert TW, Daddario-DiCaprio KM, Hickey AC, et al. Development of an acute and highly pathogenic nonhuman primate model of Nipah virus infection. PLoS ONE 2010 May 18;5(5):e0010690. [Full text]

Geisbert TW, Mire CE, Geisbert JB, et al. Therapeutic treatment of Nipah virus infection in nonhuman primates with a neutralizing human monoclonal antibody. Sci Transl Med 2014 June 25; 6(242): 242ra82 [Full text]

Georges-Corbot MC, Contamin H, Faure C, et al. Poly(I)-poly(C12U) but not ribavirin prevents death in a hamster model of Nipah virus infection. Antimicrob Agents Chemother 2016 May;50(5):1768-72. doi: 10.1127/AAC.50.5.1768-1772.2006. [Full text]

Guillaume V, Contamin H, Loth P, et al. Nipah virus: vaccination and passive protection studies in a hamster model. J Virol 2004 Jan;78(2):834-40 [Full text]

Hayman DTS, Johnson N. Nipah virus: a virus with multiple pathways of emergence. In: Johnson N, ed. The Role of Animals in Emerging Viral Diseases. Elsevier 2014; 293-315 [Abstract]

Johnston SC, Briese T, Bell TM, et al. Detailed analysis of the African green monkey model of Nipah virus disease. PLoS One 2015 Feb 23;10(2)e0117817. [Full text]

Ksiazek TG, Rota PA, Rollin PE. A review of Nipah and Hendra viruses with an historical aside. Virus Research 2011;162(1-2):173-83 [Abstract]

Kulkarni DD, Tosh C, Venkatesh G, et al. Nipah virus infection: current scenario. Indian J Virol 2013 Dec;24(3):398-408 [Full text]

Luby SP. The pandemic potential of Nipah virus. Antiviral Res 2013;100(1):38-43 [Full text]

Mathieu C, Horvat B. Henipavirus pathogenesis and antiviral approaches. Expert Rev Anti Infect Ther 2015;13(3):343-54 [Abstract]

McEachern JA, Bingham J, Crameri G, et al. A recombinant subunit vaccine formulation protects against lethal Nipah virus challenge in cats. Vaccine 2008 Jul 23;26(31):3842-52 [Abstract]

Mire CE, Satterfield BA, Geisbert JB, et al. Pathogenic differences between Nipah virus Bangladesh and Malaysia strains in primates: implications for antibody therapy. Sci Rep 2016 Aug 3;(6). doi: srep30916. [Full text]

Mire CE, Versteeg KM, Cross RW, et al. Single injection recombinant vesicular stomatitis virus vaccines protect ferrets against lethal Nipah virus disease. Virol J 2013 Dec 13. pii: 1743-422X-10-353. [Full text]

Pernet O, Schneider BS, Beaty SM, et al. Evidence for henipavirus spillover into human populations in Africa. Nat Commun. 2014 Nov 18;5:5342 [Full text]

Prescott J, de Wit E, Feldmann H, et al. The immune response to Nipah infection. Arch Virol 2012 September; 157(9): 1635–1641 [Full text]

Rahman SA. National Guideline for Management, Prevention and Control of Nipah Virus Infection including Encephalitis 2011 [Full text]

Satterfield BA, Geisbert TW, Mire CE. Inhibition of the host antiviral response by Nipah virus: current understanding and future perspectives. Future Virol 2016 Apr 15;11(5):331-34 [Abstract]

Sazzad HM, Luby SP, Ströher U, et al. Exposure-based screening for Nipah virus encephalitis, Bangladesh. Emerg Infect Dis 2015 Feb;21(2):349-51 [Full text]

Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses 2012 Feb;4(2):280-308 [Full text]

Tan KS, Tan CT, Goh KJ. Epidemiological aspects of Nipah virus infection. Neuro J Southeast Asia 1999;4:77-81 [Full text]

van den Pol AN, Mao G, Chattopadhyay A, et al. Chikungunya, Influenza, Nipah, and Semliki Forest Chimeric viruses with Vesicular Stomatitis Virus: actions in the brain. J Virol 2017 Feb 28;91(6). pii: 02154-16. [Full text]

Wang LF, Daniels P. Diagnosis of henipavirus infection: current capabilities and future directions. Curr Top Microbiol Immunol 2012;359:179-96 [Abstract]

From: Steve Luby on behalf of Steve Luby <sluby@stanford.edu>
To: Michael Osterholm
Cc: christopher.broder@usuhs.edu; chuakb@tll.org.sg; egurley1@jhu.edu; gnmillig@utmb.edu; mahmudur57@gmail.com; pyr3@cdc.gov; linfa.wang@duke-nus.edu.sg; K.Cook@wellcome.ac.uk; benassiv@who.int; kamoore@umn.edu; jto@umn.edu; johns369@umn.edu; mehr0035@umn.edu; crcruz@umn.edu; oneil005@umn.edu; preziosim@who.int
Subject: Re: Agenda and materials for the Nipah WHO R&D Roadmap Taskforce conference call to be held on Tuesday, January 9 or Wednesday January 10, depending on time zone
Date: Saturday, January 6, 2018 4:49:10 AM
Attachments: Nipah Draft Roadmap.V5 sl.docx

Mike,

Thanks so much for moving this forward. I am looking forward to the call.

I will be in Bangladesh, but I should be able to connect.

Attached are some comments on the roadmap outline.

Steve

Michael Osterholm Thursday, January 04, 2018 8:50 AM

Dear Colleagues:

ï¿½

I am writing to remind you of our Nipah Roadmap Taskforce conference call **on Tuesday, January 9 at 5:00 pm PST, 7:00 pm CST, 8:00 pm EST, and Wednesday, January 10 at 1:00 am GMT, 2:00 am CET, 7:00 am BST, and 9:00 am SGT.**

ï¿½

Each of you should have received a calendar invite with the call-in information and I have repeated that information below:

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->Conference code: 998-3378#

<!--[if !supportLists]-->oï¿½ï¿½ <!--[endif]-->USA Toll Free: 866-767-9978

<!--[if !supportLists]-->oï¿½ï¿½ <!--[endif]-->UK Toll Free: 0808 1017 535

<!--[if !supportLists]-->oï¿½ï¿½ <!--[endif]-->Switzerland Toll Free: 0800 5537 12

<!--[if !supportLists]-->oï¿½ï¿½ <!--[endif]-->Singapore Toll Free: 800 101 2395

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->For Bangladesh, we do not have a toll free line; we will contact you separately to make logistical arrangements.

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->As a BACKUP: International callers may use the following conference call information: Conference Line 205-254-8650 Conference Code: 998-3378#. However, please note that this is NOT a toll-free number.

ï¿½

This email includes the following attachments:

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->The Nipah draft roadmap outline. **Please review this document before the call** because most of our call will be devoted to discussing the document. Also, we would very much appreciate any written edits or comments that you have on the document, particularly if you are not able to participate in the call. In order to keep to our development timeline, we need to receive any written comments from you no later than Friday, **January 19.** Please send comments directly to kamoore@umn.edu.

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->A short slide deck for our call on Tuesday.

<!--[if !supportLists]-->ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ï¿½ <!--[endif]-->Supplemental materials on the MERS-CoV Roadmap as examples, including a Nature Medicine article on that Roadmap and an outline of the roadmap, similar to the outline we have developed for the Nipah Roadmap.

ï¿½

The agenda for the call is as follows (most of the time will be devoted to discussing the draft Nipah Roadmap outline):

1. Welcome and roll call
2. R&D roadmap architecture
3. Role of roadmap taskforce members
4. Roadmap development and timeline
5. Discussion of draft R&D roadmap for Nipah
6. Next steps

ï¿½

Thank you again for agreeing to be part of this important work. We look forward to speaking with you next week and to receiving your comments on the draft Roadmap outline by January 19.

ï¿½

Warm regards,

ï¿½

Mike

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Michael T. Osterholm, PhD, MPH
Regents Professor
McKnight Endowed Presidential Chair in Public Health
Director, Center for Infectious Disease Research and Policy
Distinguished University Teaching Professor
ï¿½ï¿½ï¿½ï¿½ Environmental Health Sciences, School of Public Health
Professor, Technological Leadership Institute College of Science and Engineering
Adjunct Professor, Medical School
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Science Program Review
February 08 - 10, 2017

CBEP
Cooperative Biological Engagement Program

Defense Threat Reduction Agency
United States of America

DEPARTMENT OF DEFENSE
COOPERATIVE THREAT REDUCTION PROGRAM

DEFENSE THREAT REDUCTION AGENCY
8725 JOHN J. KINGMAN ROAD, STOP 6201
FORT BELVOIR, VA 22060-6201

Dear Colleagues,

Welcome to the 9th Science Program Review of the U.S. Defense Threat Reduction Agency's Cooperative Biological Engagement Program (DTRA CBEP). The CBEP carries out its mission by collaborating with partner countries and the international community to minimize the threat of deliberate, accidental and natural infectious disease outbreaks through enhanced biosecurity, biosafety, and biosurveillance measures. Cooperative Biological Research (CBR) is an integral element of the threat reduction mission by supporting and informing operational biosurveillance, enhancing global health security, and establishing and fostering sustainable research partnerships. CBEP's achievements rely on successful collaboration with partner-country scientists, and we thank you for your dedication and cooperation in our shared efforts to better understand the biological threats that face our world today.

We have in attendance nearly 120 researchers from over 30 countries representing 70 CBR projects, and this three-day event will feature briefings by CBEP partner-country scientists and collaborators conducting CBEP-funded research. We look forward to engaging with researchers from around the globe and evaluating the spectrum of CBEP research activities, in order to strengthen the quality of projects supported by the program and ensure that current efforts meet research priorities and objectives.

A significant benefit of the CBEP Science Review is networking amongst fellow scientists. During the next few days, you will have the opportunity to meet with scientific and program colleagues from CBEP partner nations, the U.S. Department of Defense, other U.S. Government stakeholders, and international and U.S. program partners. With such a broad range of scientific expertise present, we hope that this environment will create new networks among subject matter experts and facilitate new partnerships within the scientific community.

Again, I want to thank you for your participation in this 2017 CBEP Science Program Review. I hope that you will enjoy your experience and find opportunities to expand your collaborative research network.

(b) (6)

Lance Brooks
Division Chief
Cooperative Biological Engagement
Program, J3CTB

Table of Contents

CBEP Mission

The Defense Threat Reduction Agency's Cooperative Biological Engagement Program is the Department of Defense’s premier biological nonproliferation division protecting the United States and its allies from especially dangerous pathogens by collaborating with partner countries and the international community to minimize the threat of deliberate, accidental, and natural infectious disease outbreaks through enhanced biosafety, security, and surveillance measures.

The Defense Threat Reduction Agency's Cooperative Biological Engagement Program utilizes Science Diplomacy to promote scientific and technical collaborations among partner nations and the international community in [the disciplines of] biological safety, security and surveillance to build constructive and sustainable international partnerships that address threats posed to health security from deliberate, accidental, and natural infectious disease outbreaks.

General Information

- Posters may be displayed on the available poster boards in the Foyer area. An informal networking and poster session will be held following the “Doing Science in Difficult Places” session.
    - Posters displayed in the Foyer area must be removed by 1:00 PM on Friday, February 10, 2017. Posters that are not removed by this time will not be kept by DTRA.
- A designated Prayer Room is available next to the Registration area in the Dogwood Room. Please see attendants at the Registration desk for additional information.
- If temporary luggage storage is required, please see the hotel Front Desk.
- Meeting space is available during the event for collaborative discussions. Please see the Registration desk for more information.
- Please see the Registration Desk for any questions or concerns during the event.

Acknowledgments

The following individuals were instrumental in the development and implementation of this event.

CBEP Science Program Review Steering Committee

Dr. Carl Newman
Chief Scientist, Cooperative Biological Engagement Program (CBEP)

Dr. Martha Stokes
Southeast Asia Science Manager, CBEP

Dr. Mary Lancaster
Africa Science Manager, CBEP

Contractor Support Steering Committee Members:

- Dr. Jarrad Marles
- Ms. Nicki Aleman
- Dr. Ayanna Flegler
- Dr. Dawn Defenbaugh
- Dr. Michael Patterson
- Dr. Elizabeth Waligora

Special Thank You to those who helped organize this event:

- Dr. Jeanne Fair
- Dr. Gavin Braunstein
- Mr. Andrew Kotila
- Dr. Mariam Lekveishvili
- Ms. Natalie Might
- Mr. Jeffrey Russin
- Mrs. Karla Somera
- Ms. Audrey Speer
- Ms. Danielle Wilbur

Science Program Review Panel

CBEP would like to acknowledge the following individuals for serving on the Review Panel for the 2017 CBEP Science Program Review. The Review Panel is comprised of experts with varied backgrounds in a broad range of scientific disciplines and expertise in public health security and program implementation. The Review Panel will provide CBEP with an external technical and programmatic evaluation of the CBEP-sponsored research portfolios to identify strengths and weaknesses in CBEP research engagements, along with recommendations for improving how the portfolios are designed and implemented.

Jessica Appler, PhD, is the Associate Director, Portfolio Development for the Biomedical Advanced Research and Development Authority (BARDA) Modeling and Visualization Hub within BARDA's Division of Analytic Decision Support at the U.S. Department of Health and Human Services (HHS). She oversees the development of a portfolio focused on assessing the public health and medical consequences for chemical, biological, radiological, and nuclear (CBRN) incidents, emerging infectious diseases, and pandemic influenza events. Prior to joining HHS, she was at the Deputy Undersecretary of the Army for Test and Evaluation CBRN Defense Division, where her portfolio included oversight of CBRN test and evaluation programs, as well as the testing and standards development for the Transport Isolation Systems during the 2014-2015 West African Ebola crisis. Previously, she was a member of the Chemical and Biological Defense Division of Department of Homeland Security's Science and Technology Directorate, where she provided subject matter expertise and strategic vision for existing and planned biosurveillance programs. She was part of the interagency team that identified critical needs and gaps across the government in biological detection and diagnostic technology for the National Biosurveillance Science & Technology Roadmap. Dr. Appler earned her Ph.D. in Neurobiology from Harvard University, and her B.S. in biochemistry from the University of Southern California.

Hillary H. Carter, PhD, is the Director for Countering Biological Threats on the National Security Council (NSC) staff. Dr. Carter's portfolio includes the Global Health Security Agenda, domestic and international biosecurity issues, implementation of the President's Strategy for Countering Biological Threats, biological threat reduction, and policies related to Dual Use Research of Concern. Dr. Carter is also an adjunct Professor at the George Washington University where she lectures on Global Health Diplomacy. Prior to joining NSC staff, Dr. Carter was the Senior Advisor for Biosecurity and a Team Chief in the Office of Cooperative Threat Reduction (CTR) at the U.S. Department of State. In this role, Dr. Carter led CTR's special project and management teams to develop policy and implement programs to combat global biological, chemical, and nuclear threats. Prior to this role, Dr. Carter served as the Acting Deputy Team Chief for the Biosecurity Engagement Program where she oversaw health security programs to reduce global biological risks. Dr. Carter received a Ph.D. in Cell and Developmental Biology from Vanderbilt University, where she studied intracellular transport during early development. Dr. Carter holds a B.S. in Neuroscience with a minor in Spanish from Vanderbilt University.

Marco De Nardi, DVM, has 15 years working experience in the field of animal health and epidemiology in both developed and less developed countries. He received a Degree in Veterinary Medicine (DVM) from Bologna University (IT) in 2002 and a MSc degree in Veterinary Epidemiology from the Royal Veterinary College and the London School of Hygiene and Tropical Medicine, UK in 2008. He is currently enrolled in the residency training (Population Medicine) of the European College of Veterinary Public Health. Following his DVM he practiced as a veterinary clinician in dairy farms in Italy. He then worked as a veterinary consultant and project coordinator for international organizations (non-governmental organizations and the Food and Agriculture Organization (FAO)) in East Africa from 2004 to 2007. In 2009-2013, he worked on research and capacity building programs at the "World Organization for Animal Health (OIE) and FAO Reference Laboratory for Avian Influenza" and the "FAO Reference Centre for Rabies" at Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe) in Italy. In 2011, he coordinated the European Food Safety Authority (EFSA)-funded project "Flurisk," assessing the pandemic risk posed by animal influenza viruses.

In 2013 he joined the private veterinary consultancy company SAFOSO (www.safoso.com), where he is responsible for the development and implementation of consultancy, research projects and capacity building programs in the area of risk assessment, surveillance, veterinary epidemiology, animal health and food safety. As a SAFOSO consultant he worked in different projects in Europe, U.S., Ukraine, Armenia, Georgia, Kazakhstan, Mongolia and Vietnam.

Sam Howerton, **PhD**, is as a Senior Executive at the National Science Foundation. Hearing the call to service in 2001, he escaped the bounds of a traditional scientific career by seeking out organizations undergoing change. He oversaw the development of new drugs, negotiated new international agreements, and provided entrepreneurs training on transforming their ideas into business ventures.

With a healthy appetite for risk, Sam is known for his 'first unto the breach' leadership and willingness to try most any food.

Possessed of a strong imagination and an inquisitive mind, he earned a Ph.D. in Chemistry-Environmental Toxicology and completed Air War College. Sam's most important job is raising his sons to be good Southern gentlemen.

Paul Keim, **PhD**, is the executive director of The Pathogen and Microbiome Institute, which uses genomic tools for understanding infectious diseases and the microbiome. This is a joint institute between TGen and Northern Arizona University, where Dr. Keim holds the Cowden Endowed Chair in Microbiology. His work has employed genetic and genomic analyses for understanding bacterial pathogen population structure and evolution for more than 30 years. Linking populations to their ecology has also been a critical part of his program. His laboratory served as the evidence repository and genetic analysis lab for the Federal Bureau of Investigation during the 2001 anthrax-letter investigation. He has been a leader in the field of microbial forensics which uses evolutionary analysis to understand close relationships among pathogen isolates. This work was foundational for his pursuit in public health investigations and the development of novel clinical diagnostic tests. He is an elected fellow of both the American Association for the Advancement of Science and the American Academy for Microbiology. The National Institutes of Health appointed him to the National Science Advisory Board for Biosecurity in 2004 and served as its chairman for two years. He has published over 400 scientific research articles that have been cited over 20,000 times.

Jennifer Nuzzo, DrPh, SM, is a Senior Associate at the Johns Hopkins Center for Health Security (formerly the University of Pittsburgh Medical Center for Health Security), Visiting Faculty in the Department of Environmental Health and Engineering and Associate in the Department of Epidemiology at the Johns Hopkins Bloomberg School of Public Health. An epidemiologist by training, her work focuses on international and domestic biosurveillance, infectious disease diagnostics, and disease mitigation strategies. She also has worked on issues related to the Affordable Care Act, tuberculosis control, foodborne outbreaks, and water security. Dr. Nuzzo is an Associate Editor of the peer-reviewed journal *Health Security* (formerly *Biosecurity and Bioterrorism*).

In addition to her work at the Center, Dr. Nuzzo has advised national governments and nonprofit organizations. She has served as a consultant to the National Biosurveillance Advisory Subcommittee, as a member of the US Environmental Protection Agency's National Drinking Water Advisory Council (NDWAC), and as a member of the NDWAC's Water Security Working Group. She has also served as a project advisor for the American Water Works Association Research Foundation (now called the Water Research Foundation), a primary funding organization for drinking water research in the United States. She has also been consulted on pandemic planning efforts in the Republic of Indonesia and Taiwan.

Dr. Nuzzo received a Doctor of Public Health degree (DrPH) in epidemiology from the Johns Hopkins Bloomberg School of Public Health, an SM in environmental health from Harvard University, and a BS in environmental sciences from Rutgers University.

Martina Siwek, **PhD**, a native of the Czech Republic, obtained a Bachelor of Arts (BA) in Biological Science from Goucher College and a Doctorate of Philosophy in Molecular Microbiology and Immunology from the Johns Hopkins Bloomberg School of Public Health. She has extensive research experience in HIV vaccine research and human immunity and infectious diseases, especially viral pathogens. Dr. Siwek has worked in support of the Department of Defense since 2010. She started at the Joint Program Executive Office (JPEO) as the Technical Director for the Critical Reagents Program (CRP) and as a subject matter expert in infectious diseases and especially dangerous pathogens. Dr. Siwek eventually transitioned to serving as the Liaison Officer coordinating various inter- and intra-agency efforts and organizations, including the Armed Forces Health Surveillance Branch (AFHSB), the Centers for Disease Control and Prevention (CDC), the Defense Threat Reduction Agency (DTRA), and many others. She assumed her role as the Chief Scientist for the Global Emerging Infections Surveillance (GEIS) Section at the AFHSB in 2014. There she provides scientific guidance and oversight for emerging infectious disease surveillance projects conducted by or in conjunction with US military research laboratories in continental United States (CONUS) as well as outside of the continental United States (OCONUS) settings.

Program Agenda

Wednesday, February 8, 2017		
Time	**Session**	**Notes**
1:30 PM - 2:00 PM	**CBEP EUCOM Overview**	Overview Briefs by DTRA/CBEP for Review Panel (Note: **Closed Session** for US Government Personnel and Review Panelists only)
2:00 PM - 2:30 PM	**CBEP AFRICOM Overview**	
2:30 PM - 3:00 PM	**CBEP CENTCOM Overview**	
3:00 PM - 3:30 PM	**CBEP PACOM Overview**	
3:30 PM - 4:00 PM	**Coffee Break**	Open to all Attendees
4:00 PM - 5:00 PM	**Session 1: Special Presentation** **"Doing Science in Difficult Places"**	Open to all Attendees
5:00 PM - 6:30 PM	**Poster Session & Networking Event**	Open to all Attendees

Thursday, February 9, 2017

Time	Speaker	Country	Project
8:30 AM - 8:50 AM	**Opening Remarks**		
8:50 AM - 10:10 AM	**Session 2: Chiroptera (Bats) - Important Reservoirs Hosts of Emerging Viruses**		
	Olival, Sidamonidze, & Al-Hmoud	Georgia & Jordan	(BAA) Understanding the Risk of Bat-Borne Zoonotic Disease Emergence in Western Asia
	Smith	Cambodia	(BAA) Investigating the Risk of Human Disease from Parasites of Small Mammals and Bats
	Kityo	Uganda	(Call) Arthropod-borne Viruses Associated with the Chiroptera of Uganda: Isolation and Characterization
	Mendenhall	India	(BAA) Bat Harvesting in India: Detection, Characterization and Mitigation of Emerging Infectious Disease Risk
	Ekiri	Tanzania	(BAA) Evaluating Zoonotic Viral Sharing Among Bats, Primates and People in High Risk Transmission Interface in Southern Tanzania.
10:10 AM-10:30 AM	**Coffee Break**		
10:30 AM-11:40 AM	**Session 3: Epidemiology & Biosurveillance - Group 1**		
	Chanturia	Georgia	GG-19: Epidemiology and Ecology of Human Tularemia in Georgia
	Jacobs	Azerbaijan	TAP-10: Ecological and Epidemiological Study of *Yersinia pestis* and *Francisella tularensis* in the Northern Part of Azerbaijan Regions of Gusar and Khachmaz
	Kilonzo	Tanzania	(BAA) One Health Approach to Brucellosis and Rift Valley Fever Surveillance in Tanzania
	Bhengsri	Thailand	(Call) Acute Febrile Illness Study Among Patients in Nakhon Phanom and Tak Province, Thailand
	Orynbayev	Kazakhstan	TAP-8: Especially Dangerous Pathogen Differential/Rule-Out Elimination Assays & Modeling (EDP-DREAM) of the Saiga Antelope Mortality

Thursday, February 9, 2017 (Continued)

Time	Speaker	Country	Project
11:40 AM-12:30 PM	**Session 4: Viral Pathogens of Security Concern**		
	Babuadze	Georgia	(Call) Assessing the Seroprevalence and Genetic Diversity of CCHFV and Hantaviruses in Georgia
	Eyangoh	Cameroon	Epidemiology of Emerging Viruses
	Karesh & Van Vuren	South Africa	(BAA) Understanding Rift Valley Fever in South Africa.
	Epstein	Malaysia	(BAA) Biosurveillance for Henipaviruses and Filoviruses at the Agricultural Animal-Human Interface in Malaysia
12:30 PM - 1:30 PM	**Lunch**		
1:30 PM - 2:20 PM	**Session 5: Community Outreach to Combat African Swine Fever**		
	De Nardi	Implementer	ASF Public Outreach Project Overview
	Niazyan	Armenia	TAP-A1: Community Outreach to Support Understanding of ASF Ecology and Epidemiology in Eastern Europe: Training and Implementation for Methods and Strategies for Control and Prevention
	Ninidze	Georgia	TAP-9: Community Outreach to Support Understanding of ASF Ecology and Epidemiology in Eastern Europe: Training and Implementation for Methods and Strategies for Control and Prevention
	Datsenko	Ukraine	TAP-4: Community Outreach to Support Understanding of African Swine Fever (ASF) Ecology and Epidemiology in Eastern Europe

Thursday, February 9, 2017 (Continued)

Time	Speaker	Country	Project
2:20 PM - 3:20 PM	**Session 6: Transboundary Animal Diseases**		
	Dung	Vietnam	(Call) Foot-and-Mouth Disease Virus Surveillance and Ecology in Vietnam
	Okoth	Kenya	(BAA) Whole Genome Sequencing of African Swine Fever Virus in Kenya.
	Nevolko	Ukraine	TAP-6: Analysis of the Threat of Spread of African Swine Fever and Classical Swine Fever in Wild Boar Populations in Ukraine: Improving Diagnosis, Surveillance, and Prevention
	Mwiine	Uganda	UG-2: Research and Development of Countermeasures to Support the Control of FMDV in Uganda
	Filatov	Ukraine	(USDA ARS) African Swine Fever Threat Reduction Through Surveillance in Ukraine
3:20 PM - 3:40 PM	**Coffee Break**		
3:40 PM - 5:00 PM	**Session 7: Bacterial Pathogens of Security Concern - Group 1**		
	Motin & Shikhaliyeva	Georgia & Azerbaijan	(BAA) Molecular Epidemiology and Ecology of *Yersinia* spp. in the Transboundary Plague Endemic Territory in Georgia and Azerbaijan
	Sansyzbayev	Kazakhstan	KZ-31: Effect of *Rickettsia* spp. upon Fitness of *Yersinia pestis* in Fleas that Vector Plague in the Republic of Kazakhstan
	Tarasov	Ukraine	UP-2: Development of the Epidemiological Forecasting System for Zoonotic Diseases Employing GIS Technology
	Solomonia	Georgia	GG-23: Creation of Sustainable Immunodiagnostics
	Avetisyan	Armenia	AM-1: Medical/Biological Mapping of Tularemia Natural Foci and Epidemiology using GIS in Armenia
5:00 PM - 5:10 PM	**Break**		

Thursday, February 9, 2017 (Continued)

Time	Speaker	Country	Project
5:00 PM - 6:20 PM	**Session 8: Avian Transmissible Diseases**		
	Goraychuk	Ukraine	(USDA ARS) Genomic, Epidemiological, and Biological Characterization of Newcastle Disease Virus Isolates from Ukraine
	Ateya	Kenya	(Call) Newcastle Disease: Surveillance, Molecular Epidemiology, and Control of NDV in Kenya
	Strochkov	Kazakhstan	TAP-11: Molecular Characterization and Complete Genome Sequence of Newcastle Disease Virus Isolated in Kazakhstan
	Muzyka	Ukraine	UP-4: Risk Assessment of Selected Especially Dangerous Pathogens Potentially Carried By Migratory Birds Over Ukraine
	Kapur	India	(BAA) Detection and Molecular Epidemiologic Analysis of Especially Dangerous Pathogens in Backyard Poultry, Commercial Broilers and Waterfowl in India
6:20 PM - 6:30 PM	**Day 1 Wrap-up**		

Friday, February 10, 2017

Time	Speaker	Country	Project
8:30 AM - 8:40 AM	**Opening Remarks**		
8:40 AM - 10:20 AM	**Session 9: Bacterial Pathogens of Security Concern - Group 2**		
	Chanturia	Georgia	(BAA) Characterization of NCDC Strain Repository by Next Generation Sequencing (NGS)
	Shah	Pakistan	(BAA) High Resolution Chemical Characterization of *Yersinia pestis* Cells Within Soil Matrices: Implications for Understanding Natural Foci and Telluric Reservoirs of Plague
	Bitek	Kenya	(CDC IACRO) Estimating Incidence and Socio-economic Impact of Brucellosis in Humans and Animals in Kajiado County, Kenya
	Sidamonidze	Georgia	TAP-10: Molecular Epidemiology of *B. anthracis* and *Brucella* spp. in Turkey and Georgia
	Malania	Georgia	GG-27: Regional Study of the Ecology of Anthrax Foci in Georgia and Azerbaijan
	Ismayilova	Azerbaijan	AJ TAP-11: Regional Study of the Ecology of Anthrax Foci in Georgia and Azerbaijan
	Zimmerman	Lao PDR	Environmental Surveillance of *Bukholderia pseudomallei*, Pilot Study in Lao PDR
	Limmathurotsakul	Thailand	Melioidosis Regional Coordination Network
10:20 AM- 10:40 AM	**Coffee Break**		
10:40 AM - 12:00 PM	**Session 10: Emerging Infectious Diseases**		
	Reynolds	Georgia	(Call) Enhancing Capacity for Case Detection and Diagnosis of Febrile Zoonotic-related Cutaneous Lesions in Georgia
	Gulbani	Georgia	(BAA) Enhancing Capacity for Case Detection and Diagnosis of Febrile Zoonotic-related Cutaneous Lesions in Georgia
	Abubakar	Malaysia	(Call) Multi-Year Prospective Cohort Study to Evaluation the Risk Potential of MERS-CoV
	Orynbayev	Kazakhstan	KZ-33: Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Surveillance for Distribution and Prevalence in Kazakhstan
	Buza	Tanzania	(BAA) Global Health, Emerging Infectious Diseases and Food Safety Implications of Bushmeat in Tanzania
	Sam	Malaysia	(BAA) Etiology of Severe Acute Respiratory Infections in Kuala Lumpur, Malaysia

Friday, February 10, 2017 (Continued)

Time	Speaker	Country	Project
12:00 PM - 1:00 PM	**Lunch**		
1:00 PM - 2:40 PM	**Session 11: Epidemiology & Biosurveillance - Group 2**		
	Ragan	Armenia	AM-4: One Health Surveillance for Brucellosis in Armenia
	Byarugaba	Uganda	(BAA) Acute Febrile Illness in Uganda.
	Mammadov	Azerbaijan	(Call) Infectious Etiologies of Acute Febrile Illness Among Members of the Azerbaijan Military
	Gulbani	Georgia	GG-20: Prevalence, Epidemiological Surveillance, and Laboratory Analysis of *Coxiella burnetii* in Georgia
	Sandybayev	Kazakhstan	KZ-32: Prevalence of *Brucella* Species and Bluetongue Virus Serotypes Among Domestic Livestock or Ruminants in Southern Kazakhstan
	Avetisyan	Armenia	TAP-H1: Identification of Etiology, Clinical Outcomes, Incidence, and Epidemiological Patterns of Hospitalized Febrile Patients in Armenia
	Burjanadze	Georgia	GG-21: Human Disease Epidemiology and Surveillance of Especially Dangerous Pathogens in Georgia
	Godbold	Armenia	AM-3: The Epidemiological Status of African Swine Fever in Domestic Swine Herds in the Tavush Marz Region, Republic of Armenia
2:40 PM - 2:50 PM	**Break**		
2:50 PM - 3:30 PM	**Session 12: Arthropods and Arboviruses**		
	Ismayilova	Azerbaijan	AJ TAP-13: Investigation of Mosquito and Tick-Borne Arboviruses in Southeastern Azerbaijan
	Sukhaishvili	Georgia	TAP-12: Analysis of Previously Identified *Rickettsia* Positive Georgian Ticks by Multi-locus Sequence Typing
	Khan	Pakistan	(BAA) The Role of Arboviruses as a Cause of Undifferentiated Febrile Illness in Sind, Pakistan
3:30 PM - 3:50 PM	**Closing Remarks**		

Presentation Summaries

February 8, 2017

Session 1

Doing Science in Difficult Places

PRESENTERS: Valerie DeThomas (CRDF)/ Jeffrey Skowera (CRDF)/ Robert Esler (CRDF)/ Matthew Bouton (Univ. of Michigan)/ Amir Abdulmawjood (Univ. of Veterinary Medicine Hannover – Germany)

Since 1995, CRDF Global has worked in over 40 countries using science to address priority issues in support of U.S. and foreign government agencies, foundations, and private sector organizations. Many of these countries may be considered difficult places to work due to past or ongoing war or other conflicts, political or economic instability, resource constraints, or significant differences in research capabilities, scientific norms and practices.

Application of tailored programming approaches, genuine commitment to understanding the environments in which partner country scientists operate, and flexibility form the foundation for effective science collaboration in difficult places. Ultimately, scientists are eager to engage and work together in search of solutions to the world’s problems.

In 2013-2016, CRDF Global’s work with scientists and researchers from Iraq and Afghanistan in support of CBEP included a research grant competition, science fellowships and network building and exposure programs. CRDF Global’s ability to meet program objectives required building trust and rapport with Iraqi and Afghan participants, regular communication, engagement of all necessary stakeholders, adaptability, flexibility, and creative solutions. The programs’ success is, in large part, attributed to participation of scientists in the U.S., Germany, Italy, India, Australia, Switzerland, Jordan and Malaysia, who hosted Iraqi and Afghan researchers at their labs for trainings, lab tours, exposure visits and fellowships.

Presentation Summaries

February 9, 2017

Session 2: Chiroptera (Bats) – Important Reservoir Hosts of Emerging Viruses

Understanding the Risk of Bat-Borne Zoonotic Disease Emergence in Western Asia

PRESENTERS: Kevin Olival/Ketevan Sidamonidze/Nisreen Al-Hmoud

COUNTRY: United States/Georgia/Jordan

Bats are natural reservoir hosts to several emerging viruses with pandemic potential, including Ebola, Marburg, Nipah, and SARS and MERS-coronaviruses, but current research on the distribution of bats, diversity of their viruses, and potential for zoonotic disease emergence in Western Asia is severely limited. To fill this gap and contribute to biological threat reduction, we propose a hypothesis-driven One Health research project focused on characterizing bat coronavirus diversity and the risk of bat-borne zoonotic disease emergence. This will include extensive non-lethal field sampling of bats, screening and characterization of viruses from bat specimens with two regional partner laboratories currently operating within the region, the Lugar Center in Georgia and RSS in Jordan, and modeling emerging disease risk by combining viral data with host, geographic, and ecological data. Data for risk modeling will be collated across a larger region than our field sampling will allow through the creation of a collaborative Western Asia Bat Research Network (WAB-Net) – including key researchers and public health representatives from >12 countries. Research activities will be strengthened via laboratory exchanges and annual data sharing and capacity building workshops. This integrated, multi-disciplinary approach presents a coordinated strategy to advance scientific knowledge around transboundary zoonotic disease emergence risk in Western Asia to inform early detection, diagnosis, and response to support the Global Health Security Agenda and CBEP goals.

Investigating the Risk of Human Disease from Parasites of Small Mammals and Bats

PRESENTER: Gavin Smith

COUNTRY: Cambodia

Emerging and re-emerging infectious diseases pose a significant public health challenge globally, with severe economic, social, and health consequences. It is estimated that the SARS outbreak alone cost over $50 billion dollars in lost global economic activity. The frequency of epidemics caused by newly emerging and re-emerging pathogens and the likelihood of rapid global spread have increased dramatically in recent decades, with Southeast Asia considered a hot spot for future emergence events. Small mammals and bats play an important role in the maintenance and transmission of select agents that infect humans such as *Brucella* species, coronaviruses, filoviruses, henipaviruses, hantaviruses/bunyaviruses, plague, rabies (lyssaviruses) and *Rickettsia* species. The global distribution of several species of small mammals and bats, in addition to the ever-increasing interface between humans and wildlife, ensures that cross-species transmission events will continue to occur, often with devastating effects. By proactively sampling animal populations in Cambodia to discern circulating parasitic genotypes and screening human sera for evidence of exposure, we can determine which parasites have human pathogenic potential. A standardized trapping regimen will allow us to understand which ecological and environmental variables are associated with host and parasite presence-absence, facilitating the creation of ecological niche maps and models to determine risk and inform future surveillance efforts across Southeast Asia.

Arthropod-Borne Viruses Associated with the Chiroptera of Uganda: Isolation and Characterization

PRESENTER: Robert Kityo

COUNTRY: Uganda

This project aimed to achieve a better understanding of bats, their ecology and their potential roles in virus ecology. This has been done through graduate training and research, training in field techniques of capture and processing of bats for virus detection and characterization, and a compilation of reference calls of micro-chiropteran bats for Uganda. Field biosurveillance training was held with participants from NADDEC, UVRI and Makerere University at Zika forest. A graduate student was recruited and completed an ecological study on bats in the Kaptum cave. We conducted bat surveys from around Uganda and have collected voucher calls from 28 microchiropteran bats from over 10 localities in Uganda.

From our graduate student's research, we now know there are 6 bat species (*Nycteris thebaica, Rhinolophus landeri, Rhinolophus hildebrandtii, Hipposideros caffer, Hipposideros ruber,* and *Myotis bocagei*) in Kaptum cave. Although they may mix up, these seem to have preferred roosting corners in the cave defined by slight differences in temperature and relative humidity.

Besides Kaptum Cave, we have documented the existence of many other caves around the country with bats. These caves are frequently visited by local people for various reasons. This could inevitably expose such members of the local communities to aerosols in the caves. Collectively, this project has advanced our knowledge of bat ecology in Uganda and enhanced collaborative research between US and Ugandan institutions which will promote cooperation during future biosurveillance and outbreak events.

Bat Harvesting in India: Detection, Characterization and Mitigation of Emerging Infectious Diseases Risk

PRESENTER: Ian Mendenhall

COUNTRY: India

Emerging infectious diseases pose a continual risk for humans, imparting major health and economic challenges. It is estimated that greater than 70% of these diseases originate in wildlife, which demonstrates the importance of understanding the diversity of parasites that circulate in animals. Bats are important reservoirs of several medically important viruses that have high case fatality rates, including rabies/lyssaviruses, the henipaviruses, SARS-like coronaviruses, and Ebola virus and the related filoviruses. India is one of the most biologically diverse countries in the world, however there are currently few reports of viruses detected in bats. Outbreaks will often begin from point source origins, as phylogenetic data from the recent Ebola outbreak in West Africa indicates. Therefore, studying specific interfaces where humans are exposed more frequently due to their proximity and heightened interactions with wildlife can provide critical information on exposure. Bat harvesting is a common practice across India, as concentrated efforts can yield high numbers of bats. This contact and processing of bushmeat is an opportune place for cross-species transmission to occur. By sampling the bat populations where harvesting occurs before, during and after the trapping, we can determine if bat harvests increase the shedding of viruses and what medically important viruses are circulating in these bat populations. This will allow us to generate risk models and understand which species may be natural virus reservoirs in India.

Evaluating Zoonotic Viral Sharing Among Bats, Primates, and People in a High-risk Transmission Interface in Southern Tanzania (VISHA Project)

PRESENTER: Abel Ekiri

COUNTRY: Tanzania

Through project partners, including Metabiota, University of California Davis, Sokoine University of Agriculture, and the Ifakara Health Institute, we are evaluating: 1) the risk of zoonotic virus transmission among bats, non-human primates (NHP), and people sharing a forest interface with high human-wildlife contact in Southern Tanzania; 2) the impact of bat and primate community composition on virus diversity; and 3) exposure to zoonotic viruses in high-risk human populations living at the forest interface. By investigating cross-species viral sharing at this high-risk interface, our team will increase the understanding of pathogen emergence risk and transmission between human and wildlife hosts, strengthen Tanzanian surveillance and diagnostic capacity for pathogens of pandemic and biosecurity concern, and identify key intervention points to reduce local viral spillover from wildlife into human populations. The VISHA project team is investigating the epidemiology of known and novel zoonotic viruses (including filoviruses) in bats, NHPs, and humans by: 1) characterizing forest field sites; 2) collecting bat and NHP specimens during wet and dry seasons; 3) collecting specimens from high-risk human groups near forest areas during wet and dry seasons; 4) testing wildlife and human specimens for potential zoonotic viral pathogens using virus family-level RT-PCR; 5) performing phylogenetic and other genomic analyses on all detected viruses; 6) identifying risk factors for human exposure to bat and NHP viral pathogens using spatial analyses and epidemiologic modeling; and 7) training Tanzanian scientists in surveillance, molecular diagnostics, genomic analyses, and field and laboratory biosecurity practices.

Epidemiology and Ecology of Tularemia in Georgia

PRESENTER: Gvantsa Chanturia

COUNTRY: Georgia

Our study investigated the epidemiology of human and animal tularemia in Georgia. The project included (1) the study of seroprevalence of tularemia among individuals living in geographic areas with tularemia, and healthy individuals; and (2) estimate risk factors for seropositive humans and animals. We also established active surveillance for human tularemia clinical cases with the goal of increasing the efficiency and tularemia diagnostic capability. Isolates from human cases (900 volunteers) for comparison to both current environmental and historical isolates (National Center for Disease Control and Public Health). From an environmental prevalence emphasis, we will establish active surveillance for *F. tularensis* in the environment, including a study of small rodents and associated vectors and identification of the sources of outbreaks among humans. Field sampling for active surveillance for *F. tularensis* in the environment included small rodents and associated vectors, linking with human cases and seroprevalence study among the population living in foci area. More than 60,000 vectors were collected and pooled into 6,000 collections. All strains were isolated, evaluated, and monitor patterns of antimicrobial resistance. A bacteriophage component of this project examined isolated *F. tularensis* strains by genomic sequencing, proteomics analysis (Ilia State University), and phage discovery (the Eliava Institute). Geographic information systems and genetic algorithm for rule-set production were used for pathogenic distribution. Ecological niche models were created for ectoparasite species and primary rodent vectors. Consistent differences were found by the expression of some proteins between the isolates. Real-Time PCR and Western immunoblotting further validated these differences.

Ecological and Epidemiological Study of *Yersinia pestis* and *Francisella tularensis* in the Northern Part of Azerbaijan Regions of Gusar and Khachmaz

PRESENTER: Jolanta Jacobs

COUNTRY: Azerbaijan

Robust surveillance of *Y. pestis* and *F. tularensis* was once carried out routinely, however since the collapse of the Soviet Union little funding has been allocated to such efforts, and thus almost no data are available on the current distribution of plague and tularemia foci and vectors in Azerbaijan.

TAP-10 project proposes to conduct surveillance of ectoparasite vectors of plague and tularemia in northern Azerbaijan, in an area with a historical presence of those diseases and located near known natural foci of plague and tularemia.

The project started in April 2015 and is being implemented by Khachmaz Anti-Plague Division (APD). Sample collection (arthropods: fleas and ticks) field activities were carried out over six months in spring, summer, and autumn of 2015. Sample collection was completed in total of 13 villages of Gusar and Khachmaz regions in September 2015. Collected ticks and fleas were sorted, counted, identified, pooled, homogenized and their nucleic acids were extracted. The extracted nucleic acid samples have been tested by PCR (Bio-Rad 96 instrument) for two targets for each of the pathogens. 8,216 ticks and 154 fleas have been counted, identified, and sorted. 1,269 tick pools and 55 flea pools have been created and homogenized and their DNA extracted. Primer and probe sets were optimized and testing is ongoing.

A One Health Approach to Brucellosis and Rift Valley Fever Surveillance in Tanzania

PRESENTER: Chris Kilonzo

COUNTRY: Tanzania

Our One Health team, including partners from Metabiota, the University of California, Davis, Sokoine University of Agriculture, and Ifakara Health Institute is utilizing a transdisciplinary approach to investigate the epidemiology and genomic diversity of the zoonotic pathogens Rift Valley fever virus (RVFV) and *Brucella* in south-central Tanzania. The primary objectives of the project are to evaluate the influence of risk factors such as animal contact and climatic conditions on increased RVFV and *Brucella* infection among livestock, wildlife, and humans, and to enhance in-country capacity for RVF and brucellosis surveillance, prevention, and control. To attain these goals, the team is engaged in: 1) characterization of sites with historical RVFV and *Brucella* activity in humans and animals that represent diverse climatic and animal density variables; 2) concurrent pathogen surveillance for acute and convalescent infections among humans, livestock, conspecific wildlife, and RVFV mosquito vectors to elucidate key disease transmission pathways, 3) identification of climatic conditions and temporal patterns that increase disease risk among vector and host species; 4) characterization of the RVFV and *Brucella* spp. diversity detected in mosquitos, animals, and humans; 5) identification of potential cryptic wildlife maintenance hosts using serologic evidence of prior infection, or in the case of RVFV by using blood-meal analysis of recently fed virus-infected mosquitoes, and 6) the development of integrated models to predict geographic areas of increased pathogen maintenance and transmission to identify locations for targeted intervention strategies and enhanced disease surveillance.

Acute Febrile Illness Study among Patients in Nakhon Phanom and Tak Province, Thailand

PRESENTER: Saithip Bhengsri

COUNTRY: Thailand

There are many causes of acute febrile illness (AFI) including various emerging infectious diseases in Southeast Asia and Thailand. Such diseases can be difficult to differentiate by clinical signs and symptoms, leading to misdiagnosis and possibly serious consequences for patient care. Therefore, laboratory testing is necessary to establish an accurate diagnosis. The objectives of this study are to: 1) describe the etiologies for patients hospitalized with AFI, 2) estimate incidence of specific pathogens and monitor trends over time, 3) evaluate the performance of rapid diagnostic tests (RDTs), and 4) assess laboratory diagnostic accuracy based on specimen types and testing methods for disease surveillance and outbreak identification. The study is being conducted in two Thai border provinces, Nakhon Phanom in the northwest and Tak in the east. The project is divided into 3 phases: pilot, surveillance and research phase. In the pilot phase, demographic data, clinical information and routine laboratory results will be collected from eligible patients. During the surveillance phase, patients hospitalized with undifferentiated fever will be tested for bacteremia and dengue. If dengue is not diagnosed, samples will be tested for chikungunya, *Leptospira*, *Rickettsia* species, *O. tsutsugamushi*, and Zika virus. During the research phase, new RDTs for *B. pseudomallei*, *O. tsutsugamushi*, Zika and chikungunya viruses will be evaluated. Additional testing will be performed on an expanded range of bacterial and viral pathogens. This project will provide an increased spectrum of pathogen detection, improve the timeliness of pathogen characterization, and assess promising new advanced diagnostic tests for surveillance and clinical management in the region.

Differential Diagnostics Performed by Eliminating Especially Dangerous Pathogens and Simulation (EDP-DREAM) in Cases of Saiga Antelope Mortality

PRESNTER: Mukhit Orynbayev

COUNTRY: Kazakhstan

Though several large die-offs in saiga antelope (*Saiga tatarica*) herds in Kazakhstan have been reported in the past decade, the intensity, and absolute threat to the species of the ongoing die-off in the Kostanay oblast of Kazakhstan is unprecedented. The loss of reproductive females and calves represents long-term impacts on herd recovery and may indicate an eminent threat to regional livestock health. Reports have suggested the saiga die-off may be related to pollution or plant toxins, yet it is unclear how such toxicity would result in 100% mortality of nursing calves. Such a rapid die-off could be explained by viral (or viral/bacterial) infection. Even though Foot and Mouth Disease Virus (FMDV) has been reported in saiga, relatively few viruses result in 100% mortality, particularly in calves. However, FMDV could potentially play a role in a multiple pathogen infection. This study will implement a differential diagnostic work flow to diagnose anthrax and other Especially Dangerous Pathogens (EDPs) that may be involved in the saiga die-off. The project will use high resolution GPS collar data from animals at-large during the die-off. The first field expedition began in September 2016, and experts from the lead KZ partner, the Research Institute for Biological Safety Problems (RIBSP), pursued saiga individuals of Ural population in West Kazakhstan and Atyrau Oblasts to take samples and fit them with collars. Fifty-four individuals were captured from the Ural population: fifty-two individuals in West Kazakhstan oblast, and two individuals in Atyrau Oblast. During the field expedition, the five available animal collars were placed on animals and found to provide a stable signal. All samples have been delivered to RIBSP and are being stored at the BSL-3 laboratory. Currently materials and reagents are bring procured. Dr. Jason Blackburn from University of Florida is the collaborator for the study.

Assessing the Seroprevalence and Genetic Diversity of CCHFV and Hantaviruses in Georgia

PRESENTER: Giorgi Babuadze

COUNTRY: Georgia

The proposed collaborative research project will assess the seroprevalence and genetic diversity of highly pathogenic bunyaviruses circulating in Georgia to include Crimean-Congo hemorrhagic fever virus (CCHFV) and hantaviruses causing hemorrhagic fever with renal syndrome (HFRS), such as Puumala (PUUV), Dobrava (DOBV), or Seoul (SEOV) viruses. Specific aims of the project are to: (1) Determine the prevalence and serological diversity of CCHFV and hantaviruses in patient populations using samples collected from previously funded CBEP projects, human samples (GG-21) and environmental samples (GG-19); (2) Establish multiplexed immunological and molecular diagnostic assays for detecting circulating strains of pathogenic bunyaviruses, and provide suitable training for sustainment of these assays and capabilities within Georgia; (3) Initiate active surveillance for CCHFV, and hantaviruses in the environment to include small rodents and ticks; (4) Identify and characterize the genetic diversity of CCHFV and hantaviruses detected in rodents and ticks; (5) Establish a DNA barcode reference library for local tick species, to facilitate future identification and incrimination of tick-borne disease in Georgia.

These studies will provide a baseline assessment of the potential for future outbreaks of CCHF and HFRS in this region. Rodent and tick samples will be tested by ELISA and RT-PCR for evidence of CCHFV or hantavirus infections. Selected samples will be sent to the USAMRIID for virus-specific neutralization tests in BSL-3 or BSL-4 containment laboratories.

Epidemiology of Emerging Viruses in Cameroon

PRESNTER: Sara Eyangoh

COUNTRY: Cameroon

The geographical and socio-cultural diversity of Cameroon makes it vulnerable to many emerging tropical viral infections like, Ebola, Zika, Dengue, Lassa, etc. While some studies have been done on the epidemiology of some of these viruses, these dispersed studies remain insufficient as a basis for the implementation of effective public health measures in this regard. Our objective was to assess the circulation of certain emerging viruses in the Cameroonian population in order to make recommendations to strengthen their surveillance and reinforce the rapid detection of any eventual epidemic.

In this respect, different samples were collected from 6 sites of Cameroon in different regions. These samples were serum/whole blood from 1500 blood donors, blood/oral swabs from 500 febrile patients susceptible of carrying a potential viral infection, and samples from bats to look for potential viral reservoirs. Collection of samples is complete, but analysis is ongoing at French Arbovirus National Reference Centre (Marseille, France). Preliminary data are available from blood donors. All donor blood samples were analyzed by ELISA. Positive samples underwent seroneutralisation analysis, RT-PCR (for DENV) and serotyping and phylogeny subsequently. Blood donor sample preliminary ELISA positive results were as follows: WNV(61.6%),TBE(39,9%), DENV(56,9% with 7.6% positive on RT-PCR, serotyping in progress), TOSV(7%), RIFTV(0%), ZIKV(10,4%).

Our definitive findings hope to build a framework upon which public health decisions can be made to increase preparedness for an eventual epidemic of an emerging viral infection and improve laboratory detection capabilities at the national level.

Understanding Rift Valley Fever in the Republic of South Africa

PRESENTERS: Billy Karesh & Petrus Van Vuren

COUNTRY: South Africa

Rift Valley fever virus (RVFV) is a vector-borne pathogen causing significant livestock, wildlife and human morbidity and mortality, and results in significant economic damages and food security concerns. RVFV has spread from Africa to the Arabian Peninsula, has the potential to spread to the Americas, and is considered a potential bioweapon. In South Africa, we have a multi-disciplinary team working to correlate environmental factors with vector succession and abundance, understand the role herd immunity may play in the occurrence of outbreaks and characterize the risk to people working in high-risk occupations. Initial analyses indicate that there are several vegetation and soil characteristics associated with locations of animal cases of RVF during the 2010-2011 outbreak. The baseline seroprevalence (representing herd immunity) in livestock have been established and experiments are running to understand how this may change. In people, the seroprevalence against RVFV is 0.1. Each year we hold national-level and local stakeholders' meetings to disseminate the data to national and provincial departments of agriculture, health and wildlife, as well as to farmers and workers, animal production groups and the weather service. Through this collaborative, One Health approach the project has become more robust and has strong support locally. The resultant data represents a significant step toward improving prediction of outbreaks and understanding how RVFV might spread, what effect climate change may have on the virus, how vaccination strategies may affect the risk of an epizootic and the risk of translocation to naïve countries, such as the United States.

Biosurveillance for Henipaviruses and Filoviruses at the Agricultural Animal-Human Interface in Malaysia

PRESENTER: Jonathan Epstein

COUNTRY: Malaysia

The henipaviruses and filoviruses include Hendra virus (HeV) and Nipah virus (NiV), and several species of Ebola (EBOV) and Marburg virus (MARV), respectively, which are highly pathogenic viruses and select agents capable of causing public health emergencies of international concern. Bats are recognized as reservoirs for both henipa- and filoviruses, and zoonotic transmission of these viruses from bats to humans via domestic animals has occurred in Southeast Asia. The full diversity of henipa- and filoviruses in bats and their potential to infect livestock and people is unknown. This project will enhance early detection and surveillance capacity in Malaysia by: 1) transferring Luminex-based technology with validated reagents to detect IgG antibodies against henipa- and filoviruses to Government of Malaysia partner labs in wildlife, livestock and human health sectors; 2) training laboratory personnel to develop and utilize Luminex-based assays to identify exposure to henipa- and filoviruses; 3) conduct biological surveillance in wildlife (esp. bats), livestock and people around indigenous communities that hunt wildlife and on farms in Peninsular Malaysia, where there are high levels of contact among people and animals. Based on building local capacity for hypothesis driven research and improved use of technology, this project will help characterize the distribution and spillover potential from bats of henipa- and filoviruses in Peninsular Malaysia. Activities will be coordinated with and complimentary to the USAID Emerging Pandemic Threats: PREDICT program and surveillance data will be shared with the Govt. of Malaysia (GoM). The proposed project is closely aligned with the aims of the Cooperative Biological Engagement Program in that it will support biosurveillance and capability building, engages partner-country scientists, and promotes a One-Health approach to threat reduction.

Session 5: Community Outreach to Combat African Swine Fever

ASF Public Outreach Project Overview

PRESENTER: Marco De Nardi

The Defense Threat Reduction Agency (DTRA) sponsored a four-country regional public outreach project to combat African Swine Fever (ASF). The primary objective of the project was to develop a comprehensive and sustainable regional network of knowledge, expertise, and know-how for the control and prevention of ASF emerging events through a joint-effort based Threat Agent Detection and Response (TADR) Activity Project (TAP) that combines four countries (Armenia, Georgia, Kazakhstan, and Ukraine) in a common activity-based Science plan. To achieve this goal, delegates from each country worked with project implementers to identify gaps in knowledge, legislative framework, and outbreak preparedness. As a pathogen of high bioterrorism potential, veterinary health importance, and/or responsible for major economic instability, it is of critical importance that ASF-threatened and ASF-affected countries have appropriate infrastructure to accurately and rapidly identify and report ASF activity and types to international veterinary health agencies, as well as provide follow-up concerning the spread of the virus. This project sought to identify key human resources and veterinary health systems in order to initiate implementation of solutions to address recognized gaps, thereby enhancing the capacity by which to mitigate the risk of ASF. The project used a “train-the-trainer” approach to successfully reach over 10,000 farmers, veterinarians, pig traders, rangers, and hunters among others, teaching the signs and symptoms to identify ASF along with the proper reporting measures. This project was the first four-country public outreach project sponsored by DTRA and is considered a monumental success due to the large numbers reached. The project also improved collaboration among regional partners with the hopes of additional similar outreach efforts on other pathogens and diseases of public health importance in the years to come.

Community Outreach to Support Understanding of ASF Ecology and Epidemiology in Eastern Europe: Training and Implementation for Methods and Strategies for Control and Prevention

PRESENTER: Lyudmila Niazyan

COUNTRY: Armenia

African swine fever (ASF) is a highly contagious viral disease of swine populations that can have significant economic consequence. It was successfully eradicated from most of the Eurasian continent almost 30 years ago, but was re-introduced in Georgia in 2007. Since then ASF has spread widely affecting swine in Georgia, Azerbaijan, Armenia, Ukraine, and Russia.

The primary goal of this project was to develop a comprehensive and sustainable regional network of expertise for the control and prevention of ASF through a joint-effort-based project combining four countries: Armenia, Georgia, Kazakhstan, and Ukraine. The project aims to identify and then educate persons working in the pig production chain (e.g., pig keepers, butchers, community veterinarians) to recognize clinical and epidemiological patterns of ASF. The training will allow these workers to understand (1) common routes of exposure, (2) preventative measures, (3) how to recognize clinical signs, (4) the importance of reporting to veterinary authorities, and (5) how to respond to suspected ASF cases.

In Armenia, a Knowledge and Attitude survey was implemented through veterinary authorities to assess the knowledge of the chosen target groups in ASF. For the public outreach campaign ten inspectors and ten epidemiologists were selected to train target groups, but due to the short time just 301/603 veterinarians, 1000/2412 farmers and 100/2670 hunters have been trained to date (Map 1). An additional 1000 farmers were subsequently educated by community vets during their routine work after the public outreach campaign ended. Over the course of the project and its aftermath, 3000 booklets and 150 posters were distributed and 2401 total persons informed.

This project facilitated the development of a sustainable capacity to implement outreach campaigns for future disease outbreaks in the region. Countries receiving training were taught how to run an outreach campaign including how to identify the target audience, how to produce effective educational materials, and how to effectively distribute those materials.

Community Outreach to Support Understanding of ASF Ecology and Epidemiology in Eastern Europe: Training and Implementation for Methods and Strategies for Control and Prevention

PRESENTER: Lena Ninidze

COUNTRY: Georgia

In 2007, Georgia was affected by a nationwide outbreak of African swine fever (ASF), and outbreaks were simultaneously reported in three different locations across the country. Monitoring ASFV in Eastern European countries is a top priority for the U.S. Defense Threat Reduction Agency (DTRA). The primary objective of the project was to develop a comprehensive and sustainable regional network of expertise for the control and prevention of ASF events through a collaborative project that united four countries (Armenia, Georgia, Kazakhstan, and Ukraine). Gaps in knowledge, legislative framework, and outbreak preparedness in the partner countries were identified. Additionally, this project contributed to long-term sustainability by conducting outreach campaigns for future disease outbreaks. Georgian directors were taught to lead and implement outreach campaigns, including how to identify target audiences (e.g. local farmers, veterinarians, and members of the pork industry) and efficiently create and disseminate educational materials. Over 450 veterinarians and farmers were trained, and 20,000 educational materials were distributed. Pre and post ASF campaign tests were developed to track progress; post-test scores were 16% higher than pre-test scores, which indicates that the outreach program was successful. Regional and state veterinarians, along with government agencies will be responsible for measuring the long-term success of these programs through laboratory results, monthly disease reports, and veterinarian updates. The number of ASF outbreaks are an indicator of the long-term success of the outreach program.

Community Outreach to Support Understanding of African Swine Fever (ASF) Ecology and Epidemiology in Eastern Europe

PRESENTER: Roman Datsenko

COUNTRY: Armenia

In 2015, the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE) and Institute of Veterinary Medicine (IVM) of the National Academy of Agrarian Sciences took part in an ASF outreach activity implemented within the Defense Threat Reduction Agency (DTRA) Cooperative Biological Engagement Program (CBEP) in Ukraine. The project aimed at establishing a regional alliance between Armenia, Georgia, Kazakhstan, and Ukraine to exchange experience, raise awareness, and provide education on ASF. Specialists from SSRILDVSE and IVM were trained as trainers before implementing their own outreach program in Ukraine. During in-country workshops and 14 training sessions in 14 Oblasts of Ukraine, veterinarians from 307 rayons, 531 epizootologists of state regional veterinary administrations and 4482 veterinary doctors of district animal hospitals were educated in the country. Additionally, information materials (flyer and poster) were developed. They included information about the clinical and epidemiological patterns of ASF, common sources and routes of exposure, preventative measures, how to recognize symptoms, and how to respond to suspected ASF cases. 100,000 flyers were printed and distributed among farmers and populations, as well as 1,500 posters for veterinarians in 24 Oblasts. Due to these efforts, necessary information was brought to the attention of veterinary doctors at district animal hospitals who communicate directly with farmers and persons that work with swine. This project demonstrated an approach for conducting and applying a public outreach program in Ukraine that can be used to raise awareness and help mitigate future outbreaks of ASF and other diseases.

Foot-and-Mouth Disease Virus Surveillance and Ecology in Vietnam

PRESENTER: Do Huu Dung

COUNTRY: Vietnam

The purpose of this project is to improve understanding of the epidemiology of foot-and-mouth disease virus (FMDV) in the Vietnamese setting using state of the art tools for genetic characterization of the virus in this endemic context. The intended goal is to mitigate the impact of this disease on local agriculture and improve preparedness for potential disease incursions in the US homeland with awareness of emerging virus strains.

Foot-and-mouth disease (FMD) is a contagious viral disease of domestic and wild cloven-hoofed animals, most notably cattle, pigs, sheep, buffalo and goats. Despite recent successes in controlling the disease in Europe and some parts of South America, FMD remains one of the most important infectious diseases of livestock due to the potential impact of an outbreak on trade in animals and animal products.

The project is currently operating in the second year of the (expected) four year period of performance. Five of the seven defined project objectives are either completed or well-underway. Two objectives are still in the planning phase. Current and ongoing activities in the field consist of passive surveillance of all FMDV outbreaks reported to the Vietnam Department of Animal Health and active surveillance of healthy livestock in six provinces spanning northern, central and southern Vietnam.

This collaborative endeavor has already resulted in two published, peer-reviewed scientific papers and two more papers have been submitted for peer review. Specific accomplishments achieved within the project include 1) genetic characterization of over 160 novel strains of FMDV, 2) enhanced understanding of risk factors for FMDV infection, 3) unique analyses of movement of FMDV strains across regions of Vietnam, and 4) improved understanding of the role of asymptomatic carriers in FMD epidemiology in Vietnam.

Whole Genome Sequencing of African Swine Fever Virus in Kenya

PRESENTER: Edward Okoth

COUNTRY: Kenya

African swine fever (ASF) is a lethal disease of domestic pigs caused by a large DNA virus. In endemic areas ASF virus (ASFV) circulates in asymptomatic wild pigs and ticks and can remain stable in pork products for several months. ASF is endemic to Africa and was restricted only to that continent until 1957. Since then it has spread to Europe, Latin America and Asia. It is currently endemic in Russia and the Caucasus. There is a real risk of accidental or deliberate introduction of ASF to the United States of America (US), which would be devastating to the US $1.25 billion/year pig industry. There are at present no control measures other than test and slaughter. The objective of the proposed research is to determine the genome sequences of at least 60 ASFV isolates, selected so as to include as many as possible of the major genotypes for which genome sequences are currently lacking. These will be made available to veterinary and biosecurity authorities in the US and endemic countries through GenBank. This knowledge will enable more precise identification of the source of future ASF outbreaks, both within and outside Africa. It will also underpin development of future ASF control tools including diagnostic products and vaccines. The project will build ASFV research and surveillance capacity in Africa to support future disease control efforts.

Analysis of the Threat of Spread of African Swine Fever and Classical Swine Fever in Wild Boar Populations in Ukraine: Improving Diagnosis, Surveillance, and Prevention

PRESENTER: Oleg Nevolko

COUNTRY: Ukraine

Listed as high priority pathogens by the World Organization for Animal Health (OIE), African swine fever virus (ASFV) and classical swine fever virus (CSFV) cause epizootically and economically significant animal diseases. Since 2012, the number of reported ASF outbreaks in Ukraine has increased, with 148 confirmed ASF incidents registered in 18 Oblasts of Ukraine (households - 123; wild boar populations – 24; infected object - 1) including 91 in 2016 (84 in the households and 7 in wild boar population). In order to strengthen the accuracy and effectiveness of ASF/CSF diagnostics in the country and to provide Ukrainian scientists with the ability and infrastructure to quickly and accurately monitor ASF and CSF movement, DTRA supported TAP-6 project that commenced on 1 September 2016. The aims of this project are (1) to analyze the distribution of ASFV and CSFV among wild boar populations inhabiting regions of Ukraine, which border the Russian Federation, Belarus, and Poland, and (2) to evaluate the risk of transmission to domestic pigs in the country. In addition to ELISA and PCR, monitoring targeted wild boar populations in Ukraine for ASF and CSF will be accomplished by using genomic-based biosurveillance methods and trainings on real-time PCR, molecular analysis, phylogenetic analysis, and complex sequence data analysis. This will provide an improved scientific basis to optimize current interventions and develop new tools and strategies to reduce the risk of ASFV transmission to domestic pigs. These biosurveillance efforts will facilitate development of ASF and CSF control strategies, which will contribute to limiting the spread of both infectious agents.

Research and Development of Countermeasures to Support the Control of Foot and Mouth Disease Virus (FMDV) in Uganda

PRESENTER: Frank Mwiine

COUNTRY: Uganda

Foot and Mouth Disease (FMD) virus causes an acute and the most contagious vesicular disease of livestock. The causative agent is a virus of the *Aphthovirus* genus in the *Picornaviridae* family. This disease in endemic in Uganda. Here, we report on a cross-sectional surveillance study designed to monitor and isolate FMDV serotype(s) circulating in the country divided into four regions: Northern, Western, Central and Eastern. A total of 38 representative districts from all the regions of Uganda were selected where 10,321 cattle sera and 1,300 oral-pharyngeal fluid samples were collected. All sera were analysed by the presence of antibodies directed against the virus non-structural proteins (NSP) using commercially-available kits at Makerere University. In addition, all oral-pharyngeal fluid samples were tested by rRT-PCR and virus isolation (VI) tissue culture followed by virus capsid sequencing to determine the FMDV serotypes by ARS, USDA at Plum Island Animal Disease Center. From samples collected during 2014-2015, thirty two percent of the serum samples were positive towards NSP antibodies. FMDV serotype O was isolated from Northern and Eastern regions while serotype SAT 2 was isolated from Western region of Uganda during samples collected in 2014. However, FMDV serotype SAT 1 (from the same region) and O were isolated in oral-pharyngeal fluid samples collected in 2015. The phylogenetic analysis of the P1 sequences for the viruses isolated in relation to geographical distribution of FMDV serotypes isolated during 2014-2015 in Uganda will be discussed. This information is of great importance for the improvement of disease control strategies and for vaccine strain selection for Uganda in the future.

African Swine Fever Threat Reduction Through Surveillance in Ukraine: Surveillance of Potential Arthropod Vectors

PRESENTER: Sergiy Filatov

COUNTRY: Ukraine

African swine fever (ASF) is a high-consequence viral disease threatening the pig industry in Western Europe. In its native range, ASF virus (ASFV) is transmitted to pigs and maintained in Nature by soft ticks. Eastern European nations, including Ukraine, are considered to be endemic with ASF outbreaks occurring in pigs and wild boar. However, the factors underlying ASF westward expansion and reoccurrence on the affected territories remain to be fully understood.

Because certain soft tick species of the genus *Ornithodoros* can serve as biological vectors and reservoirs of ASFV, our research group focused on the development and implementation of vector surveillance in selected regions of Ukraine to establish methods that could be scaled up to the national level. The surveys conducted updated known soft tick distribution patterns. Field samples were identified morphologically as *Ornithodoros verrucosus*, and live specimens used to establish a laboratory colony of this suspected ASFV vector at the NSC IECVM. The *O. verrucosus* colony is a valuable resource to assess the risk of soft tick involvement in the epidemiology of ASF in Eastern Europe.

Ukrainian scientists developed research capacity in soft tick biology, collection methods, rearing and colonization techniques, and vector-host-pathogen interactions at USDA-ARS locations, and collaborating universities in Texas. Results of the project were presented at five scientific national and international conferences, summarized in a book published in Ukrainian and English, and two research papers published in international peer-reviewed journals.

Molecular Epidemiology and Ecology of *Yersinia* spp in the Transboundary Plague Endemic Territory in Georgia and Azerbaijan

PRESENTER: Vladimir Motin & Sheyda Shikhaliyeva

COUNTRY: United States, Georgia, and Azerbaijan

Plague has been known in Caucasus region for many centuries, with the reports in Georgia dated back to the XI century and in Azerbaijan to VII century. The absence of recent reports can be partially explained by reduced surveillance, but also by the possibility of existence of atypical strains of *Y. pestis*, which can be missed by the testing laboratory due to presence of non-culturable strains as well as an absence of some genetic loci which would render the use of common molecular markers ineffective. The investigations of natural foci of plague in both Georgia and Azerbaijan have resulted in numerous cases of isolation of *Yersinia* species other than *Y. pestis*. The exchange of genetic material between *Yersinia* strains can challenge the detection of the strains of *Y. pestis*. We hypothesize that: 1) diverse *Yersinia* species, including *Y. pestis* and *Y. pseudotuberculosis* coexist; 2) *Yersinia* diversification is driven by host ecology; 3) *Yersinia* diversification is driven by exchange of genes within rodent populations; and 4) novel genetic methodology can improve identification of *Yersinia* species. To test these hypotheses, we propose: 1) ecological surveys targeting rodent populations in the transboundary focus located in both Georgia and Azerbaijan; 2) development of culturing and PCR-based procedures for detection of multiple strains of *Yersinia*; 3) molecular screening of the collected rodent and ectoparasite samples; 4) comparative analysis of genomes of obtained strains of *Yersinia spp*; 5) spatial analysis of distribution of strains of *Yersinia spp.* This project will result in enhancement of the plague biosurveillance capacities in the endemic regions of Georgia and Azerbaijan. This will be the first comprehensive project investigating and modeling plague foci in the South Caucasian region, which will foster improvements in national and international public health, surveillance, and biodefense efforts. This project will not overlap with national surveillance efforts.

Effect of *Rickettsia* spp. upon Fitness of *Yersinia pestis* in Fleas that Vector Plague in the Republic of Kazakhstan

PRESENTER: Yerlan Sansyzbayev

COUNTRY: Kazakhstan

The goal of this study is to strengthen surveillance of important flea-borne human infectious diseases (plague and rickettsioses) in the context of vertebrate reservoirs, invertebrate vectors (e.g. ectoparasites), and infectious disease agents. Plague and certain rickettsiae are flea-borne diseases that share similar characteristics in disease symptoms, case definitions, and association with co-infections. These data will be very important to health authorities and government agencies in Kazakhstan and the US, where they will support important DTRA objectives including: enabling a partner country to detect/identify/report disease outbreak (naturally occurring or intentional) and providing a system that is sustainable by that partner country's budget and infrastructure. From a public health standpoint, it is important to know which vertebrate species are reservoirs for particular infectious agent(s) and if they are infested with ectoparasites. The Kazakh Science Center for Quarantine & Zoonotic Diseases (KSCQZD) is the lead institute in the implementation of the project, while the anti-plague stations will be involved in providing samples for research. This project will study the effect of flea-borne rickettsial infections upon the fitness of *Yersinia pestis* (causative agent of plague) within plague vector fleas obtained from small mammals in various environmental settings of Kazakhstan. Specifically, the study hypothesizes that in austere environments, rickettsiae and *Y. pestis* will compete for limited resources within the invertebrate host, allowing one species to out compete the other.

Development of the Epidemiological Forecasting System for Zoonotic Diseases Employing GIS Technology

PRESENTER: Oleksandr Tarasov

COUNTRY: Ukraine

The project was a multi-year study focused on surveillance, mapping, and modeling the spatio-temporal and ecological patterns of *Francisella tularensis* and *Bacillus anthracis* in Ukraine. Collaborative efforts of researchers from the institutes of the Ministry of Health of Ukraine and National Academy of Agrarian Sciences of Ukraine included:

- analyses of historical tularemia and anthrax data sets;
- active surveillance for *Francisella tularensis* in small mammals and ticks and validated integration of these collections with appropriate culture and PCR-based analyses at the laboratory;
- surveillance and environmental sampling for *Bacillus anthracis*, with the integration of appropriate PCR-based detection assays in the laboratory;
- serological (ELISA) tests of sera sampled from wild boars;
- forecast pathogen outbreak using advanced spatial analyses, analyses with Geographic Information Systems (GIS) and Remote Sensing (RS) approaches to define the geographic extent of the pathogens and landscape dynamics that effect those distributions.

Throughout the period of performance, databases related to the epidemiological situation of tularemia and anthrax in Ukraine and geospatial data were developed and analyzed and areas historically at risk were determined for each disease. Ukrainians obtained extensive training in a variety of GIS and spatial analytical techniques, as well as research support at the sites and at the University of Florida. GIS and laboratory capacity at UCDCM and IVM were established. The GIS sector at IVM is now capable of sustaining basic GIS data development. Much of the work from the UP-2 project has been or will be published in the peer-reviewed literature.

Creation of Sustainable Immunodiagnostics

PRESENTER: Revaz Solomonia

COUNTRY: Georgia

The primary goal of this project was to provide Georgian scientists with critical educational tools that will help detect biological threats using immunological assays developed within their own laboratories. This included training on developing and validating immunoassays for proteins and antibodies (monoclonal [mAbs] and polyclonal). The project also provided the tools necessary to develop novel diagnostic measures against new or re-emerging biological threats. Antigenic material from *Francisella tularensis* and *Brucella* species (both endemic in Georgia) were used for the development of antibodies and subsequently for immunoassay development and testing. Collaborators from the Naval Medical Research Center (NMRC), helped develop and validate antigen production, antibody production and purification, ELISA development, optimization, and validation. Polyclonal antibodies and mAbs were raised against *F. tularensis* and *Brucella* spp. whole cell extracts. Antibodies of interest were selected by affinity, and were purified; corresponding ELISA procedures were optimized and validated. In addition, the antibodies were characterized by Western immunoblotting. Antibodies protein targets were examined on polyacrylamide gels, in-gel digestion, and liquid chromatography-mass spectrometry. Using this approach, chaperone protein DnaK and GroEL/ES from *F. tularensis* were identified as a candidate targets of MAB 3.2 and MAB 8.2. As a result, this project yielded a comprehensive suite of protocols that can be used by Georgian scientists to develop assays and scale up production of reagents for commercial production and distribution.

Medical/Biological Mapping of Tularemia Natural Foci Cases, Reservoirs, and Vectors Using GIS in Armenia

PRESENTER: Lilit Avetisyan

COUNTRY: Armenia

Background: Past studies have reported over 95% of Armenia is endemic for tularemia. The development of a Geographic Information System (GIS) database and mapping system will provide a tool for tularemia data management and introduce a sustainable technology for continued surveillance in Armenia.

Methods: A retrospective study using the archives of NCDCP centers across Armenia. For GIS data processing we used the ArcGIS 10.1. The extensions Spatial Analyst and Geostatistical Analyst from ArcView GIS were utilized to process data.

Results: In the period 1981-2012, tularemia epizootics were recorded in 27 of the 38 administrative regions of Armenia. These cases involved five species of rodents, one type of insectivore, ticks of the *Ixodidae* and *Gamasidae* genera, and three species of flea. The optimal habitat was characterized by middle mountain steppe landscape zone at 2,065 to 2,407 meters above sea level, receiving 600-800 mm yearly precipitation, and moderate climates with short cool summer and cold winters or dry warm summers with cold winters. A total of 266 human cases of tularemia were recorded in Armenia from 1996-2012. GIS mapping showed that 199 tularemia human cases were registered in the steppe vegetation zone, 224 cases- in a zone with moderate, relatively dry warm summers and cold winters (1400-2300m), 206 cases in a middle mountain steppe zone.

Discussion: The developed model showed an association ($p < 0.05$) between number of human cases and the number of epizootic sites, number of isolates, and percent of samples with positive cultures. The analysis conducted using GIS methodology delineated the risk zones with a high probability of tularemia occurrence. Identification of high-risk areas will serve public health officials in focusing surveillance efforts.

Genomic, Epidemiological, and Biological Characterization of Newcastle Disease Virus Isolates from Ukraine

PRESENTER: Iryna Goraychuk

COUNTRY: Ukraine

The genetic diversity of Newcastle Disease virus (NDV) in Ukraine was studied for the first time. Newcastle disease (ND) is caused by the virulent forms of this virus that affects a wide range of wild and domestic bird species. At least 15 genotypes cause periodic large-scale outbreaks with extensive morbidity and mortality in avian species worldwide.

We conducted passive surveillance in poultry and active surveillance in wild birds in the Southern-Eastern territories of Ukraine and bordering regions with Russia. These studies characterized viral distribution and identification of predominant circulating strains from 1967 to 2016, including the characterization of repository viruses. Genotype identification and pathotype of NDV were determined by direct sequencing of the complete F-gene and full genome using next-generation sequencing. Based on the sequence of the fusion protein cleavage site a more precise pathotyping in eggs and chickens was conducted using isolates that represent the most typical isolates of each of the geno-groups.

Migratory birds in Ukraine are part of east-to-west and north-to-south migration routes. The phylogenetic study allowed to determine the relationship of Ukrainian isolates with other viruses from Eastern Europe, Asia and Africa and a better understanding the transcontinental movement of NDV.

In addition, training programs on standard operation procedures for BSL3 laboratories, biosecurity and biosafety practices were implemented for Ukrainian scientists. Results from this collaboration were presented at three conferences and summarized in six publications in an international journal.

Newcastle Disease: Surveillance, Molecular Epidemiology, and Control of NDV in Kenya

PRESENTER: Leonard Ateya

COUNTRY: Kenya

Although Newcastle disease (ND) is a notifiable disease in Kenya, the disease is under-reported. Current ND control measures include vaccinations after hatching and outbreaks. Currently, there is no active surveillance for ND. Project objectives are to: (1) improve ND surveillance, detection, and diagnosis and reporting of pandemics and (re-) emerging pathogens; (2) facilitate & improve collaborative research amongst Kenyan institutions employing modern methods; and (3) characterize NDV strains, assess ND socio-economic impacts and identify risk factors contributing to NDV spread in Kenya. The project hypothesis is that virulent NDV reservoirs are asymptomatically maintained in wild birds and pet avian species that reside in the proximity of domestic poultry. Samples will be collected in four agro-ecological zones: (1) Zone II (tropical highlands) with poultry markets and where poultry is culturally significant; (2) Zone III (Kenyan food basket with crop-livestock farming system); (3) Zone IV (part of seasonal wild bird migratory and poultry trade routes); and (4) Zone V (free-range small-scale poultry farming). Sampling will be mainly on case-based and passive and active response surveillance. Positive samples identified by antigen detection, hemagglutination of allantoic fluids, or RT-PCR will be used to characterize and define viral strains circulating in Kenya. Ten-year (2005-2015) historical data on ND incidence and outbreaks have been analyzed and used to validate the project's sampling sites. Some of the sites have been visited and samples collected. Four MSc students, the key players in transfer of acquired technologies, have been recruited.

Molecular Characterization and Complete Genome Sequence of Newcastle Disease Virus Isolated in Kazakhstan

PRESENTER: Vitalyi Strochkov

COUNTRY: Kazakhstan

Newcastle disease virus (NDV) is a highly contagious viral infection of birds, characterized by pneumonia, encephalitis, multi-site hemorrhages and destruction of internal organs. It is considered one of the two most devastating diseases in poultry and wild birds, similar to avian influenza. Since Kazakhstan is on the main pathways of transcontinental migratory routes of many wild birds, genomic analysis of circulating NDV strains in Kazakhstan can potentially provide insights to its genetic evolution and add insight to pathogenic characteristics. Only limited studies on the genetic variability of NDV strains in Kazakhstan have been conducted. The project aims to perform full genome sequencing of five (5) isolates, conduct phylogenic analysis, and develop correlation between genotypic features and pathogenicity of five isolates from chicken flocks of previous outbreak during a one-year period. The team will retrieve full genome sequence of representative isolates from open source genomic sequence repository (GenBank), design primer sets for sequencing, and conduct sequencing using the ABI 3130xl platform at the Research Institute for Biological Safety Problems (RIBSP). Bioinformatic analysis will be performed to determine phylogenetic placement of these isolates among those in Genbank, and analyze the likelihood of introduction to and spread from Kazakhstan. The sequence data will be deposited to the National Center for Biotechnology Information (NCBI) database.

Risk Assessment of Selected Especially Dangerous Pathogens Potentially Carried By Migratory Birds over Ukraine

PRESENTER: Denys Muzyka

COUNTRY: Ukraine

Highly pathogenic avian influenza (HPAI) and Newcastle disease viruses (NDV) cause serious diseases in domestic and wild birds, and also pose additional risk due to the potential for spillover into human populations. Monitoring of orthomyxoviruses and paramyxoviruses conducted by NSC IECVM in 2010-2015 within the USDA/ARS projects revealed 168 avian influenza virus (AIV) and ND viruses, 23 antigenic combinations, and new serotypes (APMV-13). The extreme variability of AIV can lead to new genetic variants of the virus with pandemic potential that could damage people. The geographical position, natural conditions, large amount of birds and several transcontinental migration routes contribute to the re-emergence of those pathogens that was confirmed by outbreaks of HPAIV H5N1 and NDV in 2005-2008 and HPAIV H5N8 in poultry in 2016-2017. Commencing on December 1, 2016 the project aims to survey areas of Ukraine for AIV and NDV that may be harbored by wild birds associated with major northern and southern migratory flyways in the country. The project will assess the ecologic, epizootic, and epidemiologic risk of disease transmission. Bird observations, viral detection data, and Geographic Information System will be used to analyze and predict the anthropogenic impact on viral prevalence and type. The project will support local capacity building through enhancement of diagnostic capability, data management and analysis, and reporting. The project supports a One Health approach through linkage of researchers from the Institutes of the Ministry of Health, National Academy of Agrarian Sciences, and State Service for Food Safety and Consumer Protection of Ukraine.

Detection and Molecular Epidemiologic Analysis of Especially Dangerous Pathogens in Backyard Poultry, Commercial Broilers and Waterfowl in India

PRESENTER: Vivek Kapur

COUNTRY: India

Emerging and re-emerging respiratory diseases in poultry, especially velogenic viscerotropic Newcastle disease (vvNDV) and highly pathogenic avian influenza (HPAI), present a major threat to animal and public health worldwide, particularly in rapidly developing nations such as India. The core viral pathogens of human and animal concern involved in Respiratory Disease Complex of poultry have not been studied. To fill this knowledge gap, we propose to test three hypotheses. **Hypothesis 1.** The respiratory tract viromes from commercial and backyard flocks (herein referred to as domestic poultry) and waterfowl in Haryana, Odisha and Kerala are a potential source of especially dangerous pathogens (EDPs); **Hypothesis 2.** Molecular genotyping approaches will identify host- and region-specific fingerprint profiles of select agents including vvNDV and HPAI; **Hypothesis 3.** Next generation sequencing will help identify nucleic acid signatures of novel / emerging viruses in domestic poultry and waterfowl. The overall goal of this project is to discover novel viruses and enable the development of genomics-based strain-typing capability of EDPs and emerging viral pathogens from avian sources in academic research settings in India. Molecular epidemiologic analysis and comparative metagenomics investigation of respiratory viromes of domestic poultry and migratory birds, using a comprehensive and statistically robust stratified random sampling approach, will provide a strong foundation for the development of evidence-based approaches for implementing sustainable measures to control EDP. This investigation will also help generate data and tools, build sustainable capabilities, and enhance domestic and international academic partnerships critical to characterizing the zoonotic pathogens associated with domestic poultry and waterfowl in India.

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Characterization of NCDC Strain Repository by Next Generation Sequencing

PRESENTER: Gvantsa Chanturia

COUNTRY: Georgia

The three year project was launched in March 2016. It is a collaborative effort of the Los Alamos National Laboratory (LANL), NM, USA and National Center for Disease Control and Public Health of Georgia (NCDC), Tbilisi, Georgia. LANL has been working alongside the Georgia National Center for Disease Control and Public Health (NCDC) Genome Center Facility at the R. G. Lugar Center for Public Heath Research in developing Next Generation Sequencing (NGS) and analytic capabilities. The proposed project leverages the technical capabilities at both institutes to complete sequencing and characterization of the especially dangerous pathogens stored in the freezer archive at the NCDC. Up to 100 isolates of *Yersinia pestis, Bacillus anthracis, Brucella spp*., and *Francisella tularensis* from the NCDC pathogens collections will be selected for draft sequencing on the Illumina MiSeq. The draft genomes will be comparatively analyzed against strains from worldwide databases, SNPs will be discovered and subjected to phylogenetic analysis.

During the past year, ten *Brucella spp*. and twelve *F. tularensis* were have been sequenced. *F. tularensis* strains were processed for genome assembly and phylogenetic analysis on CLC-Bio, EDGE and PHAME software. Two draft genome announcement manuscripts have been drafted. A two week training for one bioinformatician from the NCDC team was held at LANL in November 2016. The sequencing of the rest of the samples is in process at NCDC. Ten isolates will be chosen to forward to LANL for PacBio sequencing. NCDC staff, as well as students involved in the project, will exercise their sequencing and bioinformatics skills on the samples and data from this project. Additional skills will be acquired through advanced training on data generated by this project. Completion of this research project will provide novel genomic characterization of the NCDC's extensive pathogen archive, cement the collaborative network between the NCDC and US collaborators, and ensure the NCDC's ability to utilize advanced sequencing technologies as independent researchers.

High Resolution Chemical Characterization of *Yersinia pestis* Cells within Soil Matrices: Implications for Understanding Natural Foci and Telluric Reservoirs of Plague

PRESENTER: Muhammad Reza Shah

COUNTRY: Pakistan

The persistence of *Yersinia pestis* in soil matrices suggests a novel, yet completely uncharacterized, environmental reservoir for plague organisms. This has critical implications for understanding natural plague foci,non-traditional transmission routes between hostsand its changing risks for humans, and finally, the detection of *Y. pestis* in environmental samples.To address this challenge, we have constructed a multidisciplinary project to examine the chemical and physical response of *Y. pestis* cells after exposure to soil habitats. The project includes high-resolution, single cell analytics to characterize the metabolism, surface chemistry, and structural changes in individual *Y. pestis* cells within this unique growth environment. Results from this work will increase basic understanding of pathogen ecology and the molecular mechanisms by which*Y. pestis* functions in environmental reservoirs outside of traditional transmission vectors.

This project is a collaboration between Virginia Commonwealth University (Richmond, VA, USA) and the HEJ Research Institute of Chemistry at the University of Karachi (Karachi, Pakistan). VCU is leading culturing efforts and surface characterization of *Y. pestis* cells using a range of high resolution microscopy and mass spectrometry techniques and the University of Karachi is developing novel techniques for chemical analysis of cell surfaces and in situ assays with nanoparticle-based probes. Training in Biosafety practices and microbiological characterization will facilitate collaborative pathogen research and build new capabilities at the University of Karachi that will complement its existing analytical strengths and create a new center in Pakistan capable of identifying unknown bacterial samples and conducting basic research on endemic pathogens.

Estimating Incidence and Socio-economic Impact of Brucellosis in Humans and Animals in Kajiado County, Kenya

PRESENTER: Austine Bitek

COUNTRY: Kenya

Brucellosis is a common bacterial zoonotic infection, but there is limited data on burden of the disease in Kenya for humans and animals. To generate data to inform prevention and control strategies, we conducted a longitudinal study to determine incidence of brucellosis in humans and animals and estimate its socio-economic impact at the household level among a pastoralist community in Kajiado.

All households and their livestock in four sub-locations in Kajiado County were enrolled for follow-up for 12 months from 2015 to 2016. A subset of their livestock were recruited in to the study after screening for *Brucella* using RBPT, and sera was collected every four months and tested for *Brucella* IgG antibodies using ELISA. Incidence in humans was calculated from the number who were determined to be acutely ill with brucellosis from among those who presented at study health facilities, while incidence in livestock was the number of livestock that sero-converted for brucella antibodies between two sampling points. To calculate economic losses, direct losses in livestock were calculated using parameters derived from the incidence study and literature, and the results were analyzed in an analytical economic model.

801 households with a total of 4,729 humans and their 5,746 livestock were recruited in the study. Of the household members enrolled, 52% (n=2475) were males. The mean household (HH) size was 6 persons (range 1 – 19). Average HH income over a 3 months period was $ 820 (range $100 - $ 16,000). Overall incidence rate of brucellosis in livestock on ELISA sero-positivity between the first and the second visit was 0.0076 (8/1,000) cases per animal; 3 months at-risk equivalent to 0.0304 (30 animals per 1,000 animal-year at risk). Total direct losses due to brucellosis in livestock was estimated to be KES 6.6 Million (USD 66,000). The losses due to abortion accounted for 54% of the total losses in livestock. Estimated out of pocket expenditures incurred by HHs was $12.2 per human case (range $2 -$100). This study reports a high burden of brucellosis in humans in a pastoral community in Kajiado with substantial economic losses arising from brucella infection in livestock at the household level.

Lugar Center Regional Integration: Kafkas University Partnering for Molecular Epidemiology *of Bacillus anthracis* and *Brucella* species in Turkey

PRESENTER: Ketevan Sidamonidze

COUNTRY: Georgia

It is vital that effective collaborative links are built between the National Center for Disease Control and Public Health (NCDC), Richard G. Lugar Center for Public Health and institutions in neighboring countries to tackle shared common bacterial threats. Kafkas University's veterinary school, located in the northeastern Turkish city of Kars, serves a primarily agricultural region adjacent to the Georgian/Turkish border; diseases including anthrax and brucellosis, are endemic in these regions. Molecular typing tools were outdated at Kafkas. A study using the high-resolution molecular tools available at NCDC Lugar Center on common bacterial strains (*Bacillus anthracis* and *Brucella* spp.) on both sides of the border was developed. To achieve this aim, a team at the Lugar Center supported by WRAIR provided training to researchers from Kafkas University in the use of the state-of-the-art capabilities available at the Center. The project consisted of three main technical elements: bacterial DNA extraction; molecular analysis of *B. anthracis*; and molecular analysis of *Brucella* spp. Overall, 60 bacterial isolates including *B. anthracis* (n=30) and *Brucella* spp. (n=30) from Turkey were analyzed at the Lugar Center using multiple locus variable number tandem repeat analysis (MLVA) and single nucleotide polymorphisms SNP. MLVA and SNP typing results show genetic homogeneity of Georgian and Turkish *B. anthracis* strains, which may be caused by migration of the pathogens across the Georgia-Turkey border over time. This study also resulted in successful capacity-building efforts for pathogen typing for the Turkish researchers from Kafkas University.

Regional Study of the Ecology of Anthrax Foci in Georgia and Azerbaijan

PRESENTER: Lile Malania

COUNTRY: Azerbaijan

Anthrax is a livestock-borne zoonotic disease that is endemic in the South Caucasus region. It is hypothesized that both human and environmental factors affect the migration of *Bacillus anthracis* strains across borders and throughout regions. This study evaluates the following: (1) identify regional foci and spatial risk factors of anthrax to improve surveillance and documentation of the disease; (2) assess the genetic relationships of environmental *B. anthracis* isolates to better understand the organism's ability to persist in the environment and to allow for improved epidemiologic "trace-back" of human and livestock infections; and 3) collaborate on regional issues related to the control and management of a re-emerging infectious disease by sharing local/regional predictions of risk and molecular profiles of the pathogen. Collaborators from both Georgia and Azerbaijan have collected and tested soil samples for the presence of *B anthracis.* Positive samples were molecularly characterized and were examined using multilocus variable number tandem repeat analysis (MLVA), as well as global and regional single nucleotide polymorphisms (SNPs), including a well-established Georgian SNP typing panel. Spatial genetic pattern analysis and risk factor mapping were conducted using geographic information system. In turn, this regional collaboration will provide the potential for the cooperative development of strategies for the control and trans-boundary management of this disease.

Regional Study of the Ecology of Anthrax Foci in Georgia and Azerbaijan

PRESENTER: Rita Ismayilova

COUNTRY: Azerbaijan

Anthrax is a livestock-borne zoonotic disease that is endemic in the South Caucasus region. Not much is known about the regional epidemiology of this pathogen nor the transboundary factors related to its persistence. The aim of this collaborative (Azerbaijan and Georgia) effort was to ascertain both human and environmental factors that may affect migration of *Bacillus anthracis* strains across borders and through regions. There are seven Azerbaijan rayons (Gazakh, Agstafa, Tovuz, Samukh, Gakh, Zagatala, and Balakan) bordering Georgia with recurring cases of both human and animal anthrax; from those, 30 villages were selected for sample collection. Samples were to be collected from known animal burial sites and areas of previous contamination, as evidenced by livestock outbreaks or human case records. Organisms isolated from the collected soil samples were to be characterized by biochemical methods (Gram stain, motility, Trypticase Soya Broth) and then by gamma phage. The nucleic acids extracted from presumptive positive samples were to be tested by PCR using Amplisense kits on BioRad.

A total of 804 soil samples have been collected from five rayons Nov 2015 - Sept 2016. From the 768 processed, over 200 samples exhibited colony morphology suggestive of *B. anthracis*; 10 were deemed positive by gamma phage. DNA extractions from 60 presumptives (by biochemistry and gamma phage) have been sent to Georgia Lugar Center for genotyping in April 2016 and were negative for *B. anthracis*. The samples should be retested by Tetracore PCR kit.

Environmental Surveillance of *Burkholderia pseudomallei,* Pilot Study in Lao PDR

PRESENTER: Rosalie Zimmerman

COUNTRY: Lao PDR

The environmental bacterium *Burkholderia pseudomallei* (*Bp)* is the causative agent of melioidosis and a Tier 1 Select Agent. *Bp* is endemic in tropical soils and has been found in surface waters. To investigate the distribution of this pathogen in Lao PDR, its occurrence in rivers, and associated environmental factors, we studied 23 rivers (including the Mekong) in the South, Center and North of the country, applying culture-based methods and a specific quantitative real-time PCR assay to water filters and streambed sediments. Geochemical measurements included turbidity, a proxy for suspended sediment load which was measured on-site using a turbidity meter and confirmed in the laboratory by dry weight measurements. *Bp* was present in 9% of the rivers in the dry season. In contrast, we found *Bp* in the water of 57% of the rivers in the rainy season, 35% of them with associated *Bp*-positive sediments. Turbidity correlated positively with *Bp* presence (p=0.01). All *Bp* positive rivers were situated in the South and Center of the country. Our preliminary results provide evidence for a heterogeneous spatial and temporal distribution of *Bp* in Lao PDR. The seasonal dynamics and predominant occurrence of *Bp* in particle-rich water and the lower yield in sediments suggest that *Bp* is washed out with eroded soil during periods of heavy rainfall and transported by rivers. These findings will contribute to improved *Bp* risk modelling and health management strategies. Further laboratory and statistical analyses, including additional geochemical factors, and land-cover data analyses using geographic information systems, are in progress.

Melioidosis Research Coordination Network

PRESENTER: Direk Limmathurotsakul

COUNTRY: Thailand

Melioidosis is caused by *Burkholderia pseudomallei,* a Tier 1 select agent and an environmental bacterium commonly found in tropical countries. It is estimated that 89,000 deaths were caused by melioidosis per year worldwide. However, melioidosis remains under-reported due to its diverse clinical manifestation, incapacity of bacterial isolation and identification in low-resource settings, and limitation of national notifiable diseases surveillance systems in low and middle-income countries (LMICs). The unawareness of the disease becomes problematic for policy makers, clinicians and researchers in many LMICs because melioidosis is not perceived as a threat.

To solve the problems, the Melioidosis Research Coordination Network (RCN) was developed. The RCN aims (1) to make data of total number of culture-confirmed melioidosis cases and deaths worldwide openly available for policy makers, clinicians, funders and researchers worldwide, and (2) to support connections and communications among those stakeholders.

The RCN working group will call for information from clinicians and researchers at any hospitals or institutions worldwide for the total number of culture-confirmed melioidosis cases and deaths they observed yearly from 2012 to 2016. We will curate and make those data openly available on www.melioidosis.info. The system will be quite similar to PROMED but better curation and better supports from funders and melioidosis research community. The RCN will make the information of data contributors clearly available so that funders and other researchers know where they can fund melioidosis research and find collaborators, respectively. The RCN will also make information about potential support from contributors available, including bacterial identification and biosafety support.

Enhancing Capacity for Case Detection and Diagnosis of Febrile Zoonotic-related Cutaneous Lesions in Georgia

PRESENTER: Mary Reynolds

COUNTRY: Georgia

In the summer of 2013 an outbreak of febrile rash illness in herders in the Akhmeta region of Georgia was linked to a novel *Orthopoxvirus* (OPXV). The unique genetic profile of this emerging zoonotic disease made it difficult to identify using the existing molecular assays for OPXV species. Two other OPXV isolates have been identified in Georgia. One was found in 1986 in a rodent in the southeastern part of the country; the other was recently identified by the National Center for Disease Control and Public Health of Georgia (NCDC) and the Centers for Disease Control and Prevention (CDC) Poxvirus laboratory in a retrospective analysis of a suspected anthrax case that occurred in 2010. Activities undertaken by CDC Headquarters in conjunction with the CDC South Caucasus Office are part of a collaborative effort comprising three complementary proposals. The goal of these combined efforts is to: 1) identify the geographic distribution and natural hosts of the known virus, 2) determine if any other such viruses occur in this region, 3) investigate the impact of OPXV infection on dairy production and to at-risk individuals, and 4) increase in-country capacity to detect, identify, and respond to future outbreaks. Activities are approached through a One Health framework, promoting timely, actionable communication among in-country counterparts representing the animal and human health sectors.

Enhancing Capacity for Case Detection and Diagnosis of Febrile Zoonotic-related Cutaneous Lesions in Georgia

PRESENTER: Ana Gulbani

COUNTRY: Georgia

The discovery of a new *Orthopoxvirus* demonstrates the need for poxvirus detection and diagnosis capacity in Georgia (country). Human illness caused by this virus has implications for differential diagnosis of cutaneous lesion-producing zoonotic infections, principally anthrax. Simultaneously, animal infection may impact agricultural productivity and food safety. Therefore, accurate detection and case diagnosis is important for both humans and domestic animals. In collaboration with the CDC Atlanta, we are working to enhance capacity to detect, diagnose and report *Orthopoxvirus* infections. Health-care workers will be trained to recognize zoonotic-related cutaneous lesions to detect human cases; NCDC personnel will be trained to collect clinical specimens from patients with such lesions and from the rodents; NFA and regional veterinarians will be trained to recognize lesions associated with orthopoxviruses among domestic and wild animals as well as field collection of pertinent animal specimens. Emphasis will be placed on field and laboratory biosafety. Additionally, CDC Atlanta Poxvirus Team members will provide training for animal (LMA) and human sample (NCDC) processing with rapid qPCR-based diagnostic tests and serologic assays for case diagnosis. Retrospective case diagnosis is needed as testing by rapid qPCR on anthrax-negative samples has already identified an orthopox positive sample, thus warranting further investigation on stored samples. New assays will be developed and assessed for detecting any new orthopoxvirus variants in humans, rodents, domestic and wild animals found in Georgia. Activities will result in improved capacity for efficient identification of emerging orthopoxviruses, as well as biosurveillance capacity for orthopoxviruses in human and animal populations.

Multi-Year Prospective Cohort Study to Evaluate the Risk Potential of MERS-CoV

PRESENTER: Sazaly Abubakar

COUNTRY: Malaysia

Since its first appearance in 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged as a serious public health threat of global concern. As of December 2016, the World Health Organization has been notified of 1,879 laboratory confirmed cases and the case fatality rate is estimated at 35%. Beyond its high fatality rate, significant concern lies in the potential for MERS-CoV to spread beyond the Middle East, as was recently witnessed in the South Korea. In collaboration with the Ministry of Health Malaysia (MoHM) and the Malaysia Hajj Pilgrims Fund Board (MHPFB), a multi-year cohort of pilgrims departing for Hajj from Malaysia has been established to assess the risk that MERS-CoV infection poses to travellers to the Middle East. Pre- and post-pilgrimage blood specimens were collected for serologic analysis to estimate MERS-CoV exposure rates, while survey data will be used to stratify the risk of exposure by factors such as age, gender, geographic regions visited during the pilgrimage. For the 2016 Hajj cohort, a total of 568 participants were enrolled and 367 (65%) submitted to post-pilgrimage blood draws and data collection upon their return from the Hajj. To date, paired blood samples (pre- and post- pilgrimage) from 318 participants have been analyzed by ELISA for the presence of IgG antibodies to MERS-CoV. Preliminary findings suggest at least three individuals showed increases in MERS-CoV specific IgG titers post-Hajj. Confirmatory microneutralization assay is ongoing and planning has started for the 2017 Hajj cohort studies.

Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Surveillance for Distribution and Prevalence in Kazakhstan

PRESENTER: Mukhit Orynbayev

COUNTRY: Kazakhstan

Middle East Respiratory Syndrome (MERS) is a viral respiratory illness caused by a recently identified zoonotic coronavirus, MERS-CoV, which is transmitted from camels to humans. The southern border regions of Kazakhstan contain more than 150,000 camels, which creates the potential for endemic circulation of the virus. Bats have also been associated with presence of the MERS-CoV, and there are 24 species of bats which may carry related coronaviruses. Due to camel trade with MERS-CoV endemic areas, the project hypothesizes that MERS-CoV is likely present in the Kazakhstani camel population. One of the objectives is to determine the sero-prevalence and epidemiology of MERS-CoV in adult and juvenile camels in seven regions of Kazakhstan to verify the prediction that camel populations in Kazakhstan have been exposed to and generated antibodies against MERS-CoV. Also, the MERS-CoV genetic diversity in camels across Kazakhstan will be determined as will the identity of coronaviruses in Kazakh bats. The project has been approved by DTRA and kick-off was held on 14 October 2016. The lead institute for the study, KZ's Research Institute for Biological Safety Problems (RIBSP), has submitted paperwork requesting permit from Ministry of Agriculture for sample collection for the study. RIBSP also reported that they have a small group of pre-existing samples from camels and bats in storage that may be of use to the project. RIBSP is currently seeking to identify the location of camel farms that have interaction with camels from the Middle East. Drs. Ian Mendenhall and Gavin Smith from Duke-NUS Medical School are collaborators for the study. Currently materials and reagents are being procured.

Global Health, Emerging Infectious Diseases, and Food Safety Implications of Bushmeat Consumption In Tanzania

PRESENTER: Joram Buza

COUNTRY: Tanzania

“Bushmeat”, the meat and organs derived from wildlife species, is a major source of protein in many parts of Africa, and is often hunted illegally and transported to markets in unsanitary conditions, thereby representing an important conduit for the transmission of zoonotic pathogens. Despite considerable evidence that select agents, including *Bacillus anthracis*, *Brucella* and *Coxiella,* are frequently found in animals harvested for consumption in Tanzania, their distribution in bushmeat and the related human health risks are not known. To fill this critical knowledge gap, we have initiated a program to map the distribution of especially dangerous pathogens in bushmeat from three major ecosystems in Tanzania (Serengeti, Ruaha-Rungwa, Selous-Mikumi) during wet and dry seasons to capture spatial and seasonal variation in pathogen prevalence.

Preliminary PCR based-analyses of more than 500 fresh and dried bushmeat samples collected from 25 villages in the Western Serengeti revealed nucleic acid signatures of *Bacillus anthracis* and *Brucella* species in bushmeat. Further, host species identification with PCR sequencing of the *cytochrome B* gene suggests initial misclassification by bushmeat traders in a significant fraction (~ 40%) of the samples. Together with 16S rDNA based microbiome profiling, our preliminary investigations reveal the presence of major zoonotic pathogens, in bushmeat in Tanzania, and provide an opportunity to discover novel emerging pathogens. In the long-term, our research is positioned to provide a rational basis for defining and mitigating the public health risk associated with the harvesting, trade, and consumption of bushmeat in Tanzania.

Etiology of Severe Acute Respiratory Infections in Kuala Lumpur, Malaysia

PRESENTER: Jamal I-Ching Sam

COUNTRY: Malaysia

The epidemiology of severe acute respiratory infections (SARI) in adults in Asia is relatively understudied, but of critical global importance. This is because of the potential emergence from this region of rare/new pathogens posing a pandemic threat or a danger as potential weapons of mass destruction. Malaysia, situated in Southeast Asia, a known hotspot for emerging diseases, has had previous imported human cases of SARS-CoV, MERS-CoV, and H7N9, and H5N1 in birds. Melioidosis, caused by *Burkholderia pseudomallei*, is also endemic in Malaysia. Kuala Lumpur, the capital, is a major travel hub for immigrants and tourists. We hypothesize that potential biological threats do cause SARI in Malaysia, but that these remain largely undiagnosed due to lack of awareness and laboratory diagnostic capacity. Therefore, we propose to study the etiology of SARI in adults admitted to a teaching hospital in Kuala Lumpur over 3 years. We will establish a comprehensive panel of molecular assays to detect respiratory viruses and bacterial select agents, supplementing existing diagnostics for bacteria and mycology. This will enable our centre to detect sporadic cases and outbreaks of respiratory agents which are potential global threats. We propose a workshop for biosafety practices in diagnostic microbiology laboratories, and a practical workshop to teach these newly established assays to other laboratories in the country, thereby enhancing national capacity to safely diagnose these agents, and contribute to threat reduction. In addition, a number of samples testing negative to known viral agents will be analysed by next-generation sequencing for potential viral pathogen discovery.

One Health Surveillance for Brucellosis in Armenia

PRESENTER: Valerie Ragan

COUNTRY: Armenia

Brucellosis is a highly infectious zoonotic disease caused by bacteria of the genus *Brucella*. It is reported to be the most common zoonotic disease worldwide. The disease causes abortion, infertility and reduction in milk production in animals and a serious, recurring febrile condition in man which may become chronic and may affect any organ of the body. Successful treatment of the chronic form is very difficult. No effective treatment for brucellosis in animals has been described. The incidence of brucellosis in humans in unknown, but is suspected to be between 10 and 25 times larger than indicated by the number of cases reported to public health agencies.

Prevalence of brucellosis in humans and animals in Armenia and neighboring countries is known to be significant. However, due to the fact that comprehensive surveillance for the disease is lacking in both the human and animal populations, and the lack of shared data between human and animal health authorities, little can be said with certainty about how widespread the disease is in either population.

A “One Health Surveillance for Brucellosis in Armenia” project has just been initiated whose objective is to strengthen coordination and collaboration between the Ministry of Health and the Ministry of Agriculture during epidemiological and outbreak investigations of brucellosis, and increase the understanding of the disease burden in Armenia. A project outcome will be a draft document outlining a comprehensive surveillance and control system for brucellosis in Armenia.

Acute Febrile Illness in Uganda

PRESENTER: Denis Byarugaba

COUNTRY: Uganda

Acute febrile illness (AFI) causes significant morbidity and mortality in the tropical countries including Uganda. While part of it is caused by malaria and other common treatable infections, a significant part is caused by unknown agents. VHFs have particularly occurred recently with more frequency in Uganda and the region. This study is undertaking systematic investigations to determine the occurrence of the select agents and other previously unknown highly pathogenic pathogens with potential for causing pandemic threats. The work leverages upon existing field and laboratory research capabilities within Makerere University Walter Reed Project (MUWRP) in the country. The project does sampling from already existing sentinel surveillance sites. We collect and test at least 26 samples from AFI patients per month from at least five geographically diverse hospital sentinel sites (Gulu, Mulago, Jinja, Bwera and Bombo Hospitals). In addition, disease vector distribution and mapping is being undertaken through vector collection and identification and climate data analysis for ecological niche modeling and risk assessment. The study is expected to generate data that contributes to create in-country capabilities for more robust systems for early detection and prediction of outbreaks and rapid response and to define risk factors and vector distributions important for long-term surveillance, early detection and rapid response, and intervention strategies in contribution to global biological threat reduction.

Infectious Etiologies of Acute Febrile Illness in the Azerbaijan Military

PRESENTER: Surkhay Mammadov

COUNTRY: Azerbaijan

Mortality rate attributable to infectious and parasitic diseases in Azerbaijan is 16.4 deaths per 100,000 population. After the collapse of Soviet Union, healthcare and public health deterioration created severe gaps in accessibility and allowed for emergence/re-emergence of infectious diseases. Epidemiological information about infectious syndromes in Azerbaijan is very limited, compounded by the lack of appropriate diagnostic assays and febrile illness knowledge, precipitating unconfirmed clinical diagnoses for most febrile illnesses. Infectious etiologies of acute febrile illness (AFI) among Azerbaijan military members have not been studied, thus causative etiologies are unknown. In the proposed study serum samples from patients with undifferentiated febrile syndrome will be tested for *Brucella* spp., Dengue, WNV, *F. tularensis*, rickettsia, Q fever, hantaviruses, CCHF and TBE using standard ELISA assays. Positive or indeterminate ELISA results will be confirmed using PCR or IFA. All laboratory analyses will be done at the Epidemiological Monitoring Station of the Ministry of Defense in Baku City. Most etiologies to be targeted are considered potential biological weapon agents. Identification of these along with incidence and probable transmission pathway will significantly increase preparedness of the Azerbaijan military medical system, develop sustainable biological agent detection capabilities, and contribute health surveillance data for Ministry of Defense (MoD) force health protection policy. This study will significantly improve laboratory skills for diagnosis to improve clinical management of AFI. In addition, findings will improve the capability to differentiate between infections with similar nonspecific clinical syndromes that are not currently diagnosed or are presumably underreported among military personnel in Azerbaijan.

Prevalence, Epidemiological Surveillance, and Laboratory Analysis of *Coxiella burnetii* in Georgia

PRESENTER: Ana Gulbani

COUNTRY: Georgia

Q fever is a zoonotic bacterial disease resulting from infection by the bacteria *Coxiella burnetii*. The goal of this study is to (1) investigate the seroprevalence of *C. burnetii* among exposed cattle and small ruminants across Georgia, (2) establish active surveillance and detection of cases of Q fever across Georgia, (3) collect isolates from veterinary cases, (4) implement and evaluate new diagnostic methods, and (5) study *C. burnetii* using GIS and DNA sequencing and genotyping. Samples were collected from nine regions by the National Food Agency (NFA) of the Ministry of Agriculture (MoA): 16,343 blood, 15,269 serum, 3,970 milk, and 5,156 swab samples were collected from these animals. Samples were tested by immunofluorescent assay (IFA), polymerase chain reaction (PCR), and bacteriology to detect *C. burnetii*. For IFA, 8,688 samples were tested: 218 were Phase I positive and 42 Phase I susceptive samples; and for Phase II 40 samples were positive, and 34 susceptive. In total, 1,311 samples were tested on ELISA: 1,218 were negatives, 76 positives, and 17 susceptive. We have tested 10,310 PCR samples and seven were positive. Bacteriological tests were performed on serologically positive/ susceptive samples, and PCR positive samples (n=340). Currently, we have isolated two cultures (confirmed by PCR). Due to the amount of sample processing, the project has been extended, and research is going.

Prevalence of *Brucella* Species and Bluetongue Virus Serotypes among Domestic Livestock or Ruminants in Southern Kazakhstan

PRESENTER: Nurlan Sandybayev

COUNTRY: Kazakhstan

Brucellosis and Bluetongue (BT) are high consequence infectious diseases that affect domestic animals in Kazakhstan and worldwide. *Brucella* spp. and an array of Bluetongue virus (BTV) serotypes are considered endemic in Southern Kazakhstan where the largest concentration of cattle, sheep and goat herds in the country are located. Southern Kazakhstan is thought to be at high risk for these two diseases. The project will determine the prevalence of circulating *Brucella* spp. and BTV serotypes over a period of two years in domestic cattle, sheep and goat holdings in Southern Kazakhstan. A disease survey is the first step to link distribution of brucellosis and bluetongue with potential risk factors. The project has been approved by DTRA and kick-off was held on 14 October 2016. The lead KZ partner, the Research Institute for Biological Safety Problems (RIBSP), has submitted paperwork requesting permit from Ministry of Agriculture for sample collection for the study. RIBSP has reported the BSL-3 laboratory has been validated and BSL-3 staff and KZ-32 participants received BSL-3 facility training. RIBSP has developed 13 SOPs focused on sample collection and transport which are currently being reviewed by the collaborators. RIBSP is working closely with collaborators and CBEP’s BTRIC (CH2M) to prioritize items for procurement of laboratory supplies. Dr. Risatti (University of Connecticut), Drs. Elzer and Hagius (Louisiana State University Agricultural Center) are collaborators for the study.

Identification of Etiology, Clinical Outcomes, Incidence, and Epidemiological Patterns of Hospitalized Febrile Patients in Armenia

PRESENTER: Lilit Avetisyan

COUNTRY: Armenia

Background: Hospitalized patients with fever represent a diagnostic challenge for physicians. Scant information is available on identification of etiology, clinical outcomes, and epidemiological patterns of hospitalized patients with fever in “Nork” Infectious Clinical Hospital. The goal of this study was to describe the most common causes, with a focus on zoonotic and arboviral infections.

Methods: Medical records of hospitalized patients with fever were retrospectively reviewed in 2014. Data were abstracted from medical charts of adults (≥ 18 years) with a fever (≥ 38°C), who were hospitalized (for ≥ 24 hours) in 2010–2012.

Results: Of the 600 patients whose charts were analyzed, 76% were from Yerevan and 51% were male; the mean age was 35.5 (± 16) years. Livestock exposure was recorded in 5% of charts. Consumption of undercooked meat and unpasteurized dairy products were reported in 11% and 8% of charts, respectively. The most common signs or symptoms reported were fatigue (97%), diarrhea (56%), nausea/vomiting (54%), shaking (52%), and abdominal pain (46%). The mean duration of hospitalization was 5.5 days. The most common physical examination findings reported were: pallor (64%), abdominal tenderness (52%), pharyngeal injection (43%), and lymphadenopathy (35%). Twenty-four percent of patients received antibiotics prior to hospital admission. Intestinal infections of known (30%) and unknown (21%) etiology were the most frequently reported final medical diagnoses, followed by diseases of the respiratory system (11%), infectious mononucleosis (9.5%), chickenpox (8.3%), brucellosis (8.3%), viral hepatitis (3.2%), and erysipelas (1.5%).

Conclusions: Half of the patients were diagnosed with enteric infections, nearly half of these had no clear etiologic agent. Brucellosis was the most frequently reported zoonotic disease. Solitary cases of anthrax, leptospirosis, FUO, imported malaria, rickettsiosis, and rat-bite fever were also reported. Further prospective studies are required to identify risk factors associated with febrile illnesses, as well as to estimate the burden of selected arthropod-borne and zoonotic infections.

Human Disease Epidemiology and Surveillance of Especially Dangerous Pathogens in Georgia

PRESENTER: Irma Burjanadze

COUNTRY: Georgia

Especially Dangerous Pathogens (EDPs), or select agents, represent a major concern for global public health. These highly pathogenic agents have the potential to be weaponized. Our project was designed to expand on the successes of the Cooperative Biological Engagement Program Collaborative Biological Research and TADR surveillance efforts, and examine the human disease incidence and prevalence of pathogens of public health and biodefense in Georgia. This project has three aims: (1) to study the epidemiology and clinical manifestations of selected pathogens among patients with undifferentiated fever and hemorrhagic fever/septic shock; (2) study the seroprevalence of selected pathogens in humans in Georgia; and (3) implement and evaluate diagnostic methods for selected pathogens and monitor patterns of antimicrobial resistance in identified bacterial infections. Laboratory surveillance for acute undifferentiated febrile illness (AUFI) was established in three major Georgian hospitals by the National Center for Disease Control and Public Health in collaboration with the U.S. Navy Medical Research Unit-3 and USAMRIID. Pathogens that are studied in the seroprevalence protocol include *Bacillus anthracis*, *Brucella* species, Crimean-Congo hemorrhagic fever virus, *Coxiella burnetii*, *Francisella tularensis*, Hantavirus, *Rickettsia* species, and tick-borne encephalitis virus. Furthermore, several other pathogens were tested to develop a comprehensive differential diagnostic algorithm and antibody prevalence of similar diseases. In patients treated at participating hospitals with an undifferentiated febrile illness were tested and involved in the developing process for a comprehensive diagnostic algorithm. Currently, 98.9% of seroprevalence tests and 69.4% of febrile studies are completed. These are preliminary results of screening trial; confirmatory testing continues.

The Epidemiological Status of African Swine Fever in Domestic Swine Herds in the Tavush Marz Region, Republic of Armenia

PRESENTER: Gene Godbold

COUNTRY: Armenia

The factors associated with the spread and persistence of African Swine Fever (ASF) in the Caucasus Region remain to be fully identified. It is assumed that large naïve domestic, free-ranging, and wild pig populations are critical to disease transmission and maintenance. Nonetheless, nine years since its epidemic introduction in the region in 2007, the virus that causes ASF is still circulating suggesting that an endemic cycle is established where contact between free ranging domestic pigs, wild pigs, and probably native *Ornithodoros* ticks serve as reservoirs. Thus, research is required to gather information on the epidemiological status of ASF in the Caucasus Region focusing on understanding modes of ASFV spread and persistence in the area

An active surveillance program was established in Armenia to determine the epidemiological status of ASF focusing on an area at high risk, Tavush marz. This region was the first to report the presence of ASF in Armenia in 2007 and 2010-2011. It is the main terrestrial point of entry for traffic into Armenia and it shares a border with Georgia where the disease was introduced. Most pigs in Tavush are kept in backyard operations and allowed to free-forage, providing contact with wild pigs and ticks.

A total of 1,506 pigs were sampled from small-scale farms clustered in 30 communities across the marz. Samples were taken from the sera, complete blood, and nasal swabs tested by ELISA, IPA, and qPCR. Fifty nine ticks were collected, but the *Ornithodoros* was not found among them. All samples were negative for ASFV and ASFV antibodies suggesting that AFSV is not circulating in the sampled population.

Since sporadic ASFV outbreaks in domestic pigs have continued to occur in the Caucasus, the capacity for long-term environmental survival should be investigated. Further research is required on the epidemiological status of ASF in ticks and swine populations deemed at high risk for ASF.

Investigation of Mosquito and Tick-Borne Arboviruses in Southeastern Azerbaijan

PRESENTER: Rita Ismayilova

COUNTRY: Azerbaijan

Twelve different arboviruses were identified between 1967 and 1980 in humans and animals in Azerbaijan. Despite the known presence of arboviruses, very little vector-borne pathogen research has been conducted within Azerbaijan since then. TAP-13 project is designed to identify selected arboviruses and their arthropod vectors in southeastern Azerbaijan (Lankaran, Masalli and Gizil-Aghaj State Reserve). The aim of the project was to facilitate the development of effective strategies for the biosurveillance, control, and mitigation of four arboviral pathogens (CCHF, TBE, Sindbis and WNV) and their arthropod vectors.

The project was initiated in August 2016 and is being implemented at Lankaran Anti-Plague Division (APD) with mentorship of the Republican Anti-Plague Station (RAPS). The UK Public Health collaborators provided two PCR trainings in Lankaran APD (November and April, 2016). Arthropods were collected from three regions in October 2015, April and May, 2016 using cloth dragging and livestock collection of ticks, as well as mosquito collection using CDC-light and BG-sentinel traps. Coordinates were recorded via GPS.

Out of 1,777 collected ticks, 1,606 ticks have been counted, identified, and sorted. 590 tick pools have been prepared; 46 pools have been homogenized, extracted, and tested for TBE, Tamdy and West Nile viruses, and all were negative. Out of 5,217 collected mosquitoes, 1,152 have been counted, identified, and sorted. 96 pools have been prepared. 37 mosquito pools were tested for West Nile and Sindbis viruses, and all were negative.

The project was temporarily halted, but will resume February 2017.

Analysis of Previously Identified *Rickettsia* Positive Georgian Ticks by Multi-locus Sequence Typing

PRESENTER: Roena Sukhiashvili

COUNTRY: Georgia

Preliminary studies have shown the presence of three spotted fever group *rickettsiae* (SFGR) species among ticks in Georgia: *R. aeschlimanii, R. raoultii*, and *R. slovaca*. The identity and prevalence of other *Rickettsia* species in ticks have not been determined. The overall goal of this project was to improve the surveillance of tick-borne pathogens in Georgia in the context of rickettsial diseases. This project used the Rickettsia-positive tick samples collected in Georgia from GG TAP-4 project. All tick samples were previously entered into a database, and pertinent sample information and GPS location data were recorded. The tick DNA preparations from the tick samples were tested using multi-locus sequence typing (MLST) to identify which Rickettsia species were present. Tick samples were not collected from the field; only tick DNA preparations already tested for Rickettsia were utilized. Overall, nine species were found among 12 different tick species from five different genera: *Ixodes, Hyalomma, Haemaphysalis, Dermacentor,* and *Rhipicephalus*. Geographical distribution maps of *Rickettsia* –infected ticks that were developed in eight regions eastern and western Georgia showed that the most common *Rickettsia* species were: *R. raoultii*, *R. slovaca*-also, *R. aeschlimannii*, and *R. monacensis*. For the first time the SFGR species *R. massiliae, R. monacensis*, *R. conorii* subsp. *conorii*, *R. hoogstraalii, R. helvetica* and *Ca. R. barbariae* were detected in ticks from Georgia. High prevalence and wide distribution of *Rickettsia* species among ticks make rickettsiosis a potential public health problem in Georgia.

The Role of Arboviruses as a Cause of Undifferentiated Febrile Illness in Sindh, Pakistan

PRESENTER: Erum Khan

COUNTRY: Pakistan

The objective of this study was to develop training in arboviral diagnostics and surveillance, to determine the burden of mosquito-borne viruses that can be used as biological agents of warfare. The Pakistani collaborators then trained other public health practitioners in Pakistan to expand arboviral diagnostics and surveillance capabilities. We recorded the presence of several co-circulating arboviruses in Pakistan. Our data show that a significant percentage of patients presenting with undifferentiated fever are afflicted with at least one arbovirus with 14.2% of patients infected with dengue or West Nile virus, 3% with chikungunya virus, and 10.7% with Japanese encephalitis virus. Diagnostic techniques developed through this project helped in the early diagnosis of Chikungunya during the Nov-Dec 2016 outbreak in Karachi. We published one paper disseminating preliminary project data and are currently drafting several more papers for publication in the coming year. Additionally, based on data from this project, a team member was awarded a 12-month Fellowship in One-Health by the National Academy of Science for 2016-2017.

During the next reporting period, we will complete validation via PRNT for all samples. In light of the global outbreak of Zika virus, we will screen our samples for Zika virus. We will present data at several conferences this year.

Event Logistics and Local Information

Venue Information

2017 Science Program Review Meeting Site

Hilton Alexandria Mark Center
5000 Seminary Road
Alexandria, Virginia 22311
(703) 845-1010

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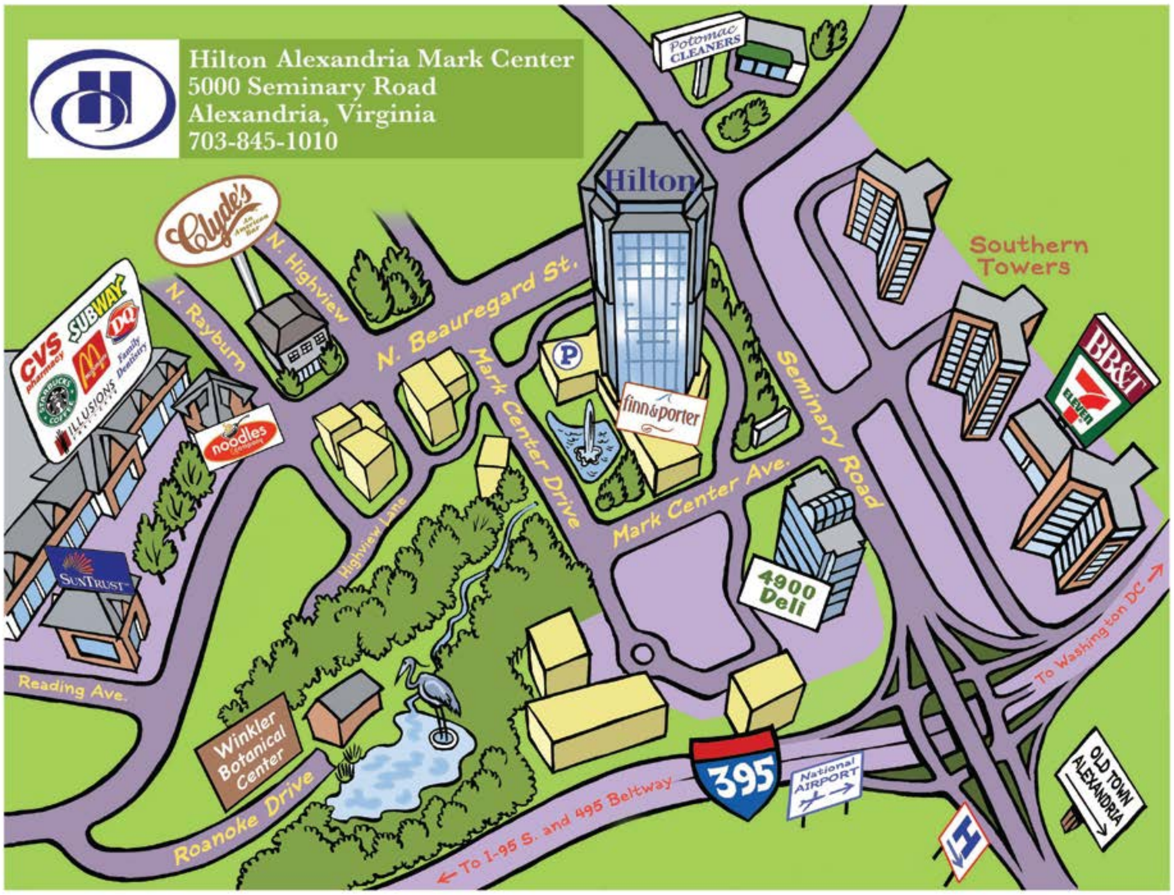
Local Information



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Uganda	Arthropod-borne Viruses Associated with the Chiroptera of Uganda: Isolation and Characterization (Call)	Kityo	Session 2: Chiroptera (Bats) - Important Reservoirs Hosts of Emerging Viruses	Thursday 8:50 AM	16
Uganda	UG-2: Research and Development of Countermeasures to Support the Control of FMDV in Uganda	Mwiine	Session 6: Transboundary Animal Diseases	Thursday 2:20 PM	35
Uganda	Acute Febrile Illness in Uganda (BAA)	Byarugaba	Session 11: Epidemiology & Biosurveillance - Group 2	Friday 1:00 PM	62

Country	Project	Speaker	Session	Day/Time	Summary Page Number
Ukraine	TAP-4: Community Outreach to Support Understanding of African Swine Fever (ASF) Ecology and Epidemiology in Eastern Europe (EE)	Datsenko	Session 5: Community Outreach to Combat African Swine Fever	Thursday 1:30 PM	31
Ukraine	TAP-6: Analysis of the Threat of Spread of African Swine Fever and Classical Swine Fever in Wild Boar Populations in Ukraine: Improving Diagnosis, Surveillance, and Prevention	Nevolko	Session 6: Transboundary Animal Diseases	Thursday 2:20 PM	34
Ukraine	African Swine Fever Threat Reduction Through Surveillance in Ukraine (USDA ARS)	Filatov	Session 6: Transboundary Animal Diseases	Thursday 2:20 PM	36
Ukraine	UP-2: Development of the Epidemiological Forecasting System for Zoonotic Diseases Employing GIS Technology	Tarasov	Session 7: Bacterial Pathogens of Security Concern - Group 1	Thursday 3:40 PM	39
Ukraine	Genomic, Epidemiological, and Biological Characterization of Newcastle Disease Virus Isolates from Ukraine (USDA ARS)	Goraychuk	Session 8: Avian Transmissible Diseases	Thursday 5:00 PM	42
Ukraine	UP-4: Risk Assessment of Selected Especially Dangerous Pathogens Potentially Carried By Migratory Birds Over Ukraine	Muzyka	Session 8: Avian Transmissible Diseases	Thursday 5:00 PM	45
Vietnam	Foot-and-Mouth Disease Virus Surveillance and Ecology in Vietnam (Call)	Dung	Session 6: Transboundary Animal Diseases	Thursday 2:20 PM	32

CURRENT ENGAGEMENTS

Africa (USAFRICOM)

- Cameroon
- Ethiopia
- Gabon
- Guinea
- Kenya
- Liberia
- Senegal
- Sierra Leone
- South Africa
- Tanzania
- Uganda

Europe (USEUCOM)

- Armenia
- Azerbaijan
- Georgia
- Ukraine

Middle East South Asia (USCENTCOM)

- Jordan
- Kazakhstan
- Iraq
- Pakistan
- Turkey
- Uzbekistan

Southeast Asia (USPACOM)

- Cambodia
- India
- Laos
- Malaysia
- Philippines
- Thailand

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COOPERATIVE BIOLOGICAL ENGAGEMENT PROGRAM

PROGRAM MISSION

The Cooperative Biological Engagement Program (CBEP) recognizes the danger to U.S. and global health security posed by the risk of outbreaks of dangerous infectious diseases. Whether natural or manmade, disease outbreaks pose a risk to the global community. CBEP strives to address this risk by promoting best practices in biological safety and security, improving partner countries' capacities to safely and rapidly detect and report dangerous infections, and establishing and enhancing international research partnerships.

PROGRAM OBJECTIVES

1. *Secure and consolidate collections* of especially dangerous pathogens (EDPs) and their associated research at a minimum number of secure facilities
2. Enhance partner country/region's capability to prevent the sale, theft, diversion, or accidental release of biological weapons (BW)-related materials, technology, and expertise *by improving biological safety and security standards*
3. Enhance partner country/region's capability to *detect, diagnose, and report* endemic and epidemic, man-made or natural EDPs, bio-terror attacks, and potential pandemics
4. Ensure the developed capabilities are designed to be *sustainable*
5. Facilitate engagement of partner country/region's scientific and technical personnel in *research areas of interest* to both the partner country/region and the United States
6. *Eliminate any BW-related infrastructure* and technologies encountered

CORE PROGRAM AREAS

Two core program areas contribute to CBEP mission success: Biosafety and Biosecurity, and Biosurveillance.

BIOSAFETY AND BIOSECURITY

Strengthen biosafety and biosecurity standards and practices. CBEP activities improve the capability of global partners to handle, store, and account for EDPs safely and securely in accordance with U.S. and international standards, guidelines, and best practices.

Objectives

- Assess existing biosafety and biosecurity capabilities for facilities, procedures, and personnel involved with EDPs
- Provide sustainable solutions to increase biosafety and consolidate and secure pathogens, to include: facility upgrades, engineering controls, training and mentorship, and personal protective equipment
- Facilitate collaborative development of country-specific biosafety and biosecurity standards and guidelines that are sustainable and promote international scientific cooperation

BIOSURVEILLANCE

Enhance Country Disease Surveillance, Detection, Diagnostics, and Reporting Capacity. CBEP works with partner countries across human and clinical, veterinary, epidemiological, and laboratory communities to enhance partner country disease surveillance, detection, diagnostics, and reporting capabilities related to EDPs.

Objectives

- Develop disease surveillance and diagnostic laboratory networks at the national, regional, and district levels to facilitate near real-time reporting of outbreak data to national authorities
- Survey suspicious disease outbreaks, analyze epidemics, and collect disease reports from veterinarians, clinicians, and epidemiologists
- Aid partner capability to comply with the World Health Organization's International Health Regulations and World Organization for Animal Health's reporting guidelines

PROGRAM ENABLERS

CBEP-sponsored research is an important enabling tool. CBEP funds and conducts research that involves partner-country scientists and directly advances strategic policy objectives.

RESEARCH

Facilitate strategic research partnerships. CBEP establishes international research partnerships and engages scientists to enhance epidemiological and diagnostic capacities related to EDPs. Engagement focuses on research areas of interest to both the partner country and the United States.

Objectives

- Prevent the proliferation of dual-use expertise
- Increase transparency and encourage high standards of openness, ethics, and conduct
- Integrate scientists and institutes into the international scientific community
- Improve partner's understanding of endemic and emerging diseases
- Share unique pathogen strains/data with the scientific community for joint research

CURRENT ENGAGEMENTS

Africa (USAFRICOM)

- Cameroon
- Ethiopia
- Guinea
- Kenya
- Liberia
- Senegal
- Sierra Leone
- South Africa
- Tanzania
- Uganda

Europe (USEUCOM)

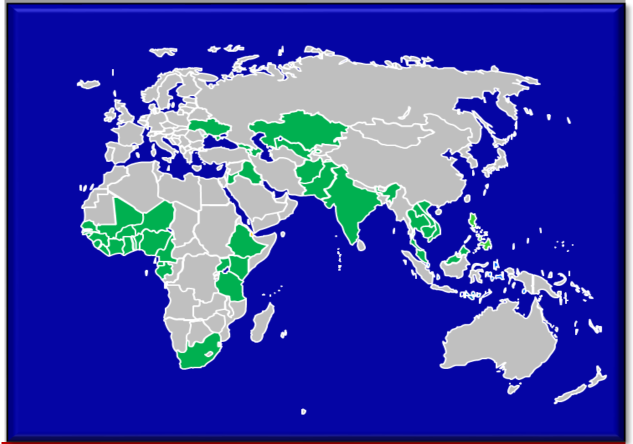
- Armenia
- Azerbaijan
- Georgia
- Ukraine

Middle East South Asia (USCENTCOM)

- Jordan
- Kazakhstan
- Iraq
- Pakistan
- Turkey
- Uzbekistan

Southeast Asia (USPACOM)

- Cambodia
- India
- Laos
- Malaysia
- Philippines
- Thailand
- Vietnam



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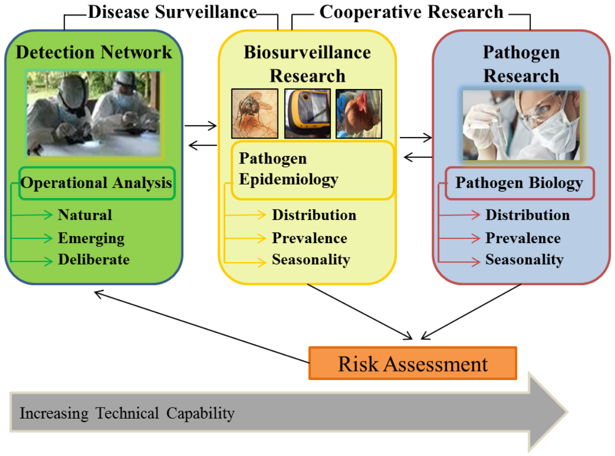
Applying for Research Funding from the Cooperative Biological Engagement Program

RESEARCH PROGRAM MISSION AND VISION:

The Cooperative Biological Engagement Program (CBEP) seeks to use cooperative international research partnerships to support its mission to reduce the threat to the U.S. and to global health security from the spread of pathogens of security concern, which includes U.S. Biological Select Agents and pathogens of pandemic potential, emerging, and reemerging infectious diseases. CBEP aims to establish and maintain these partnerships to inform and enhance operational biosurveillance systems, enhance global health security, and foster safe, secure, and sustainable bioscience capability with partner countries.

COOPERATIVE BIOLOGICAL RESEARCH

The CBEP uses Cooperative Biological Research (CBR) projects to support, enhance, and inform biosurveillance (BSV) and biosafety and biosecurity (BS&S) capabilities that reduce the threat of pathogens of security concern, while sustainably addressing infectious disease priorities of the CBEP and its partner countries.



RESEARCH FIELDS AND PRIORITIES

CBR supports and informs operational biosurveillance through an improved understanding of pathogens and their risk to global health security. In order to remain relevant, agile, and sustainable, the scope of the CBEP's research priorities include:

1. Understanding the ecology and epidemiology of pathogens of security concern
2. Differentiating pathogens of security concern

RESEARCH GOALS

The CBEP is committed to fair and open competition of research topics that enhance biosurveillance, mutually benefit the U.S. and global partner institutes, and achieve the goals outlined below:

1. Support biosurveillance and biosafety and security (BS&S) capability building efforts
2. Engage partner country scientists in ethical hypothesis-driven research resulting in high-quality data and active participation in professional societies
3. Foster sustainable partnerships with key national and international stakeholders, as well as advance partner country sustainment of global health security and one-health initiatives.

PROJECT SCOPE

The CBEP is most likely to fund projects demonstrating:

- Clear relationships to pathogens of security concern
- Links to the CBEP's threat reduction mission
- Support of BS&S and BSV capabilities
- Alignment with the CBEP and partner country priorities
- Use of sustainable techniques for partner countries in appropriate facilities
- Ethical, Hypothesis-Driven research aims

Projects focusing on or likely to result in Dual Use Research of Concern (DURC) will not be supported. Additionally, the CBEP research objectives do not include diagnostic assay development, medical countermeasures, or research focused on non-infectious diseases.

APPLYING FOR FUNDING

Research projects supported by the CBEP must align with the CBEP's mission and vision and are expected to produce results suitable for scientific publication. The CBEP welcomes research funding applications from the following domestic and foreign entities:

- Academic institutions, NGOs, industry, foreign laboratories, and private sector members through the Broad Agency Announcement (BAA)
- U.S. government partners and federally funded Research and Development Centers through the Government Service Call (Call).

ROADMAP FOR APPLICATIONS

Submitting your proposal to the BAA or the Call involves three key steps: Pre-coordination, Phase I, and Phase II. Note, each step has its own rules and requirements. A summary of each step is provided as follows:

1. Pre-coordination: This phase involves discussion and coordination between proposal offerors and the CBEP Country/Regional Manager and/or a Science Manager to ensure the proposed work is within the CBEP scope and meets CBEP priorities. Abstracts are to be emailed to the appropriate administrative email listed below. The abstract must be reviewed favorably by the CBEP prior to continuing on to a Phase I white paper. Prior to the official white paper request from CBEP, direct contact and communication is allowed. Following an invitation to submit a Phase 1 white paper, all communications must be conducted through the appropriate administrative email address listed below.
2. Phase I: Upload the Phase 1 submission with application package to www.grants.gov (BAA) or www.dtrasubmission.net (Call). Following favorable review by the CBEP, a Phase II full proposal may be requested.
3. Phase II: If invited to submit a full proposal, develop the proposal incorporating any comments from the Phase I debrief summary. Submit completed Phase II information and attachments to www.grants.gov or www.dtrasubmission.net as appropriate. Applicants must complete additional appropriate documents for BS&S review as well as documentation if research involves human or animal use.

More detailed instructions and requirements can be found through the solicitation links at www.grants.gov.

WHERE TO APPLY

For abstract submission to the BAA (HDTRA1-14-24-FRCWMD-BAA) or Service Call (HDTRA1-12-17-FRCWMD-Call) please use: HDTRA1-FRCWMD-TA6@mail.mil

For administrative questions for the BAA, please use: HDTRA1-FRCWMD-A@mail.mil

For administrative questions for the Service Call , please use: HDTRA1-FRCWMD-C@mail.mil

Navigating the Defense Threat Reduction Agency and United States Strategic Command Center for Combating Weapons of Mass Destruction Cooperative Biological Engagement Program Proposal Submission Process

Thank you for your interest in working with the Defense Threat Reduction Agency and United States Strategic Command Center for Combating Weapons of Mass Destruction's (DTRA/SCC-WMD) Cooperative Biological Engagement Program (CBEP). CBEP often works with universities, U.S. Government agencies, non-profit organizations, foreign laboratory equivalent entities, and many other organizations to implement the biological threat reduction mission through research projects.

This correspondence is intended to assist offerors in applying for DTRA/SCC-WMD CBEP funding. Information contained herein is intended to supplement, not replace, official documents including:

- DTRA/SCC-WMD's Broad Agency Announcement (BAA): HDTRA1-14-24-FRCWMD-BAA (*BAA*)
- DTRA/SCC-WMD's Government Service Call (Call): HDTRA1-12-17-FRCWMD-Call (*Call*)

These documents can be found through the solicitation links at www.dtrasubmission.net/portal/.

CBEP recommends all offerors read the applicable official document, but frequent references and citations are provided below in red to facilitate understanding of the process. Information here will help offerors answer specific questions and ease the initial stages of project/proposal development and application submission.

Frequently Asked Questions:

Question One: *Is my project appropriate for the BAA or the Call?*

The BAA and the Call both enable the same types of projects; the differentiating factor is the applying entity.

Eligible applicants under the BAA include: accredited degree-granting colleges, universities, and academic institutions; industrial and commercial entities, including small businesses with a portfolio predominantly in research; non-government organizations; not-for-profit entities with a portfolio predominantly in research; and foreign government laboratories. *BAA Section 3, pp 12- 13.*

Eligible applicants under the Call include: Federal laboratories to include Department of Defense (DoD), Department of Energy (National Labs), Department of Homeland Security (National Biodefense Analysis and Countermeasures Center, Plum Island Animal Disease Center), Health and Human Services (Centers for Disease Control and Prevention, National Institutes of Health), and U.S. Department of Agriculture (Agriculture Research Service, Animal Plant and Health Inspection Service); and DoD sponsored Federally Funded Research and Development Centers as specified in

Defense Federal Acquisition Regulation Supplement 235.017-1 and Federal Acquisition Regulation 35.017-1. *Call Section 4, pp 9.*

Question Two: *Is my project appropriate for Thrust Area 6?*

Thrust Area 6 (TA6) is intended to receive exploratory basic and applied research project abstracts, white papers, and proposals. Projects that are hypothesis driven and involve data generation in a laboratory or field environment are considered research projects and are applicable to TA6. This also includes projects that plan to display or present an analysis of data in a peer-reviewed publication or presentation. *BAA Section 1.5.6, pp 7-9; Call Section 2.1.6, pp 5-7.*

Question Three: *How can I ensure my proposal is aligned with CBEP's mission?*

At its highest level, CBEP strives to address the risk of outbreaks of dangerous infectious diseases by promoting biological safety and security, improving partner country capacity to detect and report dangerous diseases, and establishing and enhancing international research partnerships. The contribution to threat reduction is critically important to a successful white paper/proposal. Applicants have the opportunity, and are encouraged, to reach out to CBEP and coordinate project scope through submission of an abstract or concept note before applying to the BAA or Call. *BAA Section 1.5.6, pp 7-9, Section 4.2.1, pp 14; Call Section 2.1.6, pp 5-7, Section 9.2, pp 27.*

Understanding the process:

Question Four: *How long should I expect the review and award process to take?*

Receiving an award through the BAA or Service Call requires a number of key steps. Each step involves a number of factors that can influence the timeline from initial submission to award. Generally, the average length of time between an official white paper submission and project award is 12 months for both BAA and Service Call submissions. Factors influencing the process include: internal review processes, external interagency review processes, offeror submission of revised proposal documents, and contract/grant award processes. The specific timelines and deadlines for submission of documents related to each application phase will be provided in the official correspondence during the submission process.

Question Five: *What is the application process?*

Submitting your proposal to the BAA or the Service Call involves three key steps: Pre-coordination, Phase I, and Phase II. Note each step has its own rules and requirements. A summary of each step is provided in the chart on the following page.

	Thrust Area 6
Pre-Coordination	• Initiate direct communication with the Regional/Country Lead and Science Manager to determine project viability and align proposed research scope with CBEP's objectives. o *BAA Section 1.5.6, pp 7-9* o *Call Section 2.1.6, pp 5-7* • Develop an abstract of the research project and submit in the body of an email to HDTRA1-FRCWMD-TA6@mail.mil. o *BAA Section 4.2.1, pp 14; Section 7, pg 37* o *Call Section 5.1, pp 9; Section 9.2, pg 27* • Abstract must be reviewed favorably by CBEP prior to the submission of the Phase I white paper. Applicant must receive an invitation to continue on to Phase I white paper. o *BAA Section 4.2.1, pp 14-15* o *Call Section 5.1, pp 9*
Phase I – **Pre-Application White Paper**	• Please note, following an invitation to submit a Phase I pre-application white paper (white paper), communications must be conducted via the appropriate administrative email address: o *BAA: HDTRA1-FRCWMD-A@mail.mil* o *Call: HDTRA1-FRCWMD-C@mail.mil* • Develop a Phase I white paper. o *BAA Section 4.2.4, pp 14-16* o *Call Section 5.1.4, pp 10; 5.3, pp 11-12* • BAA only o Register at www.grants.gov. o Submit a completed application to www.grants.gov. o *BAA Section 4.2.4, pp 14-16* • Call only o Ensure to register with the DTRA/SCC-WMD submission website www.dtrasubmission.net. o Submit the completed application to www.dtrasubmission.net. o *Call Section 5.1-5.2, pp 9-11*
Phase II – **Full Proposal**	• Upon receipt of an invitation for a Phase II full proposal, develop a proposal and incorporate any comments from DTRA/SCC-WMD's Phase I debrief summary. o *BAA Section 4.2.6., pp 16-22* o *Call Section 5.5, pp 12-18* • All proposals involving infectious or potentially infectious materials must include a completed and signed Protocol Risk Assessment Tool (PRAT). The purpose of this form is to ensure laboratory work sponsored by CBEP is conducted safely, securely, and responsibly. The PRAT should be completed and signed by the Principal Investigator. A blank form is available via the www.dtrasubmission.net Document and Template library. • All TA6 research Phase II proposals (BAA and Call) that involve human or animal use must provide Institutional Review Board (IRB) and/or Institutional Animal Care and Use Committee (IACUC) protocols and provide provisional protocol numbers as well as IRB/IACUC point of contact information. No human/animal is permitted until BOTH the institutional review and U.S. Department of Defense approval authorities grant approval. DTRA/SCC-WMD sends all proposals through its internal Research Oversight Board and an interagency review to ensure use of best practices and a safe and secure research environment for all participants. Awardees will be notified via email when permission to begin human/animal work has been granted. This process may increase the timeline between Phase II review, funding approval, and project start. • Submit completed Phase II information including all attachments to www.grants.gov (BAA), or www.dtrasubmission.net (Call), as applicable and instructed in BAA and Call instructions. Note that Protocol Risk Assessment Tool attachments must be emailed to HDTRA1-FRCWMD-A@mail.mil (BAA) or HDTRA1-FRCWMD-C@mail.mil (Call) and NOT attached to the www.grants.gov (BAA) or www.dtrasubmission.net (Call) submission. o *BAA Section 4.2.6, pp 22* o *Call Section 5.5.3, pp 17*

CBEP Points of Contact

For clarifications or concerns, please reach out to the following CBEP representatives:

Dr. Carl Newman, Chief Scientist – carl.i.newman.civ@mail.mil
Mrs. Janet Weir, Acting Deputy Chief Scientist – janet.c.weir.civ@mail.mil
Dr. Mary Lancaster, Africa Science Manager – mary.j.lancaster5.civ@mail.mil
Dr. Gavin Braunstein, Europe & Middle East South Asia Science Manager – gavin.m.braunstein.civ@mail.mil
Dr. Marty Stokes, Southeast Asia Science Manager – martha.m.stokes.civ@mail.mil

From: Lancaster, Mary J CIV (US) on behalf of Lancaster, Mary J CIV (US) <mary.j.lancaster5.civ@mail.mil>
To: Brett, David M CIV (US); Tuttle, R Emerson CIV DTRA J3-7 (US); Marles, Jarrad M CIV (US); Stokes, Martha M CIV (US)
Cc: Christopher Broder; Laing, Eric D CTR (US); Lancaster, Mary J CIV (US)
Subject: RE: [Non-DoD Source] USU and biothreat reduction
Date: Thursday, January 4, 2018 8:18:09 AM
Attachments: 2017 CBEP SPR Program Book Final.pdf
CBEP Trifold Brochure-PAO Approved.pdf
CBEP Research Trifold Dec 2017.pdf
CBEP RD-BA Roadmap OCT2016 PAO approved.pdf

Hi David,
Thanks for arranging everything and taking the time to meet with us and provide an overview of the work you've been doing.

Attached are the updated tri-fold brochures that I handed out yesterday. I'm also attaching the Roadmap document Jarrad mentioned and the book of abstracts from the 2017 CBEP Science Program Review. The abstracts will give you an idea of the kinds of projects we currently have underway.

If you or any of your colleagues have questions, feel free to give any of us a shout and we can get the inquiry sent on to the right folks.

Cheers,
Mary

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Mary.j.lancaster5.civ@mail.mil

-----Original Message-----
From: (b) (6)
Sent: Wednesday, January 3, 2018 4:10 PM
To: Lancaster, Mary J CIV (US) <mary.j.lancaster5.civ@mail.mil>; Tuttle, R Emerson CIV DTRA J3-7 (US) <robert.e.tuttle37.civ@mail.mil>; Marles, Jarrad M CIV (US) <jarrad.m.marles.civ@mail.mil>; Stokes, Martha M CIV (US) <martha.m.stokes.civ@mail.mil>
Cc: Christopher Broder <christopher.broder@usuhs.edu>; Laing, Eric D CTR (US) <eric.laing.ctr@usuhs.edu>
Subject: [Non-DoD Source] USU and biothreat reduction

Many thanks for making the drive and your time! I attach a PDF of the slide set I used today. Best wishes (b)

(b) (6)

Sanford Chair in Tropical Medicine

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Department of Preventive Medicine and Biostatistics F. Edward Hébert School of Medicine Uniformed Services
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